THE UNIVERSITY OF
SYDNEY

BACTERAEMIA DUE TO DENTAL FLOSSING

by

Kenneth Crasta

B.D.S. (Hons)

University of Sydney

A treatise submitted in partial fulfilment of the requirements for the degree of

Master of Dental Science

Discipline of Periodontics
Faculty of Dentistry
University of Sydney

2008

CANDIDATE'S CERTIFICATE

This is to certify that the work presented in this thesis was carried out by the candidate in the Faculty of Dentistry, University of Sydney; the Periodontics clinics, Westmead Centre for Oral Health and the Institute of Clinical Pathology and Medical Research, Westmead Hospital. Any contribution made to the research by others with whom I have worked is explicitly acknowledged in the treatise. The work presented in this treatise has been submitted only to the University of Sydney for a higher degree.

Kenneth Crasta

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Associate Professor Chris Daly, for his unrelenting guidance, infinite patience and dry wit whilst steering me towards completing this research.

I would like to thank Mrs. Rachel Ma and Mrs. Angelita Bugayong at the Westmead Centre for Oral Health for their assistance in the venepuncture and blood sampling procedures and also acknowledge the work of Mr. Tom Olma and his staff from the Institute of Clinical Pathology and Medical Research, Westmead Hospital.

TABLE OF CONTENTS

LIST OF TABLES

PART A: REVIEW OF THE LITERATURE

1. Introduction

Infective endocarditis is a potentially life-threatening condition with a high risk of mortality (Netzer et al. 2000). Dental procedures have been identified as a cause of bacteraemia which can lead to infective endocarditis in at-risk patients (Roberts et al. 2000). Bacteria of oral origin are consistently implicated in the aetiology of infective endocarditis (Moreillon & Que 2004). The American Heart Association (AHA) recommends administration of antibiotic prophylaxis prior to the provision of invasive dental procedures such as scaling and root planing and extractions for at-risk individuals (Wilson et al. 2007). However, most of the evidence relating dental procedures to infective endocarditis has been based on circumstantial evidence such as case reports and it is likely that there is an overestimation of the link between dental procedures and infective endocarditis. Most studies associating dental treatment with infective endocarditis do not take into account the incubation time necessary for infective endocarditis and draw a relationship between the two events even though a long lag time may intervene. Further, just because a dental procedure precedes the onset of infective endocarditis does not mean that it actually causes it. Despite prophylactic antibiotics being used almost universally prior to at-risk dental procedures in at-risk individuals, the incidence of infective endocarditis has not altered significantly (Durack 1995). It has been suggested by several authors that low-grade bacteraemia caused by everyday physiological and patient-performed procedures may be of more importance than dental procedures in the aetiology of infective endocarditis (Guntheroth 1984, Durack 1995, Seymour et al. 2000). Whilst it is possible that invasive dental procedures will cause bacteraemia of a greater magnitude than that caused by everyday procedures, the total exposure to these

procedures by a patient in a given year is far less when compared to patient-performed procedures.

Several everyday patient-performed oral hygiene procedures such as toothbrushing, flossing, use of oral irrigation devices and use of woodsticks have been previously shown to cause bacteraemia (Lineberger & De Marco 1973, Berger et al. 1974, Carroll & Sebor 1980, Kinane et al. 2005). Most of these studies have focused on toothbrushing and any associated bacteraemia. However, only four studies thus far have attempted to ascertain the link between flossing and bacteraemia (Lineberger & De Marco 1973, Ramadan et al. 1975, Wank et al. 1976, Carroll & Sebor 1980). There are several methodological problems associated with these studies. All of them had too few patients to demonstrate any results of statistical significance with regards to bacteraemia following flossing. It was also unclear exactly how many people actually had their teeth flossed (Ramadan et al. 1975) or who did the flossing (Ramadan et al. 1975, Lineberger & De Marco 1973). Some studies made use of partial recording measures when determining the amount of periodontal disease present (Wank et al. 1976) and there was also a time gap of a week or more between measuring various indices and the actual performance of the flossing exercise (Wank et al. 1976, Carroll & Sebor 1980). On the other hand, some authors did not mention at what point they performed their periodontal data collection (Lineberger & De Marco 1973, Ramadan et al. 1975) or did not report the periodontal status of their patients after gathering all the data (Wank et al. 1976). Some studies also included skin contaminants as positive results in their bacteraemia analysis (Ramadan et al. 1975, Wank et al. 1976) or did not report the type of microbes associated with the bacteraemia (Carroll & Sebor 1980).

None of the four studies adequately addressed the issue of comparing the incidence of bacteraemia following flossing in a periodontally healthy group versus a periodontitis group. Wank et al. (1976) compared bacteraemia following flossing in the same group of patients before and after periodontal therapy was instituted but did not assess or state whether the treatment had eliminated or improved their periodontal disease status and so it is unclear whether the comparison was between a periodontally healthy and a non-healthy group. Lineberger & De Marco (1973) did have a healthy control group but did not evaluate bacteraemia following flossing in this group because they felt that it was unnecessary to do so, given the low rate of bacteraemia following flossing that they obtained in the periodontitis group. The study designs of Ramadan et al. (1975) and Carroll & Sebor (1980) precluded comparison between a periodontitis group and a periodontally healthy group.

Dental flossing is considered the most common method of interproximal cleaning recommended by dentists to their patients and the most common method of interproximal cleaning utilised by patients (Warren & Chater 1996). It should be noted that most individuals do not floss as an isolated procedure. Instead, flossing is generally performed in conjunction with toothbrushing. However, studies analysing the incidence of bacteraemia after flossing can estimate how much of a bacteraemia risk is contributed to by the action of flossing. Given the paucity of data on bacteraemia caused by flossing, there is a need for further investigations to clarify whether patients with untreated periodontitis are more at risk of oral bacteraemia due to flossing than are patients with a healthy periodontium. If it can be demonstrated that the incidence of bacteraemia after flossing is increased in individuals with

periodontitis then it may be possible to reduce this rate of flossing-induced bacteraemia by encouraging and maintaining good oral hygiene practices. Some evidence for this comes from a case-controlled study that showed that daily flossing was associated with a lower incidence of infective endocarditis (Strom et al. 2000). All these statements are, however, slightly conjectural as no study has adequately demonstrated if a difference exists in the rates of bacteraemia caused by flossing in patients with differing levels of periodontal inflammation. There remains a need to assess what incidence of bacteraemia is associated with dental flossing and if there is a difference in the incidence of flossing-induced bacteraemia between patients with periodontitis and those with periodontal health.

2. Oral bacteraemia

A bacteraemia is the presence of viable bacteria in the bloodstream. Invasive dental procedures that traumatise the periodontium such as periodontal probing, scaling and root planing, subgingival irrigation, periodontal surgery, rubber dam placement, matrix band placement and extractions can allow microbes in the gingival sulcus/pocket to gain entry into the systemic circulation and cause bacteraemia (Lineberger & De Marco 1973, Sconyers et al. 1973, Berger et al. 1974, Baumgartner et al. 1976, Lofthus et al. 1991, Roberts et al. 2000, Daly et al. 2001, Kinane et al. 2005, Forner et al. 2006, Cherry et al. 2007). It is thought that the more invasive and the longer the duration of the procedure, the greater the incidence of bacteraemia detected (Lockhart 2000). The density and volume of the microbial flora and the degree of inflammation at the site of trauma may also determine the incidence of bacteraemia. However, studies have also shown that patient-performed daily procedures such as toothbrushing, flossing and use of oral irrigation devices can also produce a bacteraemia (Cobe 1954, Berger et al. 1974, Silver et al. 1977, Carroll & Sebor 1980, Schlein et al.1991, Kinane et al. 2005, Forner et al. 2006).

It has been estimated that physiologic procedures such as chewing and everyday patient-performed events such as oral hygiene procedures combined can account for up to 5370 minutes of potential bacteraemia exposure in a month whereas a dental extraction may only account for six minutes of potential bacteraemia exposure (Guntheroth 1984), and as a consequence ‘everyday bacteraemia’ poses about a 1,000 times greater risk for bacteraemia than a single extraction. However, the magnitude of this statement should be questioned as it has recently been shown that chewing does

not cause bacteraemia (Murphy et al. 2006). Further, Guntheroth postulated that "random bacteraemia" accounted for 11% of all everyday bacteraemia but provided no evidence for its existence. Both these factors would have caused an overestimation of the bacteraemia exposure caused by everyday procedures as reported by Guntheroth (1984). Another study modified the calculations of Guntheroth (1984) and evaluated the cumulative exposure of these procedures in the general population and estimated that twice daily toothbrushing posed a 154,000 times greater risk of bacteraemia exposure than a single dental extraction over one year (Roberts 1999). However, it should be noted that the Roberts (1999) data was based on unpublished data carried out on children. Children have a greater incidence and magnitude of bacteraemia following oral manipulations (see Section 10). Roberts (1999) also assumed a baseline or background bacteraemia occurring on average once every hour which would have increased his calculations of cumulative exposure. As such, the calculations should be interpreted with caution.

3. Extra-oral sources of bacteraemia

Table 1 – Extra-oral sources of bacteraemia

Procedure	Incidence of bacteraemia (range)
Gastrointestinal tract procedures	
Oesophagogastroduodenoscopy	0-8%
Oesophageal dilation	0-54%
Endoscopic variceal ligation	3-9%
Endoscopic irrigation sclerotherapy	10-50%
Endoscopic mucosal resection	5%
Upper gastrointestinal tract endoscopy	3-12%
Colonoscopy	3-6%
Rigid proctosigmoidoscopy	0-12%
Flexible sigmoidoscopy	0-1%
Endoscopic retrograde cholangiopancreatography	6-27%
Transrectal ultrasound guided prostate biopsy	16%
Transoesophageal echocardiography	0-13%
Percutaneous needle biopsy of the liver	0-14%
Polypectomy	0-4%
Barium enema	5-11%
Genitourinary tract procedures	
Catheter insertion or removal	0-17%
Sterile urine prostatectomy	12-13%
Infected urine prostatectomy	60-82%
Transurethral prostatic resection	12-76%
Dilation of genito-urinary strictures	18-33%
Cytoscopy	0-26%
Parturition	0-5%
Caesarean delivery	11-14%
Respiratory tract procedures	
Orotracheal intubation	<10%
Nasotracheal intubation	16%
Rigid bronchoscopy	13-15%
Tonsillectomy	27-38%
Extra corporeal shock wave lithotripsy	9%

Table 1 provides a summary of the incidences of bacteraemia that have been reported following extra-oral procedures in several review articles (Everett & Hirschmann 1977, Durack 1995, Dajani et al. 1997, Durack et al. 1994, Nelson 2003a, Nelson 2003b, Gould et al. 2006a). Non-oral sources of bacteraemia include gastrointestinal,

genitourinary and respiratory tract procedures. All of these procedures with the exception of parturition and defecation are medical procedures.

The frequency of bacteraemia is considered to be low for procedures involving the gastrointestinal tract, intermediate for procedures involving the genitourinary tract and high for oral procedures (Durack 1995). The genitourinary tract is second only to the oral cavity as a potential source of bacteria that can cause infective endocarditis (Dajani et al. 1997). Current American Heart Association (AHA) guidelines state that conclusive links have not been demonstrated between the respiratory tract and infective endocarditis and that the possible association with infective endocarditis of gastrointestinal and genitourinary tract procedures has not been studied extensively (Wilson et al. 2007). The British Society for Antimicrobial Chemotherapy guidelines note that there is a lack of good epidemiological data on the impact of non-dental bacteraemia on the risk of developing infective endocarditis (Gould et al. 2006a). The British Cardiac Society guidelines note that evidence for significant bacteraemia after many gastrointestinal, genitourinary, respiratory or cardiac procedures has not been proven, although it notes that cases of infective endocarditis have been reported to follow these procedures (Ramsdale et al. 2004).

4. Infective endocarditis

Infective endocarditis is a life threatening condition where the mortality rate has been estimated to vary from 6-37% (Bayliss et al. 1983, Nissen et al. 1992, van der Meer et al. 1992a, Dwyer et al. 1994, Sandre & Shafran 1996, Netzer et al. 2000, Moreillon & Que 2004). Its morbidity and mortality remain high despite prolonged treatment with intravenous antibiotics and cardiac surgery. It is a relatively rare condition, the overall incidence of which has either stayed the same or slightly increased over the last few decades. Its incidence has been estimated to vary between 0.7 and 6.8 per 100,000 patient-years and increases with age (Drangsholt 1998, Moreillon & Que 2004). Its distribution is roughly even between the sexes and there is increased risk of developing infective endocarditis with increasing age (Nissen et al. 1992).

The most common and important complication of infective endocarditis is heart failure due to the direct effects of the proliferating vegetations on the heart valves leading to valve destruction. Severe aortic or mitral valve regurgitation and formation of fistulas and aneurysms are not uncommon sequelae. Infection of prosthetic heart valves or the endocardium can also cause thrombus formation. Fragments of this thrombus can dislodge and these emboli can lodge in other areas such as the brain, lung, coronary arteries, gastro-intestinal tract, retina, spleen and the extremities of the limbs where they may cause further complications (Ramsdale et al. 2004).

5. Bacteraemia and infective endocarditis

There is a complex interaction between bacteria, matrix components and platelets that leads to the development of infective endocarditis. The following is the sequence of events that is currently thought to lead to infective endocarditis (Freedman 1987): -

1) Damage to the endocardial surface leading to adherence of platelets and fibrin and subsequent development of a nonbacterial thrombotic vegetation (NBTV).
2) A discharge of bacteria into the blood from a local site leading to a transient bacteraemia.
3) Adherence of micro-organisms to the NBTV with further deposition of fibrin and platelets.
4) Multiplication and proliferation of organisms within the vegetation.
5) Development of local and systemic sequelae of infective endocarditis.

The entry of bacteria into the bloodstream is a necessary step in the pathogenesis of infective endocarditis but does not determine whether the patient will develop the condition. Establishing a link between a bacteraemia and infective endocarditis is difficult as although bacteraemias in infective endocarditis-susceptible individuals are common, infective endocarditis is not (Martin 2003). It is thus difficult to establish which bacteraemia-causing interventional procedures are associated with an increased risk of infective endocarditis. It is important to note that most bacteraemia in most people occurs without clinical significance and is eliminated by host defences.

6. Dental procedures and infective endocarditis

There have been no clinical trials which have provided evidence that dental bacteraemia causes infective endocarditis in humans. The evidence that dental procedures cause infective endocarditis is considered to be circumstantial and a causal association is often assumed on the basis of a temporal relation with dental treatment (Durack 1995). Most of the information regarding dental bacteraemia and infective endocarditis has been obtained from animal studies.

6.1 Animal models

Animal studies have shown a direct link between dental bacteraemia and infective endocarditis. Studies on rabbits with surgically altered heart valves found the same streptococci that had been seeded in the rabbits' mouths were isolated in their endocardium after periodontal manipulation and tooth extraction (Durack & Beeson 1972, McGowan & Hardie 1974). A study on rats with sterile aortic valve vegetations, induced via a transaortic catheter, showed that 48% of these rats developed infective endocarditis after tooth extractions if they had ligature-induced periodontitis, whereas only 6% of rats developed infective endocarditis if they had periodontal health (Overholser et al. 1987). When no extractions were performed, none of the rats developed infective endocarditis. These animal studies support the concept that endogenous bacteria in the oral cavity are able to spread via the bloodstream and cause infective endocarditis following extraction of teeth. In all studies, it was concluded that trauma to the periodontium during tooth extraction allowed ingress of bacteria.

Large numbers of bacteria are required to induce infective endocarditis in animal studies (Pelletier et al. 1975). The intensity of bacteraemia necessary to induce experimental infective endocarditis in small animals is of the order of 1 x 10^6 to 1 x 10^7 per mL (Bahn et al. 1978). However, the intensity of bacteraemia in humans following dental procedures is of the order of 1 x 10^1 to 1 x 10^2 per mL and these are considered to be much too low to cause infective endocarditis (Roberts 1999). Rather then infective endocarditis in humans being caused by one single dental procedure, it is more likely that repeated bacteraemias of lower intensity are important aetiologically.

6.2 Human studies

In humans, it is estimated that the temporal window between a specific bacteraemic event and the onset of infective endocarditis symptoms, in most cases, is less than two to four weeks (Starkebaum 1977, Durack 1995, Dajani et al. 1997, Drangsholt 1998). This depends in part on the specific bacteria involved, the magnitude of the bacterial inoculum and the immunological and cardiovascular status of the patient (Huber & Terezhalmy 2005). In a review of nine reports comprising 1,322 patients published between 1934 and 1979 associating dental extractions and infective endocarditis, it was estimated that dental extraction procedures accounted for 4% of all cases of infective endocarditis assuming an incubation period of no more that two months (Guntheroth 1984) whereas Drangsholt (1998), in a review of 116 case series from 1930 to 1996, estimated the rate of infective endocarditis associated with a recent dental procedure to be 7.5%. The risk of developing endocarditis after a dental procedure was estimated to be between one per 1500 to one per 4000 high risk dental procedures such as extractions and was considered to be very low (Drangsholt 1998).

In a large case-control study involving 273 random subjects diagnosed with community-acquired infective endocarditis not associated with intravenous drug use, dental treatment was found not to be a risk factor for infective endocarditis (Strom et al. 1998). Dental treatment during the preceding three months prior to admission in hospital was no more frequent among case subjects than among controls. Further, no specific dental treatment, with the possible exception of extractions, was associated with developing infective endocarditis. In this study, few participants had received antibiotic prophylaxis at the time of treatment and so the effect of dental procedures was not masked. This study questioned the significance of dental treatment as a risk factor for infective endocarditis.

Similar findings to the Strom et al. (1998) study were reported in another case-control study involving 342 patients where dental treatment of any sort was not found to be significant as a risk factor for infective endocarditis, assuming an incubation period of three months or less between the date of treatment and onset of infective endocarditis symptoms (Lacassin et al. 1995). In an earlier study, 132 cases of infective endocarditis diagnosed in Denmark during the 1980s were studied (Nissen et al. 1992). In 33% of the cases, a possible bacteraemia-inducing procedure occurred during the previous 30 days prior to the onset of infective endocarditis symptoms. However, none of these procedures were of a dental nature. It should be noted though that in this study, the population surveyed may have had a high level of oral hygiene and this may have confounded the results of this study.

The lack of association between dental treatment and infective endocarditis has also been reported in a prospective epidemiological study involving 427 patients in the Netherlands (van der Meer et al. 1992b). Only 11% of infective endocarditis cases underwent any potential bacteraemia-causing medical or dental procedures in the preceding 30 days. This low incidence could not be ascribed to good compliance with antibiotic prophylaxis measures. Another study concluded that as many as 60-75% of infective endocarditis cases occurred in patients in whom there was no recent history of dental or non-dental interventional procedures (Steckelberg & Wilson 1993). In yet another study of 544 episodes of infective endocarditis, 19% of cases were deemed probably dental in origin because the patient had undergone a dental procedure or had dental sepsis with or without antibiotic prophylaxis during the previous three months (Bayliss et al. 1983). However, the authors noted that this dental background was similar to those enjoying normal health. Further, in 60% of the cases the cause of bacteraemia could not be determined. This was despite streptococci being the main organism isolated in 63% of cases. No consideration was given to patient-performed procedures such as toothbrushing or flossing as possible portals for bacterial entry into the systemic circulation.

It is often difficult to determine the source of the bacteraemia that leads to infective endocarditis. It was reported in a review article that in over 50% of cases, the precipitating cause of infective endocarditis could not be determined and most cases did not have any associations with prior procedures or infectious episodes in the previous three months (Drangsholt 1998). Many retrospective studies linking infective endocarditis with dental treatment have inferred an association despite dental treatment having been performed several months prior to the onset of symptoms. An

analysis of successful infective endocarditis litigation cases in the United Kingdom revealed that, in these cases, the average incubation time from dental treatment to development of symptoms of infective endocarditis was only nine days (Martin et al. 2007). Another review of transient bacteraemia and antibiotic prophylaxis concluded that the focus on a potential cause and effect relationship between dental treatment and infective endocarditis was misplaced and paled in comparison to the more realistic association between infective endocarditis and the patient's day-to-day activities and underlying oral health (Huber & Terezhalmy 2005). It has been suggested that transient, asymptomatic bacteraemia caused by patient-performed procedures such as toothbrushing and flossing may also be important in causing oral bacteria to enter the bloodstream and subsequently lead to infective endocarditis in susceptible patients (Roberts 1999, Moreillon & Que 2004).

A population based case-control study found no association between frequency of routine dental care within the previous year, toothbrushing (use or frequency) or use of a toothpick and infective endocarditis (Strom et al. 2000). Nevertheless, the authors did find a decreased risk of infective endocarditis when the subjects flossed once a day or more compared with no use of dental floss (odds ratio of 0.64; 95% confidence interval 0.39-1.04) suggesting a protective effect from this oral hygiene practice. There was also an increased risk associated with having teeth as compared with being edentulous (odds ratio of 7.02; 95% confidence interval 1.25-39.6) when the infective endocarditis was caused by oral flora. This suggests that the periodontium is a possible route of entry for oral microflora into the bloodstream. There may also be fewer bacteria resident in the mouths of edentulous individuals. Unfortunately, this

study did not assess the periodontal status nor the presence or absence of gingival inflammation in its subjects.

It is unlikely that randomised controlled trials of infective endocarditis and dental procedures will ever be available due to the large numbers of subjects necessary. It has been estimated that over 6000 patients with cardiac disease would be required to provide enough statistical power to address this issue (Durack 1995). Although ethical concerns would once have been associated with such a project, it is increasingly evident that there now exists enough doubt for such a prospective study to be ethically acceptable (Gould et al. 2006b). Most of the recent evidence from case-control studies indicates that dental procedures are not among the chief causes of infective endocarditis. There may not be a significant difference in the frequency, magnitude, duration and nature of bacteraemia caused by a dental procedure compared with that caused by everyday patient performed procedures. The most recent guidelines for infective endocarditis prevention published by the AHA conclude that everyday bacteraemia from physiological events and patient-performed procedures may be more important in the aetiology of infective endocarditis than dental treatment (Wilson et al. 2007).

7. Antibiotic prophylaxis and infective endocarditis

No placebo-controlled, randomised clinical trial has ever been performed in humans to assess the efficacy of antibiotic prophylaxis in preventing infective endocarditis. Ideally, this level of evidence is necessary to conclusively demonstrate the need, or lack thereof, for antibiotic prophylaxis prior to dental or other invasive procedures.

Historically, due to equivocal evidence about the need for antibiotic prophylaxis prior to dental treatment, recommendations have been published by various committees in order to guide clinicians when treating patients considered susceptible for infective endocarditis. These guidelines are essentially expert opinions based on their interpretation of the best available evidence. They have been updated and amended periodically as new evidence has come to light and perspectives among the scientific community have changed.

Previously, the AHA stated that at-risk patients undergoing invasive procedures should be stratified into high, moderate and negligible risk groups before determining whether antibiotic prophylaxis was necessary prior to performing an invasive dental procedure (Dajani et al. 1997). However, the AHA recently modified these guidelines (Wilson et al. 2007) by reducing the list of cardiac conditions considered at-risk for infective endocarditis. Antibiotic prophylaxis is now restricted to those patients with the highest risk of adverse outcomes after infective endocarditis rather than those with the highest risk of acquiring infective endocarditis.

In 2006, the British Society for Antimicrobial Chemotherapy released amended infective endocarditis prophylaxis guidelines that limited the number of patients for whom antibiotic prophylaxis prior to dental procedures was considered necessary (Gould et al. 2006a). The main reason cited for this was that everyday bacteraemia may be more important in the aetiology of infective endocarditis than any bacteraemia resulting from a specific dental procedure. Indeed, in subsequent correspondence, the authors of these guidelines stated that they initially concluded that dental prophylaxis could be abandoned for all patients with cardiac risk factors, based on the available evidence, but that a stepwise approach was probably prudent in implementing any amended infective endocarditis prophylaxis guidelines (Gould et al. 2006b).

Very recently, in March 2008, the Guideline Development Group for the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom released guidelines that recommended that no antibiotic prophylaxis was necessary prior to dental procedures in any patient, regardless of the dental procedure being performed or whether the patient was at risk of infective endocarditis (National Institute for Health and Clinical Excellence Guideline Development Group 2008). The reasons cited for this paradigm shift were, once again, that everyday oral hygiene procedures presented a greater risk of infective endocarditis than a single dental procedure. In addition, the authors stated that the clinical effectiveness of antibiotic prophylaxis was not proven; there was no consistent association between having a dental procedure and the development of infective endocarditis; current antibiotic prophylaxis regimens would result in a greater loss of life through fatal anaphylaxis than a strategy of no prophylaxis; and that current antibiotic prophylaxis regimens were not cost-effective.

It should be pointed out that these recent guidelines have not received universal support and contention exists as to their merits (Ramsdale 2007).

The new Australian guidelines (Infective Endocarditis Prophylaxis Expert Group 2008**)** are similar to the current AHA Guidelines in that antibiotic prophylaxis is recommended only for those with the highest risk of adverse outcomes from infective endocarditis. Consequently, the categories of patients for whom prophylaxis are recommended have been reduced with prophylaxis no longer being recommended for those with valvular or structural heart disease, including mitral valve prolapse. In addition, indigenous Australians with a history of rheumatic heart disease are considered a special population at high risk for adverse outcomes of infective endocarditis and so antibiotic prophylaxis has been recommended for this group of patients. For dental procedures, key risk factors are the state of periodontal health of the patient and the nature and duration of the procedure. Prophylaxis is always recommended for those procedures with a high incidence of bacteraemia (>70%) whereas it is not for those with a low incidence of bacteraemia.

The protective mechanism of antibiotics in prophylaxis against infective endocarditis is not fully understood. Antibiotics may directly kill bacteria or damage them so that they can be eliminated by the host defences. These actions may be carried out when the bacteria are still in the bloodstream or after they adhere to surfaces such as the lining of the heart (Moreillon et al. 1986). Animal experimental infective endocarditis studies have shown that antibiotic administration reduces the risk of endocarditis on damaged heart valves following high levels of bacteraemia (Wright et al. 1982). In humans, administration of antibiotics has been shown to decrease the frequency of

bacteraemia detected following dental treatment (Roberts et al. 1987, Baltch et al. 1988), although it does not completely eliminate bacteraemia (Wahlmann et al. 1999). In contrast, other studies have shown that the frequency of bacteraemia is not reduced after administration of antibiotic prophylaxis (Hall et al. 1993, Hall et al. 1999). Despite the differences in results, it is currently accepted that antibiotic prophylaxis prior to dental procedures may significantly affect the nature, incidence, magnitude and duration of bacteraemia but does not completely eliminate bacteraemia (Lockhart et al. 2004).

Although antibiotic prophylaxis may significantly reduce the rate of bacteraemia detected following dental procedures, it may not have an impact on the rate of infective endocarditis following dental treatment (Imperiale & Horwitz 1990, Hall et al. 1993). A recent systematic review in the Cochrane database on the use of penicillins for the prophylaxis of infective endocarditis stated that previous studies suggesting a benefit from antibiotic prophylaxis in reducing the incidence of infective endocarditis had too small a sample size or had too long an incubation time between the dental procedure and onset of infective endocarditis symptoms (Oliver et al. 2006). The Cochrane report concluded that there was no clear evidence to support the use of prophylactic penicillins to prevent endocarditis in invasive dental procedures.

In a prospective epidemiological study over two years involving 427 patients in the Netherlands who developed infective endocarditis, it was calculated that a theoretical maximum of only 6% of infective endocarditis cases could have been prevented by antibiotic prophylaxis prior to at-risk procedures, whether dental or non-dental in nature, in at-risk patients, even if the antibiotic prophylaxis was 100% effective (van

der Meer et al. 1992b). This was assuming an incubation period of less than or equal to 30 days intervening between the procedure and the onset of infective endocarditis symptoms. The same authors have also reported that antibiotic prophylaxis was not 100% effective as five out of 25 individuals who developed infective endocarditis due to a dental procedure did so despite receiving antibiotic prophylaxis (van der Meer et al. 1992c). A more recent one-year epidemiological study of 2805 individuals in France evaluated the risk of infective endocarditis in adults with predisposing cardiac conditions who underwent dental procedures with and without antibiotic prophylaxis and concluded that a large number of prophylaxis doses would be necessary to prevent a very low number of cases of infective endocarditis (Duval et al. 2006). The risk of developing infective endocarditis was estimated at 1 in 46,000 after a dental procedure without antibiotic prophylaxis and 1 in 150,000 after a dental procedure with antibiotic prophylaxis. The authors also estimated that only 2.7% of cases, or 37 cases per year in France, could be attributed to dental procedures.

It has been reported that the advent of antibiotic prophylaxis has not reduced the overall rate of infective endocarditis in the community (Bayliss et al. 1983, Dajani et al. 1997) and that infective endocarditis may develop after a procedure even if antibiotic prophylaxis has been given (Durack et al. 1983), suggesting that antibiotic prophylaxis may not be 100% effective. To complicate matters, it appears that there is limited compliance with antibiotic prophylaxis guidelines with low numbers of patients who require antibiotic prophylaxis actually taking or receiving antibiotics (Buckingham et al. 1992) and high numbers of patients who do not require antibiotic prophylaxis actually receiving it (Epstein et al. 2000, Seto et al. 2000).

Antibiotic prophylaxis may not be 100% effective partly due to the fact that several organisms may be resistant to the antibiotic. Antibiotic resistance rates among viridans group streptococci have been increasing in the general community (Doern et al. 1996, American Dental Association Council on Scientific Affairs 2004). A recent study compared the drug resistance patterns of viridans streptococci recovered from patients diagnosed with infective endocarditis during 1971 to 1986 with those diagnosed during 1994 to 2002 (Prabhu et al. 2004). None of the strains of viridans streptococci were penicillin resistant in the earlier period whereas 13% of strains were resistant during the later period. It was also found that macrolide resistance increased from 11% to 26% and clindamycin resistance increased from 0 to 4%.

The risk of adverse effects from antibiotic use may be far greater than any benefit. Widespread antibiotic use may lead to the emergence of drug-resistant strains of bacteria, although the extent to which a single dose of antibiotic prophylaxis could be implicated in the selection of resistant microbes is unknown (Duval & Leport 2008). Antibiotic use is commonly associated with side effects such as gastrointestinal upsets, rash, diarrhoea and anaphylaxis. Fatalities due to anaphylactic reactions following a dose of penicillin have been reported, although only rough estimates exist as to their true incidence. It has been estimated that antibiotic prophylaxis may result in up to five times more deaths from anaphylactic reactions than would result from infective endocarditis if no prophylaxis was used (Bor & Himmelstein 1984, Pallasch 1989, Seymour et al. 2000). A hospital-based case-control study from Europe and India estimated the incidence of anaphylaxis related to oral Amoxycillin administration to be six cases per 1000 (Kaufman & Kelly 2003) although this rate is likely to be lower in doses administered orally for one or two doses (Cerny et al.

2000). The rate of fatal anaphylaxis following prophylaxis for a dental procedure has been reported to be 0.9 per million (Clemens & Ransohoff 1984). These authors calculated that the cost-benefit ratio of penicillin prophylaxis against infective endocarditis in patients with mitral valve prolapse would be more than $US 1,300,000 per year of life saved (Clemens & Ransohoff 1984). This is due to the fact that an extremely low number of infective endocarditis cases may be prevented even if antibiotic prophylaxis is 100% effective (Duval & Leport 2008). Antibiotic prophylaxis may also pose a barrier to patients electing to undergo dental treatment (Daly 1995). Thus, whilst antibiotic prophylaxis may be beneficial in individual cases, it may not be an effective method of reducing the incidence of infective endocarditis in the community (Morris 2007).

In conclusion, due to the lack of evidence for effectiveness of antibiotic prophylaxis and the problems associated with the development of antibiotic-resistant strains, all current guidelines have seen a reduction in the number of risk groups or, as in the United Kingdom, a paradigm shift of opinion to no requirement for antibiotics. Whilst the current AHA and Australian guidelines might seem prudent in abolishing antibiotic prophylaxis for all but those at greatest risk of adverse effects of infective endocarditis, the British guidelines are probably the most apt. Given the available evidence, completely abolishing the need for antibiotic prophylaxis would seem appropriate.

8. Dental bacteraemia and other medical conditions

Periodontal micro-organisms such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* have been isolated from atheromas (Leinonen & Saikku 2002). It has been postulated that these micro-organisms may create a pro-inflammatory and pro-coagulant environment in the vessel wall and so foster and accelerate the formation of atheromas and render the heart susceptible for later adherence and colonisation by other microbes (Li et al. 2002). It has also been hypothesised that periodontal infections serve as reservoirs for Gram-negative anaerobic organisms and lipopolysaccharide which may be a potential threat to the foetal placental unit and so lead to pre-term low birth weight babies (Offenbacher et al. 1998). Over 30% of septicaemias in acute leukemia patients originate from oral sources, particularly periodontal pockets and pericoronal infections (Greenberg 1990, Peterson & Overholser 1981). In myelosuppressed cancer patients, there are often acute exacerbations of periodontal disease that cause elevated numbers of bacteria to enter the bloodstream and cause septicaemia (Overholser et al. 1982, Peterson 1990).

Although bacteraemia of oral origin has been implicated in infections of prosthetic joints, commensal microbes found on the skin predominate in these types of infections. In two separate reviews of the literature it was concluded that dental treatment did not significantly contribute to the development of infections of these joints (Wahl 1995, Scott et al. 2005).

9. Duration of bacteraemia and bacterial clearance

The role of bacteraemia duration in the development of infective endocarditis is unknown. The duration of bacteraemia most likely reflects the volume and nature of micro-organisms that originally enter the bloodstream as well as multiple host resistance factors (Lockhart 1996). Any bacteraemia is cleared by the cells of the reticuloendothelial system consisting of phagocytic cells located in reticular connective tissue, primarily monocytes and macrophages. These cells also include the alveolar macrophages, Kupffer cells of the liver, and tissue histiocytes (Yagupsky & Nolte 1990).

It was initially believed that the reticuloendothelial cells in the lungs were of primary importance in clearance of bacteraemia (Touroff 1942). If this were the case then one would expect to find a greater rate of infective endocarditis in the right side of the heart. However, infective endocarditis is more frequently associated with the mitral and aortic valves, which are located in the left side of the heart (Delahaye et al. 1995). This might indicate that the lungs may not be very important in the clearance of bacteria or, more probably, that other factors such as disturbances in blood flow may be more important than the amount of bacteria in the bloodstream in determining which valves succumb to infective endocarditis. Evidence for this can be seen in intravenous drug users who develop infective endocarditis in the absence of pre-existing cardiac defects. Intravenous drug users often introduce microbes such as *Staphylococcus aureus* and *Staphylococcus epidermidis* into their veins when injecting themselves with non-sterile drug needles and these bacteria eventually make their way to the superior vena cava (Moreillon & Que 2004). The incidence of

infective endocarditis is much higher in the right side heart valves (almost exclusively the tricuspid) than the left side heart valves in these patients (Moss & Munt 2003), presumably because of the protection that the lungs afford to the left side of the heart via their bacterial clearance mechanisms.

The liver is also an important organ in the clearance of microbes from the bloodstream. In a classic experiment conducted in the pre-antibiotic area, it was found that bacterial levels were considerably lower in the hepatic veins when compared to arterial blood or bacterial levels in other veins, suggesting that the liver was a key organ in eliminating bacteria (Beeson et al. 1945). More evidence for the importance of the liver can be seen in that alcoholics and those with liver disease experience increased bacteraemia caused by organisms that are normally found in the gastro-intestinal tract (Filice et al. 1980, Rimola et al. 1984, Barnes et al. 1988). It is thought that the Kupffer cells in the liver phagocytose bacteria as they pass through the liver (Beeson et al. 1945). Blood from the gastro-intestinal tract enters the liver via the hepatic portal vein prior to entering the systemic circulation. Gastro-intestinal commensals are usually cleared by the liver but in the absence of optimal liver function they may cause a bacteraemia.

The spleen is also an important organ in the removal of bacteraemia by phagocytosis. Whereas bacteria are eliminated mainly via phagocytosis in the liver and the lungs, the spleen may have an important immune function to play in the removal of bacteraemia (Bohnsack & Brown 1986). The spleen is involved in the production of opsonising antibodies, which are important for the rapid and efficient removal of bacteria from the bloodstream. Although the liver appears to remove the majority of

well-opsonised bacteria from the bloodstream, the spleen also plays an important role through its ability to sequester bacteria that are not as well opsonised and this is of critical importance in the non-immune host response (Bohnsack & Brown 1986). There is an increased incidence of bacteraemia in patients with splenectomies (Filice et al. 1980).

Gram negative micro-organisms tend to be eliminated via the actions of antibody and complement mediated lysis whereas Gram positive micro-organisms are eliminated via the actions of phagocytic cells (Seymour et al. 1995). In a canine study, aerobic and anaerobic oral commensals from the oral cavity were inoculated into the bloodstream of 12 anaesthetised dogs via the external jugular vein or superficial hind limb vein (Silver et al. 1975). Blood was then collected at a superficial fore limb and the resultant bacteraemia was measured at time intervals varying from within 30 seconds to 20 minutes after this inoculation. It was reported that the maximum magnitude of bacteraemia occurred between 30 and 60 seconds and that the circulation was usually free of bacteria after ten minutes and always within 20 minutes. Blood samples taken prior to 30 seconds always showed lower bacterial counts. This was probably due to the fact that the bacteria had not had a chance to evenly disperse in the short time period since the inoculation or were still present in the lymphatics.

By injecting fluorescein into a vein and timing its appearance in the corresponding vein on the other side of the body, the blood circulation time in a human body can be calculated. The time taken for one complete circulation of the blood from the right atrium, through the pulmonary circulation, back to the left ventricle, through the

systemic circulation down to the foot and back to the right atrium is about 28 seconds whereas the corresponding time for the jugular vein is 23 seconds when the heart is beating at 70 beats per minute (Scott 1996). It has also been determined that one minute is ample time for bacteria to reach the antecubital fossa after being inoculated into the bloodstream from the oral cavity (Lazansky et al. 1949). The types of bacteria present do not seem to affect the rate of clearance from the bloodstream (Silver et al. 1975).

In a study on bacteraemia following dental extractions under general anaesthesia in 500 children with a mean age of 7.6 years, blood samples were taken at 10 seconds, 30 seconds, 1 minute, 2 minutes, 4 minutes, 7.5 minutes, 15 minutes, 30 minutes, 45 minutes and 60 minutes following tooth extraction (Roberts et al. 2006). It was reported that the duration of most bacteraemia following extractions was greater than 7.5 minutes but less than 15 minutes. Most bacteraemias were eliminated within 12 minutes. This data was derived from children and may not be relevant to adults.

Adult studies have detected bacteraemias from 10 to 30 minutes after completion of procedures such as scaling and root planing and extractions (Lofthus et al. 1991, Messini et al. 1999, Rajasuo et al. 2004, Lafaurie et al. 2007, Tomas et al. 2008). Another study evaluating bacteraemia after extractions performed under general anaesthesia detected a bacteraemia rate of 20% one hour after the dental procedure (Tomas et al. 2007). However, the authors included Staphylococci as positives in their detection rate although these may have been skin contaminants (Cockerill et al. 1997) and it was not clear whether the removal of the nasotracheal tube in these subjects

may have caused the bacteraemia which was detected in the blood samples that were taken after an hour.

Bacteraemia induced by dental procedures are generally of short duration and peak within the first minute. Following this peak, the levels of bacteria in the bloodstream decline, probably due to clearing mechanisms from the host defence system (Pallasch & Slots 1996) so that the majority of these detectable, dental procedure-induced bacteraemias are eliminated within ten minutes of being induced (Silver et al. 1975, Lofthus et al. 1991, Durack 1995). However, there are no available data regarding the effectiveness of the immune system in eliminating a continual, low level bacteraemia. This would be of particular relevance when considering possible repeated, low grade bacteraemias caused by physiological and patient-performed events.

The duration of bacteraemia is of importance when conducting experimental studies because it affects the timing of bacteraemia measurement. If blood samples are taken too early or too late the level of bacteraemia could be too low to be detected by normal culturing methods, or may be non-existent. This would lead to an underestimation of the rate of bacteraemia caused by a given dental procedure.

It should also be noted that leaving a catheter in a vein for a significant amount of time prior to the taking of a blood sample may result in increased magnitudes and/or incidences of bacteraemia that are detected. This is because the catheter may act as a focal point for the adherence of bacteria. A blood clot may form at the tip of the catheter and this too may serve to adhere bacteria. Thus, when a blood sample is taken from an indwelling catheter it will not only provide a sample of the magnitude of

bacteria flowing through the vein at that time, but also any bacteria that have adhered to the inner lumen of the catheter since its insertion (Yagupske & Nolte 1990). This adherence of bacteria to the catheter also has implications for the duration of bacteraemia that is measured. A blood sample taken from an indwelling catheter at a given time after a test procedure may provide a false-positive result for bacteraemia as any bacteria isolated may not have been physically flowing through the vein at that time but may have adhered earlier to the inner surface of the catheter.

10. Magnitude of bacteraemia

The magnitude of bacteraemia may be of importance in infective endocarditis although there are no conclusive data showing that bacteraemia of larger magnitude lead to a higher incidence of infective endocarditis. According to animal studies, large inocula of bacteria are required to produce infective endocarditis (Pelletier et al. 1975, Glauser & Francioli 1987). The intensity of bacteraemia necessary to induce experimental infective endocarditis in animals is of the order of 1×10^6 to 1×10^7 colony forming units (CFU) per mL (Bahn et al. 1978). A dentally associated bacteraemia in adult humans is usually of a low-grade intensity and of the order of <10 CFU per mL of blood (Reinhardt et al. 1982, Forner et al. 2006, Cherry et al. 2007).

Few studies exist as to the magnitude of bacteraemia detected in humans following dental intra-oral procedures or daily intra-oral activities and methodological variations in measurements make it difficult to compare the results of these studies to each other. The magnitude of bacteraemia has ranged from 0.11-1.11 CFU/mL following full-mouth hand and sonic scaling in gingivitis subjects (Forner et al. 2006) to 0.0-1.1 CFU/mL following ultrasonic scaling of a quadrant of six teeth (Reinhardt et al. 1982) to 0.1-0.7 CFU/mL following two minutes of ultrasonic scaling of five teeth in subjects with gingivitis (Cherry et al. 2007). Larger magnitudes of bacteraemia have been observed following full-mouth scaling in severe periodontitis subjects; 0.11-2.67 CFU/mL (Forner et al. 2006) and even greater magnitudes have been detected in adult subjects following extractions; an average of 2.5 CFU/mL (Hockett et al. 1977).

Bacteraemia following use of silk dental floss and interdental woodsticks has been reported to range from <1 to 11 CFU/mL (Ramadan et al. 1975) whilst a study in children has reported bacteraemia rates ranging from 0-1666 CFU/mL (average of 32.2 CFU/mL) following toothbrushing, 0-557 CFU/mL (average of 15.9 CFU/mL) following polishing, 0-93 CFU/mL (average of 2.2) following scaling and 0-4 CFU/mL (average of 0.23 CFU/mL) following extractions (Lucas & Roberts 2000). Roberts et al. (2000) also reported bacteraemia rates of 0-8CFU/mL (average of 0.3) following use of a slow drill, 0-90CFU/mL (average of 1.9) following use of a fast drill, 0-128 CFU/mL (average of 4.8) following matrix band and wedge placement and a surprisingly high 0-100,000 CFU/mL (average of 1,962) following rubber dam placement. However, the results of these latter two studies should be interpreted with caution as they were performed on children.

The age of the patient may impact on the magnitude of the bacteraemia. In children, the magnitude of bacteraemia is usually much higher than in adults and is generally inversely related to the child's age (Yagupsky and Nolte 1990). This may be due partially to the fact that children have a smaller blood volume and so any bacteraemia that they experience is automatically of a higher concentration in their blood than in the blood of an adult experiencing the same bacteraemia. There may also be a difference in the rate of bacteraemia clearance between children and adults that may account for this difference in magnitude of bacteraemia from a given exposure.

It has been hypothesised that more invasive procedures such as dental extractions produce a larger magnitude of bacteraemia than less intrusive methods such as scaling which in turn produce larger magnitudes than oral hygiene procedures such as

toothbrushing. However, there is insufficient evidence to corroborate this. The degree of inflammation at the operative site may also affect the magnitude of bacteraemia. The number of oral bacteria isolated from blood samples where bleeding was present was statistically greater than when there was no bleeding (Roberts 1999).

The timing of blood sampling directly impacts on the magnitude of the bacteraemia detected. A blood sample taken a considerable period after the performance of the bacteraemia-inducing procedure would detect a lower incidence and magnitude of bacteraemia than one taken immediately after the procedure as the host defences may have cleared most or all of the bacteraemia by then.

The site of blood collection is of importance in influencing the magnitude of bacteraemia that is detected. A peripheral site such as the antecubital fossa drains blood from the skin and superficial tissues. If a patient feels cold then their blood is preferentially diverted away from the periphery and to the core areas of the body. Similarly, if a patient fells stressed then the release of catecholamines sees vasoconstriction occur in the peripheral areas and blood is diverted to the central areas. Further, the blood that is diverted to peripheral areas preferentially goes to deeper tissues such as muscles and bone marrow which are better at eliminating bacteria than superficial tissues such as the skin and submucosal tissues (Beeson et al. 1945). The above situations may decrease the magnitude of bacteraemia detected by a cannula in a peripheral region of the body, such as the antecubital fossa, compared with a more central vein. Conversely, much of the blood to the hands often passes through special arteriovenous shunts without passing into the tissues, especially if the subject's hands are warm (Beeson et al. 1945). This may decrease the opportunity for

removal of bacteria and thus the magnitude of bacteraemia detected in the antecubital fossa may be greater due to this phenomenon.

The site of blood collection is also of importance in another aspect. The magnitude of bacteraemia experienced by the right side of the heart is greater than that experienced by a site such as the antecubital fossa since the bacteria have not traversed the lung's reticuloendothelial system when they first arrive at the right side of the heart. Thus, when the blood returns from the lungs to the left side of the heart and is pumped out again, eventually returning through the veins where the sampling site is present, the bacterial levels in the blood are considerably less. Consequently, the bacteraemia levels detected at a site such as the antecubital fossa may underestimate the magnitude of the bacteraemia shower experienced by the heart. It is the levels of bacteria experienced by the heart that are ultimately important in the risk of developing infective endocarditis.

The magnitude of bacteraemia detected is also affected by the timing of blood sampling after a procedure as it takes about 30 seconds for the blood to traverse through the cardiovascular system to reach the antecubital fossa. In a study involving 229 children, blood samples were taken at 10, 30, 60, 90, 120, 180 and 600 seconds after tooth extraction and the incidence of bacteraemia detected at these times compared (Roberts et al. 1992). It was found that the highest incidence of bacteraemia occurred when the blood sample was collected at 30 seconds after the extraction. At 30 seconds, 56% of the blood samples tested positive for bacteraemia whereas at 90 seconds and 600 seconds, 38% and 28% respectively of blood samples tested positive for bacteraemia. This suggests that the number of bacteria in the bloodstream is

rapidly reduced, probably due to the actions of the reticulo-endothelial system. If there is a considerable delay between a procedure and the collection of a blood sample, then a lower magnitude of bacteraemia may be detected. However, the maximal magnitude of bacteraemia (and therefore the maximum chance of detecting a bacteraemia) may not necessarily always occur 30 seconds after a given procedure but could occur 30 seconds after the point in a procedure when the largest bacterial inoculum reaches the bloodstream. This could occur at any given time during the procedure and if bacteraemia is assessed at 30 seconds after cessation of the whole procedure, most of this large inoculum of bacteria might have been cleared away by the host defences and a lower level of bacteraemia will be detected.

11. Nature of bacteraemia.

The "nature" of bacteraemia refers to the microbial profile of a bacteraemia. In humans, it has been shown that some of the most common microbes associated with infective endocarditis are commensals found in the oral cavity. In an early study, it was demonstrated that *Serratia marcesens* bacteria introduced into the gingival crevice were transported into the bloodstream during traumatic dental procedures such as extractions (Burket & Burn 1937).

Viridans streptococci are the most commonly isolated microbes from community acquired native valve infective endocarditis vegetations not associated with intravenous drug use (Fowler et al. 2005). Fiehn et al. (1995) isolated and identified the same streptococcal strains from the oral cavity and bloodstream of infective endocarditis patients. However, it should be noted that these bacteria are also found in other parts of the body such as the skin, respiratory tract and the gastro-intestinal tract and their presence in infective endocarditis lesions does not necessarily mean that they had an oral origin (Durack 2000).

Viridans streptococci are considered to be important in the pathogenesis of infective endocarditis because of their ability to attach themselves to the surface of cells and to aggregate platelets (Manning et al. 1994, Herzberg & Meyer 1996). They possess numerous adherence factors that may mediate their binding to extracellular matrix proteins such as fibronectin in the endocardium (Jenkinson & Lamont 1997). Anaerobic microbes are commonly associated with periodontal inflammation and are

often associated with bacteraemia. However, they are rarely associated with infective endocarditis (Pallasch 2003).

Streptococci, along with staphylococci and enterococci species, have been identified as the main pathogens of infective endocarditis, accounting for about 80% of cases (Barco 1991, Dwyer et al. 1994, Delahaye et al. 1995, Netzer et al. 2000, Moreillon & Que 2004) and the viridans streptococci account for 30% of cases of infective endocarditis (Dwyer et al. 1994, Netzer et al. 2000).

Bacteria such as Staphylococci and Propionibacterium species are often isolated in studies dealing with oral bacteraemia. The consensus is to attribute their detection to contamination from the microflora of the skin since a contamination rate of 2-3% can be expected in experiments involving bacteraemia (Pierce et al. 1986). However, some authors have considered the detection of these organisms in the blood following oral procedures as true oral bacteraemia (Tomas et al. 2007). Staphylococci have been detected in more than half the samples isolated from the gingival sulcus, palate and floor of mouth in subjects with no periodontal disease and in 71% of samples taken from those with periodontal disease (Murdoch et al. 2004). If skin contaminants are suspected, then quantification may also be necessary to distinguish them from true bacteraemia. Any bacteraemia caused by skin contaminants is of low magnitude and is generally of <1CFU/mL (Dorn et al. 1981). However, no quantitative criterion can adequately distinguish true bacteraemia from contamination (Yagupsky & Nolte 1990).

12. Effect of periodontal inflammation on oral bacteraemia

There seems to be conflicting evidence as to whether increased levels of inflammation in the periodontium are associated with increased levels of bacteraemia. It is assumed that the extent of periodontal disease correlates with the frequency, nature, magnitude and duration of bacteraemia but this statement may be presumptuous. However, it cannot be assumed that manipulation of a healthy mouth reduces the risk of a bacteraemia compared to a mouth with periodontal disease (Wilson et al. 2007, National Institute for Health and Clinical Excellence Guideline Development Group 2008). Animal studies tend to indicate that the presence of periodontitis models is associated with an increased chance of developing infective endocarditis. Further, the number of microbes entering the bloodstream may not be as important as other factors such as the ability of microbes to adhere to the heart valves (Glauser & Francioli 1987). Maintaining a healthy dentition and periodontium through proper oral hygiene practices plays an important part in preventing endocarditis in at-risk patients (Horstkotte et al. 2004, Gould et al. 2006b, Wilson et al. 2007) but this has yet to be conclusively demonstrated.

It has been observed as early as 1935 that individuals with gingival or periodontal inflammation may be at far higher risk of bacteraemia than those with periodontal health (Okell et al. 1935). The number of bacteria associated with shallow, healthy sulci has been estimated to be in the magnitude of 1×10^3 whereas the number associated with periodontal pockets has been estimated to be greater than 1×10^8 (Socransky & Haffajee 1997). There is also a qualitative change in the microflora that occurs in the transition from periodontal health to periodontal disease. However, this

may not be relevant in determining the type of bacteria associated with a bacteraemia as the overall numbers of the initial colonisers such as Streptococci increase as well. Indeed, as mentioned earlier, most of the bacteria detected in the bloodstream after oral procedures are Streptococci or Staphylococci (Pallasch & Slots 1996).

The total dentogingival surface area of an individual with gingival health is estimated to be about 5 cm^2 whereas in individuals with periodontitis, the mean dentogingival surface area is far greater and ranges from 8 cm^2 to 20 cm^2 (Hujoel et al. 2001). This is smaller than the range of 50 to 200 cm^2 previously assumed (Offenbacher et al. 1998, Scannapieco 1998). However, it still presents a significant surface area for the ingress of bacteria into the systemic circulation. Furthermore, a significant proportion of this area in an individual with periodontitis is composed of ulcerated epithelium and the integrity of the epithelium is decreased through alteration of intercellular substance by various enzymes. Penetration of the epithelial barrier is probably associated with the vast extent of intercellular spaces and the lack of desmosomes, especially in the junctional epithelium (Saito et al. 1981). This lack of epithelial barrier function aids the ingress of bacteria into the underlying tissues.

With inflammation, there is an increase in the number of capillaries and an increase in the amount of blood present in these capillary beds. It has been hypothesised that these engorged capillaries may be torn or perforated by oral hygiene procedures, thus creating negative pressures and allowing microbes to be aspirated into the bloodstream (Romans & App 1971, Roberts 1999). The gingival margin also does not adapt to the tooth as tightly as in health and is easily separated from the tooth surface during oral hygiene procedures.

Previous AHA guidelines recommended antibiotic prophylaxis for procedures for which bleeding may be anticipated (Dajani et al. 1997). However, there is no conclusive evidence to indicate that the presence of bleeding during a procedure may be a reliable indicator of bacteraemia. In an animal study, it was shown that bacteria can enter the circulation via the lymphatic system rather than direct invasion of the blood system (Barnes & Trueta 1941). Capillaries exert a positive pressure gradient that can flush bacteria away from capillary leaks. Thus, the presence of bleeding may be less relevant than factors such as muscle contraction which enhances lymphatic flow (Guntheroth 1984).

In a study evaluating the incidence of bacteraemia after periodontal probing in 40 patients, there was a 40% incidence of bacteraemia in individuals with periodontitis compared with a 10% incidence of bacteraemia in individuals with gingivitis (Daly et al. 2001). This amounted to an odds ratio of 6.0 (95% confidence interval 1.081 to 33.215) for bacteraemia in the periodontitis group compared to the gingivitis group. The incidence of bacteraemia caused by toothbrushing was also shown to increase significantly when gingival inflammation increased (Silver et al. 1977). Madsen et al. (1974) showed that there was a significant reduction in bacteraemia following toothbrushing and use of a toothpick once patients with periodontal disease were treated and returned to periodontal health. Forner et al. (2006) reported a positive but weak correlation between gingival inflammation levels and the incidence of bacteraemia following scaling.

Gingival bleeding caused by oral hygiene measures such as toothbrushing has been identified as a mechanism for bacterial penetration into the underlying tissues (Sconyers et al. 1973). This is especially the case in individuals with poor oral hygiene and gingival inflammation, and is thought to be due to minor trauma to the gingival tissues (Schlein et al. 1991).

Flossing, like toothbrushing, also causes minor trauma to the gingiva and disrupts the junctional epithelium attachment (Waerhaug 1981). Flossing may have more of a potential for introducing bacteria into the circulation than toothbrushing since the flossing action is more similar to rubber dam placement. In a study evaluating the incidence and magnitude of bacteraemia in children, it was noted that the incidence of bacteraemia following rubber dam placement was 31.4% (Roberts et al. 2000). The authors ascribed this unexpectedly high incidence following rubber dam placement to the mechanisms inherent to rubber dam placement, which involve "interproximal rubber being forced down between the teeth and at the same time pushing the dental plaque ahead of it and then squashing it onto the inflamed gingivae". The act of flossing involves a similar action and so a high rate of bacteraemia might also be expected following flossing.

New epithelial attachment is evident from three days onward after flossing and the flossed junctional epithelium only resembles the unflossed junctional epithelium at two weeks post-flossing. If an individual flosses daily then the junctional epithelium is kept in a continuous state of disruption and healing without ever being fully healed (Waerhaug 1981). The significance of this, however, is not clear and it may be that

the disruption of the junctional epithelium may be classified as a physiological event rather than a pathological condition (Waerhaug 1981).

Inflammation may also extend the duration of bacteraemia. A study on mentally handicapped children found that the duration for a dentally induced bacteraemia extended beyond 30 minutes (Messini et al. 1999). The authors ascribed the increased duration of these bacteraemias to the poor level of oral hygiene evident in these children. However, no assessment of plaque or gingival status was undertaken in this study to corroborate this statement and therefore the effect of increased inflammation on the duration of bacteraemia is unclear.

On the other hand, the risk of bacteraemia may not be increased in subjects with periodontal disease. There was no clear association between severity of periodontal disease and detection of bacteraemia following toothbrushing (Kinane et al. 2005) and extractions (Lazansky et al. 1949). In a prospective, randomised, controlled trial of 59 subjects, it was found that oral hygiene and periodontal status did not significantly affect the incidence of bacteraemia following extractions (Wahlmann et al. 1999). Subjects with the deepest pockets were not necessarily those in whom bacteraemia was detected. A higher frequency of bacteraemia after scaling and root planing was detected in aggressive periodontitis subjects than in chronic periodontitis subjects even though the former had lower levels of gingival inflammation than the latter (Lafaurie et al. 2007). A recent study reported no correlations between periodontal indices and the incidence of bacteraemia following surgical extractions of third molars (Tomas et al. 2008).

Croxson et al. (1971) demonstrated eight cases of viridans Streptococci endocarditis in edentulous patients, suggesting that these bacteria of oral origin entered the bloodstream through other means. Bacteraemia can occur in patients with healthy gingiva as well as in edentulous patients and the periodontal ligament may not be the only portal of entry for oral bacteria. Gingival trauma and inflammation may be only a few of the many factors contributing to the incidence of bacteraemia. The presence of oral inflammation alone correlates poorly with an increased risk of bacteraemia-induced complications and other factors such as the underlying health and immune status of the patient, the site and extent of mucosal exposure and the quantitative and qualitative nature of the oral microflora may be more important in determining the risk of infective endocarditis (Lockhart & Schmidtke 1994, Lockhart 1996).

13. Oral hygiene aids and bacteraemia.

Procedures such as toothbrushing, flossing, use of woodsticks and oral irrigation devices may cause trauma to the gingival epithelium and allow bacteria to gain access to the underlying tissues (Sconyers et al. 1973, Wank et al. 1976, Kinane et al. 2005, Forner et al. 2006). Roberts (1999) has estimated the cumulative exposure to bacteraemia generated by various dental procedures by taking into account the total time exposure as well as the intensity of the inoculum and the estimated number of procedures experienced over a one year period. The potential cumulative exposure in the community of twice a day toothbrushing was found to be 154,219 times greater than that of a simple extraction in children whereas the cumulative exposure due to daily flossing in adults was found to be 267,070 times greater than that of a simple extraction of a permanent tooth. Although problems exist with the calculations (discussed in Section 2), the cumulative bacteraemia exposure from oral hygiene procedures still far outweighs that from dental procedures.

When contaminants are excluded, the types of bacteria isolated and cultured from blood samples after oral hygiene manipulations are found to be normal inhabitants of the gingival sulcus (Romans & App 1971, Wank et al. 1976, Kinane et al. 2005). Oral hygiene aids such as toothbrushes, woodsticks, oral irrigation devices and dental floss have been shown to cause bacteraemia. Table 2 summarises the methodology of several studies evaluating oral hygiene aids and bacteraemia. It should be noted that significant variations exist in the clinical and laboratory protocols between all these studies and these may partly account for the wide variation in the results obtained.

Table 2 – Summary of studies evaluating oral hygiene aids and bacteraemia

Authors	Oral hygiene method studied	Number of subjects	Periodontal status of subjects	Clinical criteria assessed for correlation	Time taken for oral hygiene procedure	Timing of blood sampling	Microbiological method used	Bacteremia level identified
Cobe et al 1954	Brushing	305	Not stated	None used	Not stated	Not stated	Aerobic and anaerobic culturing	24.20%
Tamimi et al 1969	Brushing & oral irrigation device.	30	10 each with no gingival inflammation, gingivitis and periodontitis	Full mouth radiographs, PPD.	Not stated	2 minutes and 10-15 minutes after brushing.	Aerobic and anaerobic culturing	0%
Felix et al 1971	Oral irrigation device	30	Generalised periodontitis of at least 10 teeth	Not stated	1 minute	60 seconds use of oral irrigation device	Aerobic and anaerobic culturing	50%
Romans & App 1971	Oral irrigation device	30	All had chronic gingivitis	BOP, PPD 3mm or less, PI (Silness & Loe 1964), GI (Loe & Silness 1963)	1 minute	1 minute after use of oral irrigation device	Aerobic and anaerobic culturing	7%
Sconyers et al 1973	Brushing	30	Periodontitis	PPD, mobility, PI (Silness & Loe 1964), radiographic bone loss	4 minutes	During the 4th minute of brushing	Aerobic and anaerobic culturing	16.7% in periodontitis patients
Lineberger & de Marco 1973	Flossing & interdental sticks	21	Generalised periodontitis with PPD of > 3mm	PPD	2 minutes + 30 seconds extra during blood collection	2 minutes after start of flossing or use of stimudent	Aerobic and anaerobic culturing	36%
Berger et al 1974	Oral irrigation device & brushing	30	No gingivitis by direct examination, no history of periodontitis	Visual assessment of gingivitis	1 minute	60 seconds after use of oral irrigation	Aerobic and anaerobic culturing	27% after oral irrigation, 0% after brushing
Madsen 1974	Brushing & toothpicks	29	16 with gingivitis and 13 with periodontitis	Full mouth radiographs, PPD, GI (Loe & Silness 1963), PI (Silness & Loe 1964) - done 2 days prior to procedure	2.5 mins brushing followed by 2.5 mins toothpick	Within 1 minute after procedure	Aerobic and anaerobic culturing	21.8%
Ramadan et al 1975	Flossing & interdental sticks	50	All had at least 1 PPD of 4mm or more	All had PPD of 4mm or more	Not stated	10 minutes after flossing or interdental stimulation	Aerobic and anaerobic culturing	18%
Wank et al 1976	Brushing, flossing & perio-aid	21	Varying degrees of periodontitis or periodontal health	PPD, Gingival periodontal index, Irritant Index	Brushing for 5 mins. Not stated for flossing and perio-aid	Within 90 seconds following the procedure	Aerobic and anaerobic culturing	10% for brushing, 22% for flossing 14% for perio-aid

PPD – Periodontal probing depth, BOP – Bleeding on probing, PI – Plaque index, GI – Gingival Index

Authors	Oral hygiene method studied	Number of subjects	Periodontal status of subjects	Clinical criteria assessed for correlation	Time taken for oral hygiene procedure	Timing of blood sampling	Microbiological method used	Bacteremia level identified
Silver et al 1977	Electric brushing	96	Not mentioned	Modified PI (Silness & Loe 1964) and modified GI (Loe & Silness 1963) - both performed without probing	2 minutes	Last 30 to 60 seconds of brushing	Aerobic and anaerobic culturing	42.7%
Sconyers et al 1979	Brushing	50	"Orally healthy". PPD<4mm. No BOP	PPD, Navy Periodontal Disease Index, Navy Plaque Index	4 minutes	During the 4th minute of brushing	Aerobic and anaerobic culturing	0%
Silver et al 1979	Brushing	36	No evidence of clinical inflammation	Modified PI (Silness & Loe 1964) and modified GI (Loe & Silness 1963) - both performed without probing	2 minutes	Last 30 seconds of brushing	Aerobic and anaerobic culturing	8.33%
Carroll & Sebor 1980	Flossing	4	2 with no oral disease, 2 with marginal gingivitis	Not stated	3 minutes	3 minutes after start of flossing	Culturing - method not detailed	37.5%
Chung et al 1986	Brushing	16	11 with good oral hygiene , 5 with poor oral hygiene	GI (Loe & Silness 1963), PI (Silness & Loe 1964)	2 minutes	15 minutes after brushing	Aerobic and anaerobic culturing	62.5% prior to brushing, 18.75% after brushing.
Schlein et al 1991	Brushing	20	Not stated	PI (Silness & Loe 1964) performed least 20 minutes before procedure. GI in response to probing.	2 minutes	3 minutes after brushing	Aerobic and anaerobic culturing	25%
Roberts et al 1997	Brushing	52	Not assessed	None used	1 minute	30 seconds after brushing	Bactec 760 and Bactec radiometric	39%
Bhanji et al 2002	Brushing & electric brushing	50	Not stated - most had only mild inflammation	Modified GI (Loe & Silness 1963), PI (Silness & Loe 1964)	1 minute	30 seconds after brushing	Bactec	46% after manual brushing 78% after electric brushing
Hartzell et al 2005	Brushing	30	Low gingival index scores	Periodontal screening & recording (PSR)	1 minute	30s and 20 minutes after brushing	Bactec	0%
Kinane et al 2005	Brushing	30	Untreated periodontitis (no criteria stated)	PPD, BOP, mobility, 6PPC - all recorded at a previous visit.	2 minutes	Within 3 minutes after brushing	Bactec and PCR	3.33% detected by Bactec 13.33% detected by PCR.
Forner et al 2006	Brushing	60	20 each with good oral health, gingivitis and periodontitis.	PPD, GI (Loe & Silness 1963), PI (Silness & Loe 1964), BOP, CAL - performed 1 week prior to procedure.	2 minutes	0.5, 10 and 30 minutes after brushing	Lysis filtration under anaerobic conditions	3.33% overall (10% of periodontitis patients, 0% of healthy & gingivitis patients).

PPD – Periodontal probing depth, BOP – Bleeding on probing, PI – Plaque index, GI – Gingival Index

14. Oral irrigation devices and bacteraemia

Four studies have evaluated the rate of bacteraemia following the use of oral irrigation devices (Table 3). The rate of bacteraemia detected according to these four studies ranged from 0-50%.

Table 3 – Rate of bacteraemia following use of an oral irrigation device

Authors	(N)	Rate of bacteraemia
Tamimi et al. 1969	30	0%
Felix et al. 1971	30	50%
Romans & App 1971	30	7%
Berger et al. 1974	30	27%

Tamimi et al. (1969) found no evidence of bacteraemia following use of an oral irrigation device (Water-Pik, Aqua Tec Corp, Colorado) after toothbrushing in adults with periodontal health (n=10), gingivitis (n=10) or periodontitis (n=10). The incidence of bacteraemia was compared with bacteraemia after toothbrushing alone. However, there was no evaluation of bacteraemia after use of an oral irrigation device alone which would have been beneficial in order to ascertain the bacteraemia contribution made solely by the oral irrigation device. More detailed analysis of this study is presented in Section 15 that deals with toothbrushing and bacteraemia.

Felix et al. (1971) reported a rate of bacteraemia of 50% after use of an oral irrigation device in 30 adults with untreated generalised periodontitis. All subjects had at least

ten teeth that were affected by periodontitis, although the authors did not state what they considered to constitute periodontitis, nor did they report use of any indices to assess this. The authors reported no correlation between age, sex, smoking habits or blood pressure and the incidence of bacteraemia. After an initial instruction session on proper usage, the subjects used the oral irrigation device (Water-Pik) themselves with the pressure gauge set at five for approximately one minute. There would have been considerable variation in the method of oral irrigation device use by the subjects, especially considering the fact that they had never used an oral irrigation device before. The blood sample was taken 60 seconds after completion of irrigation. Detection via microbial culturing revealed that 53% of microbial isolates were Streptococci and 18% were Staphylococci. The authors did not exclude the Staphylococci isolates as contaminants as they stated that these were among the most common inhabitants in the gingival crevice. However, Staphylococci are also present on skin surfaces, and their presence in the positive samples here may have indicated that the blood samples were contaminated by bacteria found on the skin during collection procedures. If the Staphylococci isolates had been excluded, then the rate of bacteraemia would have been lower than the reported 50%. However, due to the lack of data provided by the authors, it is not possible to calculate what this lowered incidence would have been. The authors concluded by advocating that caution should be used when recommending the use of oral irrigators to patients at risk for infective endocarditis.

The same group of researchers found a non-significant post-oral irrigation bacteraemia rate of 7% in 30 dental students with generalised mild chronic gingivitis (Romans & App 1971). This was defined as patients who had probing depths of ≤

3mm and slight haemorrhage from the papilla upon gentle probing but without haemorrhage from the facial or lingual crevicular areas. Periodontal probing was performed at least 15 minutes prior to the experimental procedure in order to minimise the chances of a positive control blood sample. The oral irrigation procedure was performed by the subjects themselves for one minute after an instruction session on proper standardised usage. A Water-Pik oral irrigator was utilised on a dial setting of five. The authors did not report exactly when this instruction was provided. A post-irrigation blood sample was taken at 60 seconds after cessation of irrigation. Only two subjects tested positive for a bacteraemia post-irrigation. Once again, as per their previous study, there was no correlation between age, sex, blood pressure or smoking habits and any detectable bacteraemia. Neither of these studies utilised subjects with periodontal health for comparison.

Berger et al. (1974) evaluated bacteraemia in 30 adults with periodontal health and reported an incidence of 27% at 60 seconds after the use of an oral irrigation device (Water-Pik). They concluded that the use of oral irrigation devices were contra-indicated in patients at risk for infective endocarditis. However, the level of periodontal health in their subjects was assessed only by visual examination to exclude gingivitis and by ascertaining that the subjects had no previous recorded history of periodontitis. This screening procedure may have potentially underestimated the levels of periodontal disease in this subject population. Subjects in this study were also instructed to abstain from toothbrushing on the morning of the study. The subjects used the oral irrigation device on themselves for 60 seconds after minimal instruction and so standardisation of the use of the irrigation device may not have been achieved in this study. To compound this, the setting used on the oral

irrigation device varied between the subjects, with the middle and maximum settings being used randomly from subject to subject. No data was provided regarding whether it was the maximum setting that was associated with any gingival bleeding, or indeed any bacteraemia. The authors found a statistically significant correlation between the presence of gingival bleeding caused by oral irrigation and the occurrence of a bacteraemia. However, it should be pointed out that the fact that gingival bleeding occurred in 12 of the 30 subjects during use of the oral irrigation device suggests that not all of the subjects may have had healthy gingivae. Bleeding caused by irrigation was observed by the investigators by standing next to the subject and observing them and no intra-oral examination was carried out. It would be difficult to observe any bleeding using this method, especially from posterior areas of the mouth, and not all bleeding on use of the irrigation device may have been detected. The stream of water emanating from the oral irrigation may have also washed away any blood and so made bleeding difficult to detect.

Bacterial counts, as assessed from pour plates after microbial culturing, from the positive samples ranged from 1-4CFU/mL of blood. The organisms isolated from the eight positive subjects were *Streptococcus mitis* (four cultures), *Bacteroides melaninogenicus, Streptococcus sanguis, Peptostreptococcus intermedius* and *S. epidermidis* (one culture each). *S. epidermidis* is considered to be a bacterium that resides on the skin and would be considered by many, but not the authors of this study, to be a contaminant.

From these four studies with different methodologies, it may be concluded that use of oral irrigation devices is associated with bacteraemia and that the incidence appears to

slightly increase as the level of periodontal disease worsens. It should be noted that the type of oral irrigation device and the device setting also varied from study to study. The degree of the subjects' periodontal health also varied between studies. Different authors cultured their blood samples for different amounts of time before either discarding or subculturing them. This would have affected the incidence rate of bacteraemia as the longer that blood cultures are observed for, the greater the bacterial detection rate (Kara et al. 2004).

15. Toothbrushing and bacteraemia

Toothbrushing is the most common oral hygiene procedure performed by patients (Axelsson et al. 1998). The rate of bacteraemia following toothbrushing has been evaluated in 17 studies to date (Table 4). The results of these studies show a bacteraemia rate range of 0% to 62% following toothbrushing. If studies including children and teenagers are excluded, then the bacteraemia rate ranges from 0% to 43%. When assessing studies with children and teenagers, the bacteraemia rate ranges from 19% to 62% whereas if studies including children only are included, it ranges from 39% to 62%.

Cobe (1954) assessed bacteraemia following brushing in 305 subjects and found that 24.2% of subjects tested positive for bacteraemia. The duration of the brushing was not stated nor was it mentioned if it was standardised between individuals. There was no significant difference in the incidence of bacteraemia between a group needing periodontal treatment and a group not needing treatment. However, the criteria used to categorise these groups were not stated. Pre-procedure control blood samples were discontinued after the first 100 such samples proved to be sterile. Since a control sample is necessary in each subject in order to interpret a positive blood sample after a test procedure, the reported results of this study must be interpreted with caution.

Rise et al. (1969) assessed bacteraemia following brushing in 50 dental students with ‘clean mouths’ and reported that 26% of their subjects experienced a detectable bacteraemia. However, no definition or data were provided as to what the authors

Table 4– Rate of bacteraemia following toothbrushing

Authors	(N)	Rate of bacteraemia
Cobe et al. 1954	305	24%
Rise et al. 1969	50 dental students	26%
Tamimi et al. 1969	30	0%
Sconyers et al. 1973	30	17%
Madsen 1974 *	29	22%
Berger et al. 1974	30	0%
Wank et al. 1976	21	10%
Silver et al. 1977	96	43%
Sconyers et al. 1979	50	0%
Silver et al. 1979	36	8%
Chung et al. 1986	16 teenagers	19%
Schlein et al. 1991	20 teenagers	25%
Roberts et al. 1997	52 children	39%
Bhanji et al. 2002	50 children	62%
Hartzell et al. 2005	30	0%
Kinane et al. 2005	30	3%
Forner et al. 2006	60	2%

* Bacteraemia following brushing and use of toothpicks.

All subjects were adults unless stated otherwise

thought constituted a clean mouth. There was also no information provided about the experimental protocol.

Tamimi et al. (1969) measured the incidence of bacteraemia following brushing in three groups of only ten individuals each and reported no incidence of bacteraemia. However, they excluded 19 positive cultures, out of a grand total of 2160, because they contained contaminants and mould colonies. Duplicate plates inoculated from the same specimen showed no growth of microbes. The study was of a crossover design, comparing the rate of bacteraemia following use of an oral irrigation device with that following brushing. The three groups were comprised of subjects with gingival health, gingivitis or periodontitis. However, the authors did not state what criteria they used to define these groups. The subjects brushed their teeth by themselves and no attempt was made to standardise the amount of time spent brushing. Blood samples were taken prior to brushing and at two and ten minutes after brushing. The experimental protocol was repeated at 15, 45 and 60 days, during which time the subject continued to brush twice daily as instructed as part of their oral hygiene regimen. Comparison of the latter samples between groups may not be valid as several of the subjects would have had resolution of their disease due to the daily regimen of oral hygiene that was part of the study. There would have been little correlation between the periodontal status of the subjects at the time of data collection and at 60 days, for instance. The authors did not assess disease levels at any appointment subsequent to the initial one.

Sconyers et al. (1973) found that five out of 30 subjects, or 17% of participants, developed bacteraemia after use of a powered toothbrush. The investigators assessed plaque levels, periodontal pocket depths and tooth mobility but, surprisingly, did not assess gingival inflammation. It was not stated when this information was recorded. Brushing was performed by the investigators utilising a standardised brushing technique for one minute. The authors found no correlation between the degree of

plaque accumulation and bacteraemia. Culturing techniques were used for microbial identification. Two species of bacteria were isolated following brushing in the positive samples – *S. mitis* and *Streptococcus mutans*. A quantitative analysis indicated that there was 1CFU/mL and 0.3CFU/mL in two of the positive samples. The remaining three positive samples had less than 0.3CFU/mL.

Madsen (1974) performed a longitudinal cohort study on 16 subjects with gingivitis, defined as a score of between 0.5 and 1.5 according to the Gingival Index (GI) (Löe & Silness 1963) and having periodontal pocket depths ≤3mm, and 13 subjects with marginal periodontitis, defined as subjects having gingival inflammation associated with pocket depths >3mm and having at least one area of 'interdental ulceration' in each quadrant. Subjects in both groups had an oral hygiene procedure, comprising brushing for 2.5 minutes followed by use of toothpicks for 2.5 minutes. It was unclear as to whether the investigator or the subject performed these procedures. Blood samples were taken before and within one minute after the oral hygiene procedure. Following this initial appointment, the subjects rinsed daily with 0.2% chlorhexidine twice daily, instead of their regular home oral hygiene regimen, for the next seven days before having the experimental procedure performed on them again. Periodontal treatment was then carried out for varying periods of time in order to bring the subjects to an optimal periodontal status with healthy gingivae and no pocket depths >3mm. The experimental procedure was then carried out one final time. In this study, all baseline data was captured one to two days before the experimental procedure in order to eliminate the potential for instrumentation to cause bacteraemia. However, this may invalidate any correlations made between bacteraemia and this data, as the

subject's gingival health, plaque levels and periodontal condition could have changed in the intervening days between data collection and the experimental procedure.

Madsen (1974) detected an overall Streptococcal bacteraemia in 14.6% of his gingivitis subjects and 30.7% of his periodontitis subjects following brushing and use of a toothpick. It should be noted that he obtained a significant number of positive cultures for *Staphylococcus albus* and Bacillus spp. (42 out of 174 cultures), which he classified as contaminants associated with laboratory processes. These contaminants were present at roughly the same frequency throughout the experiment, both before and after the oral hygiene procedure, and were discounted by the researcher in his final analysis of true bacteraemia, which he defined as Streptococcal bacteraemia. It must also be borne in mind that this study assessed the combined effects of toothbrushing and use of toothpicks on bacteraemia and the effects of each of these procedures on bacteraemia cannot be evaluated separately from the study design. The study found no correlation between bacteraemia and the degree of plaque accumulation or gingival inflammation. Substitution of chlorhexidine rinsing for normal home oral hygiene procedures did not affect the incidence of bacteraemia. The author, however, reported a significant reduction in the detection of bacteraemia following periodontal treatment so that none of the periodontitis subjects who underwent periodontal therapy experienced a bacteraemia after brushing once they achieved optimum periodontal health. The author concluded that bacteraemia can be avoided by having an optimal periodontal status. However, he considered that this would be impossible to maintain over an extended period, although no reason was given for this belief. Thus, he recommended complete dental clearance in patients at risk of infective endocarditis. However, it should be remembered that the

periodontium may not be the only portal of entry for bacteraemias. Croxson et al. (1971) demonstrated eight cases of viridans Streptococci endocarditis in edentulous patients, suggesting that these bacteria of oral origin entered the bloodstream through other means.

In a study that primarily evaluated the use of an oral irrigation device, 30 subjects were evaluated for detectable bacteraemia after toothbrushing (Berger et al. 1974). The brushing was performed by the subjects themselves for a duration of one minute. The authors reported that there was no incidence of bacteraemia after toothbrushing. However, one person in the toothbrushing group did develop a bacteraemia during the study. This was conveniently excluded by the researchers who stated that the individual had to be dropped from the study because of periodontal infection that became evident the day after the experiment. It should be noted that the researchers did this retrospective exclusion after conducting the initial screening process which was supposed to have excluded subjects with periodontitis in the first place. The screening process for subjects with oral disease was by direct examination only and no periodontal probing or instrumentation of any sort was performed. This method of screening lacks sensitivity as well as specificity and so this undermines the assessment of the oral health status of all the subjects in this study.

Wank et al. (1976) detected an overall 10% incidence of bacteraemia in 21 subjects with periodontal health or periodontitis following brushing. Their subjects were assessed for brushing-induced bacteraemia both before and after initial periodontal therapy and a five to six week plaque control program. Brushing was performed by the subjects and was carried out for five minutes. There were two instances of

brushing-induced bacteraemia prior to and two instances upon completion of initial periodontal therapy and the plaque control program. This did not include one instance of a positive culture prior to the program that was identified to be *Propionibacterium acnes*. They concluded that the incidence of bacteraemia following brushing was not significantly different before or after initial periodontal therapy and a plaque control program. This was despite significant improvements in the Gingival-Periodontal Index and the Irritant Index that they utilised. The authors suggested that there may have been a lack of significance due to their small sample size and low rate of bacteraemia experienced in their study. Further critical analysis of this paper is detailed in Section 17 which discusses studies dealing with flossing and bacteraemia.

Silver et al. (1977) assessed bacteraemia following an investigator's use of a powered brush for two minutes on 96 subjects categorised into four different groups and detected a high overall incidence of bacteraemia (42.7%). They attributed this high figure to the specific brushing procedure and the stringent culturing procedures that they utilised. However, in arriving at this result, they included two individuals who tested positive for bacteraemia prior to the brushing procedure being performed. They justified this inclusion by stating that the number of species of bacteria isolated from these two individuals was 'markedly higher' after brushing had been performed. No further data was provided regarding these individuals except for that fact that they had high plaque scores. Data from these two individuals should have been omitted from the results of this study. The groups in this study were categorised according to their modified GI scores and their modified Plaque Index (PI) scores, these being the GI (Löe & Silness 1963) and the PI (Silness & Löe 1964) scores evaluated without instrumentation of the gingival sulcus. The researchers then correlated any detectable

bacteraemia in the subjects with these scores. They could find no correlation between the PI scores and the incidence of bacteraemia in their subjects. However, the percentage of subjects with bacteraemia following brushing increased with increasing severity of gingival inflammation.

The authors found that the higher the GI in a person, the greater the chance of isolating more species of organisms. It should be noted though that the authors also assessed bleeding on brushing during the experimental procedure and stated that any bleeding on brushing resulted in the assignation of a score of 2 for the GI. This, seemingly, was irrespective of what GI score was assigned initially. It is not clear, for instance, whether individuals who had been initially assigned a higher GI score had that score reduced to a 2 following any bleeding on brushing. The number of sites that bled on brushing was not reported. This modification of the score undermines the validity of the initial assessment of the GI that was carried out, and so undermines the authors' finding of a correlation between bacteraemia and severity of gingival inflammation. It should also be noted that the GI they used involved assessing the inflammation around six predetermined teeth rather than the whole mouth. Further, the authors did not mention how or when they assessed any bleeding on brushing nor did they provide any data on how many individuals had their GI categorisation changed because of bleeding on brushing. Nevertheless, if the findings of this study are valid, it implies that absence of gingival inflammation significantly reduces the likelihood of bacteraemia following brushing. The lack of correlation between the PI and bacteraemia suggests that the amount of plaque present on teeth may not be as important as the severity of inflammation in the adjacent gingiva as a risk factor for bacteraemia.

Silver et al. (1979) investigated the incidence of bacteraemia following brushing for two minutes in 36 subjects with healthy gingivae and found that only three individuals had detectable bacteraemia (8.67%). It was not stated whether the subjects or the authors performed the brushing nor was it evident whether a manual or powered toothbrush was used. All subjects in the study had gingival inflammation scores of zero, indicating gingival health, according to a modification of the GI (Löe & Silness 1963). The modifications were that there was no instrumentation of the gingival sulcus and assessment of inflammation was made at all sites.

The experimental protocol utilised by the researchers for this study was similar to that of their previous study (Silver et al. 1977). However, the authors did not take any baseline blood samples prior to the brushing procedure as their previous study had revealed only two positive blood samples, both in subjects with severe gingival inflammation. Notwithstanding this, a baseline blood sample would have been useful in this study in order to attribute any bacteraemia detected solely to the experimental procedure. The microbes identified in the positive samples were Propionibacterium spp. in two subjects and Actinomyces spp., *S. sanguis* and *S. mitis* in the third subject. Whilst Propionibacterium spp. are a common skin contaminant, the authors stated that they are also present in the mouth and so they did not consider its presence to be a contaminant in this study. As with their earlier study, no correlation was found between PI and incidence of bacteraemia. The authors also stated that the fact that probing was not utilised in assessing the GI could have meant that some subjects were falsely categorised as having gingival health when in fact they may have had a degree of gingival inflammation.

Sconyers et al. (1979) evaluated bacteraemia following brushing with a powered toothbrush in 50 subjects with healthy gingivae and reported no incidence of bacteraemia in any of their subjects. The brushing was performed by the authors and was for four minutes. All subjects had periodontal pocket probing depths of ≤3mm and had no bleeding on probing. An experimental protocol similar to their earlier study (Sconyers et al. 1973) was utilised for this study. Further, all subjects received a prophylaxis prior to their participation and were free of any detectable plaque on all teeth. The prophylaxis was performed far enough in advance so that it could not influence a detectable bacteraemia at the time of testing. However, the clinical relevance of performing a toothbrushing procedure on teeth that were already rendered plaque-free after a prophylaxis, and then testing for bacteraemia, could be questioned. The study did show, though, that if subjects possessed healthy gingivae then there was no significant possibility of detecting a bacteraemia after brushing.

Chung et al. (1986) investigated bacteraemia following self-performed brushing for two minutes in 16 teenagers (average age of 14 years and ranging from 12 to 19 years of age) with orthodontic appliances. They reported an incidence of 63% bacteraemia prior to brushing and 19% after brushing. Indeed, some of their subjects tested positive for bacteraemia before brushing and tested negative after brushing. This surprising result may be explained by a number of factors. The authors stated that several of the subjects may have brushed or eaten prior to the experimental procedure and so bacteraemia resulting from these procedures may have tainted their results. The failure of the investigators to advise their subjects to refrain from any oral manipulations immediately prior to the experimental procedure may be viewed as a

flaw in the study design. The authors also utilised a GI (Löe & Silness 1963) and PI (Silness & Löe 1964) prior to the brushing procedure. Utilisation of both these indices involves probing the marginal gingivae, a procedure that can generate bacteraemia (Daly et al. 1997). This too may have accounted for the high rate of bacteraemia detected prior to the brushing procedure.

The authors reported that they isolated no facultative anaerobes in their positive samples and that all their isolates were obligate anaerobes. However, they mentioned that they only collected 5mL of blood before and after the experimental procedure and this proved insufficient for effective testing for organisms. They were forced to subculture two to three days after incubation into the medium bottles and most of the facultative anaerobic bacteria could have become non-viable by then. Given the serious flaws associated with this study, the results should be interpreted with caution.

Schlein et al. (1991) evaluated bacteraemia following brushing in 20 subjects with fixed orthodontic appliances. Brushing was performed by the subjects without dentifrice for two minutes. This study was performed by the same group of examiners as the study by Chung et al. (1986), albeit with a far more refined methodology and significantly improved sampling and culturing techniques. In contrast to their previous study, they reported no instances of bacteraemia prior to brushing and a 25% incidence of bacteraemia after brushing. Both aerobic and anaerobic microbes were isolated. The authors were careful to ensure that no oral manipulations were performed by the subject prior to the appointment and they allowed 20 minutes to elapse between assessment of the PI (Silness & Löe 1964) and GI (Löe & Silness

1963) and commencement of the experimental procedure in order to eliminate the possibility of these procedures causing any detectable bacteraemia.

Roberts et al. (1997) detected a bacteraemia on average 39% of the time following brushing in 52 children aged 2-16 years of age whilst they were under general anaesthesia. Nasotracheal intubation was used for the general anaesthesia and it should be noted that this could have led to an increased incidence of bacteraemia. Brushing was carried out by the dentist for one minute with normal vigour. No reference was made with regards to what the authors considered normal. The authors did not indicate what the periodontal status of the subjects was and there were no exclusion criteria when the subjects were selected for the study. This may have led to selection of individuals who may have been more or less susceptible to bacteraemia, such as those with periapical infections, desquamative oral lesions or recent antibiotic use. Additional information on this group of children was provided in a subsequent paper (Lucas & Roberts 2000). The magnitude of the bacteraemia experienced after toothbrushing was reported here and was found to range from 0-1666 CFU/mL with a mean of 32.2 CFU/mL. There was no significant difference reported between this result and the magnitude of bacteraemia reported after polishing or scaling nor was there any significant difference in the types of bacteria detected and the incidence of bacteraemia after the different procedures. It was concluded that patients were as likely to experience bacteraemia from everyday procedures such as brushing when compared to dental procedures such as polishing, scaling and extractions.

Bhanji et al. (2002) compared the incidence of bacteraemia in children after manual brushing and use of a powered toothbrush and reported these to be 46% and 78%

respectively. Their sample population consisted of 25 children utilising the manual toothbrush and 25 children utilising the powered toothbrush (Sonicare, Phillips Oral Healthcare Corporation). There were no significant differences in the non-intrusive gingival and plaque indices between the two groups. Plaque levels in this study were evaluated following disclosing with a plaque disclosing solution prior to the toothbrushing procedure being carried out. The children had the experimental procedure performed on them whilst they were under general anaesthetic and their teeth were cleaned for them by the researcher for one minute. Blood samples were drawn before and after the brushing and assessed for bacterial growth. The authors reported no correlation between bacteraemia and the plaque and gingival indices. However, they did mention that there was limited time to perform the gingival and plaque indices in the operating room and the operator's ability to distinguish subtle changes in gingival colour may have been compromised. It should also be noted that even though an orotracheal intubation procedure was used for the general anaesthetic instead of a nasotracheal technique in an attempt to minimise the chances of a bacteraemia (Berry et al. 1973), the orotracheal intubation procedure could still have caused a bacteraemia due to abrasion of the oral or tracheal mucosa by the intubation apparatus (Hansen et al. 1989). This may have partially accounted for the high rate of bacteraemia in this study when compared with other studies. Further, the powered toothbrush used in this study was a high frequency brush that operated at 31,000 brush strokes per minutes and utilised sonic waves to dislodge plaque bacteria. This too may have caused increased trauma to the gingivae, when compared with previous studies evaluating bacteraemia following use of a powered toothbrush, and so led to an increased rate of detectable bacteraemia in this study.

The BACTEC aerobic and anaerobic culture system was used to identify positive isolates, which were then Gram stained and inoculated on agar plates. The authors essentially only knew whether the isolate was an aerobic one, an anaerobic one or both. Colony morphology and Gram stain status was also used to try and elucidate the nature of the isolate. As a result, several contaminants may have been included in the positive samples due to the absence of subculturing to species or genus levels. The authors also stated that brushing in an anaesthetised child whilst manoeuvring around an orotracheal tube, in the absence of water and toothpaste, may have affected the rate of bacteraemia detected.

Hartzell et al. (2005) found no incidence of bacteraemia following brushing in 30 healthy adults with low Periodontal Screening and Recording (PSR) scores (Nasi 1994). Blood samples were drawn prior to brushing and then 30 seconds and 20 minutes after brushing and were tested for bacteraemia using aerobic and anaerobic blood culture bottles. Brushing was performed using a manual toothbrush and was performed by the subjects themselves for one minute. They reported a 0% incidence of bacteraemia after toothbrushing. The PSR procedure was conducted at an appointment subsequent to the experimental procedure. The exact duration of this time lag was not stated and there is the possibility that the subject's periodontal parameters could have changed significantly between appointments. Further, only 17 of the 30 subjects attended the PSR appointment and so even though the authors stated that the average PSR score was 9.8, they based this on data that was obtained from only 57% of their subjects. Although the authors reported that none of their subjects tested positive for bacteraemia, both at 30 seconds and 20 minutes after cessation of brushing, they excluded three blood culture bottles, from three different

subjects, which were positive for growth of *P. acnes* as they deemed this to be a skin contaminant and thus not representative of a true bacteraemia. However, Silver et al. (1979) included their samples that tested positive for Propionibacterium spp. in their results, because they believed it possible for this species to be derived from the oral cavity. Hartzell et al. (2005) also did not provide any data on the periodontal condition of the three individuals who tested positive for *P. acnes*, quite possibly because they were not able to collect the data for one or more of the subjects. The authors concluded that bacteraemia after brushing, in a healthy population, was an uncommon occurrence. The lower incidence of bacteraemia detected in this study, when compared to ones conducted previously, may reflect the fact that subjects used a manual toothbrush and brushed their own teeth unlike the other studies conducted in the 1970s. There were also different methods of microbial detection used as the experiments were conducted roughly 30 years apart. The indices used to categorise subjects were also different in these studies.

Kinane et al. (2005) evaluated bacteraemia following self-performed brushing for two minutes in 30 subjects with untreated periodontitis and reported an incidence of 3% when a conventional microbial culturing method (BACTEC) was used and an incidence of 13% when a Polymerase Chain Reaction (PCR) was used to detect microbes. The higher incidence of bacteraemia detected by PCR is probably due to the fact that PCR is much more sensitive at detecting organisms than the BACTEC system. Several microbes are currently uncultivable and so may only be detected via PCR, although this does not seem to be an issue for oral microbes associated with infective endocarditis. However, it should be borne in mind that PCR does not differentiate between viable and non-viable microbes in the bloodstream. The authors

suggested that the relatively low incidence of bacteraemia when compared to that found in previous studies assessing brushing and bacteraemia may have been due to insufficient sensitivity of the detection methods used. The authors found no significant correlation between average periodontal pocket depth and incidence of bacteraemia, although they did report a slight trend towards this when PCR was the method of detection utilised. They suggested that this may be due to the possibility that patients with periodontitis may have much more efficient processes developed to prevent or eliminate bacteraemias. The low number of subjects in this study also hampered any significant results being obtained in this study. There was no correlation between bleeding on probing and incidence of bacteraemia. All subjects had at least one pocket depth of ≥6mm. Baseline measurements of gingival and periodontal indices were taken at a first appointment. However, the experimental brushing procedure was performed at a subsequent appointment and the time difference between these two appointments was not stated. The subjects' periodontal condition may well have changed in the intervening period and any attempts to correlate bacteraemia following brushing with periodontal indices is hampered by this.

Another concern with this study was that at no point was the plaque level of the subjects assessed. It is possible that those subjects with larger amounts of plaque may have had higher incidences of bacteraemia as bacteria located on the teeth may have been seeded into the gingivae during the toothbrushing procedure. The authors also reported that it was not necessarily the same subjects who tested positive for bacteraemia when the two different methods of bacterial detection were compared. In fact, only two samples tested positive for both PCR and BACTEC culture. This serves

to illustrate the fallibility of the microbial detection methods that are currently utilised. There were two positive blood samples at baseline when the BACTEC detection method was used in this study. However, subculturing of these isolates did not result in the growth of any bacteria. The authors postulated that there could have been bacteria that used up all of the nutrients in the BACTEC bottles and then died before subculturing was attempted. There may also have been an incompatibility between the type of media used for subculturing and the type of organism present. The authors also stated that high levels of white blood cells may have caused a false positive signal in the BACTEC bottle.

Forner et al. (2006) evaluated bacteraemia following self-performed brushing for two minutes in 60 individuals and reported no incidences of bacteraemia in subjects with periodontal health and gingivitis but a 5% incidence (in one subject) of bacteraemia in those with periodontitis. However, in making this statement they excluded another subject whose blood samples tested positive 30 minutes after brushing but were negative 30 seconds and 15 minutes after brushing. They also detected no significant correlation between brushing-induced bacteraemia and gingival inflammation, periodontal pocket depth, bleeding on probing or plaque levels. It should be noted though that collection of all periodontal data was performed at least two weeks before the brushing procedure and so the periodontal status of the subjects may have changed significantly when the brushing was carried out. All the subjects performed their own brushing, under supervision, after thorough instruction in a standardised technique. The authors used a lysis filtration technique, which is claimed to increase sensitivity when compared to use of BACTEC cultures, to detect any bacteraemia. This technique also enabled them to estimate the magnitude of any bacteraemia. However,

it has been shown that the BACTEC and the lysis filtration methods of microbial identification are equivalent in detecting post-procedural bacteraemia (Lucas et al. 2002). Following brushing, in the one subject that had a positive bacteraemia sample, a magnitude of 0.11 colony forming units (CFU) per mL was detected.

It should be noted that some studies (Sconyers et al. 1973, Silver et al. 1977, Sconyers et al. 1979) utilised powered toothbrushes whereas others (Hartzell et al. 2005) used a manual toothbrush. The investigators performed the brushing procedure in some studies (Sconyers et al. 1973, Silver et al. 1977, Sconyers et al. 1979) whereas in other studies the subjects brushed their own teeth (Hartzell et al. 2005, Forner et al. 2006). Having the subjects brush their own teeth is more representative of what patients experience in their daily lives. However, it does have the disadvantage of a lack of standardisation of the brushing technique.

The duration of toothbrushing also varied from one minute (Bhanji et al. 2002) to five minutes (Wank et al. 1976) to anything that the subject felt was appropriate (Tamimi et al. 1969). This variance in the length of the test procedure could have affected the rate of bacteraemia detected. Some studies utilised pre-sterilised brush heads (Silver et al. 1977) whereas most studies did not specify whether new or old toothbrushes were used. Previously used toothbrushes may harbour bacteria on their heads which may then act as a source of bacteria detected in bacteraemia (Glass & Lare 1986, Kennedy et al. 2002) whereas pre-sterilised brush heads are generally not utilised by patients and so their use may not be relevant to clinical research. Some researchers used toothpaste (Hartzell et al. 2005) during the test procedure whereas others did not (Chung et al. 1986, Schlein et al. 1991). It is not known if the antibacterial ingredients

of dentifrices would sufficiently impact on the incidence and magnitude of bacteraemia. It should also be noted that there were differences in the timing of blood collection in the various studies which ranged from the last 30-60 seconds of brushing (Sconyers et al. 1973, Silver et al. 1977) to 30 minutes after brushing (Forner et al. 2006). Some studies collected multiple post-brushing blood samples in order to test for bacteraemia at different time intervals (Hartzell et al. 2005, Forner et al. 2006). These variances in the duration of brushing and the timing and number of post-brushing samples must be taken into account when assessing the findings of the different studies.

Some studies utilised children as their subjects whereas others utilised adults. Caution should be used when comparing the results of bacteraemia in children with those in adults. In children, blood is generally collected during a general anaesthetic procedure and it should be borne in mind that the act of intubation may in itself produce a bacteraemia (Baltch et al. 1982). A varying bacteraemia incidence of 5.5-17% may be expected from nasotracheal intubation (McShane & Hone 1986, Dinner et al. 1987). Rates of bacteraemia clearance in children are also thought to be considerably higher than those in adults although no study has specifically addressed this issue.

The methods of culturing utilised by the different studies also varied significantly. The toothbrushing studies listed in Table 4 all span a period of about 50 years. Microbial identification and culturing techniques have advanced and changed significantly during this time and what was considered the gold standard 50 years ago is not necessarily considered to be so now. Further, different groups of researchers may have chosen different methods of microbial cultures in order to best answer their

specific aims. Nevertheless, there was a wide variance in the type of microbial identification methods used. Anaerobic culturing in assessing bacteraemia following toothbrushing was first used by Sconyers et al. (1973) and then by Silver et al. (1977) whereas PCR was used by others (Kinane et al. 2005). Later studies on the effects of toothbrushing-induced bacteraemia have used more stringent culturing procedures resulting in higher numbers of positive cultures (Schlein et al. 1991, Roberts et al. 1997). The more recent studies have used the BACTEC system of microbial identification (Bhanji et al. 2002, Kinane et al. 2005, Hartzell et al. 2005).

Noting all the above-mentioned differences, it is not surprising that there has been a wide variance in the incidence of bacteraemia reported subsequent to toothbrushing (0% to 78%). Even when manual toothbrushing is considered, the rates vary from 0% to 46%. The higher incidence of bacteraemia detected by latter studies may be ascribed to better detection techniques for anaerobic microbes as technology has advanced.

Only two studies attempted a quantification of the bacteraemia caused by toothbrushing. These results comprise only six patients that tested positive for bacteraemia. The five patients that tested positive for bacteraemia following use of a powered toothbrush (Sconyers et al. 1973) had magnitudes of 1.0 CFU/mL, 0.3 CFU/mL (one subject each) and <0.3CFU/ML (three subjects) and the one patient that tested positive for a bacteraemia following use of a manual toothbrush (Forner et al. 2006) had a magnitude of 0.11CFU/mL. The low numbers of patients that tested positive in these two studies mean that this quantification of toothbrush-induced bacteraemia should be interpreted with caution.

16. Interdental sticks and bacteraemia

Only four studies have assessed the rate of bacteraemia following the use of interdental woodsticks. These are presented in Table 5. The weighted mean rate of bacteraemia from these studies is 20% with a range from 14-30%.

Table 5 – Rate of bacteraemia following use of woodsticks

Authors	(N)	Rate of bacteraemia
Lineberger & De Marco 1973	10	30%
Madsen et al. 1974 #	29	22%
Ramadan et al. 1975	50*	18%
Wank et al. 1976	21	14%

* - Unclear how many subjects had their teeth cleaned with an interdental stimulator
# - Bacteraemia following brushing and use of toothpicks.

These four studies used different types of interdental sticks to clean the interdental areas of the teeth (plaque-aid, toothpick, interdental stimulator and interdental woodstick). However, these aids are all essentially the same shape, are all made of wood and are used in the same manner and so, for the purposes of this literature review, have been assessed together. Due to the fact that all four studies also evaluated the use of other oral hygiene aids besides interdental sticks, varying aspects of these studies have been critiqued in other parts of this literature review and so only the parts pertaining to interdental sticks will be discussed here.

Lineberger & De Marco (1973) used an interdental woodstick (Stimudents) for two minutes on ten of their subjects and reported a bacteraemia rate of 30%. It is unclear whether the subjects used the interdental woodsticks themselves or whether they were used on them by the investigators. Wank et al. (1976) evaluated bacteraemia following use of a perio-aid (Marquis Dental Mfg, Colorado - an instrument used to hold a toothpick) and found an overall bacteraemia incidence of 14%. The time that the perio-aid was used for was not reported. Madsen et al. (1974), as discussed earlier, reported an overall 22% rate of bacteraemia following an oral hygiene procedure involving brushing and use of a toothpick. The toothpick was used for two and a half minutes after two and a half minutes of brushing and so the bacteraemia caused by the toothpick alone could not be assessed. Ramadan et al. (1975) used interdental stimulators for unspecified periods of time and reported a bacteraemia rate of 18% after ten minutes. However, as mentioned earlier, it was unclear how many of their subjects actually had interdental stimulators used on their teeth.

Thus, the rate of bacteraemia caused by use of interdental sticks has been shown to vary from 14% to 30%. However, as is the case with other studies dealing with detection of bacteraemia, variances in methodology, including that of timing of bacteraemia, complicate comparisons between the different studies.

17. Flossing and bacteraemia

It has been reported in the Australian medical literature that flossing by a patient in a risk-group for infective endocarditis, who also had inflamed gingivae, resulted in that patient developing infective endocarditis (Jenney et al. 2001). The authors advocated that individuals at risk of infective endocarditis should avoid flossing unless their "gums were healthy". No diagnosis of that particular patient's periodontal condition was given, nor was any data presented on the presence or extent of gingival inflammation or plaque. The authors' recommendation that patients at risk of infective endocarditis should avoid flossing is at odds with the evidence that dental flossing is necessary to remove dental plaque from interdental sites (Egelberg and Claffey 1998).

Some evidence that flossing may be important for prevention of infective endocarditis may be gleaned from a large population-based case-control study where use of dental floss once a day or more was one of the factors associated with a borderline decreased risk of developing infective endocarditis, which suggests a benefit from this oral hygiene practice (Strom et al. 2000). However, it should be noted that individuals with increased oral health awareness may also have increased general health awareness and thus this association may have confounding factors. Spurious associations, such as an increased risk of obesity due to lack of flossing, may be inferred when analysing the results of association studies (Hujoel et al. 2006).

Only four studies have previously sought to assess the relationship between flossing and bacteraemia (Lineberger & De Marco 1973, Ramadan et al. 1975, Wank et al. 1976, Carroll & Sebor 1980). The results of these studies are summarised in Table 6.

Following thereafter is a critical analysis of these four studies. They have been cited 67 times previously in the literature ('Web of Science' online search 22 Feb 2007). The weighted mean rate of bacteraemia following flossing according to these studies is 25% with a range from 18-38%.

Table 6 – Rate of bacteraemia following flossing

Authors	(N)	Rate of bacteraemia
Lineberger & De Marco 1973	10	20%
Ramadan et al. 1975	50*	18%
Wank et al. 1976	21	22%
Carroll & Sebor 1980	4	38%

* Unclear as to how many subjects actually had their teeth flossed

Lineberger & De Marco (1973) reported a 20% rate of bacteraemia following use of unwaxed dental floss in ten subjects aged 21-67 years with untreated chronic generalised periodontitis, which was defined as presence of pocket depths greater than 3mm in all quadrants. However, no correlation was found between age or gender and flossing-induced bacteraemia. This study also evaluated the incidence of bacteraemia after use of interdental stimulators and periodontal surgery. It had been intended by the authors to carry out the procedure on subjects treated for periodontitis as well. However, the low incidence of bacteraemia detected in the untreated subjects caused them to abandon these plans. The authors did not state when probing and periodontal assessments were carried out. Clinical attachment levels were not evaluated. They also stated that the subjects did not have any residual antibiotics from previous or

current administration but no definition was provided as to what they considered residual levels.

Pre-flossing control blood samples were taken 20 minutes prior to flossing. It was unclear whether the subjects performed the flossing procedure themselves or if this was performed by the authors. The test blood sample was taken after the authors had judged when maximal trauma had occurred from the flossing procedure (adjudged to be approximately two minutes), but while the flossing was still being carried out. This was to ensure continued potential for bacteraemia. Blood cultures were cultured both aerobically and anaerobically and were monitored for seven days for a negative result before being discarded. Positive cultures were subcultured. The authors stated that all the microbes detected in this study could be considered as oral commensals even though 13.6% of all positive results were *S. epidermidis* and 4.5% were *B. subtilis*. These organisms are usually regarded as skin contaminants. Although the authors listed the organisms that were isolated, they did not provide a breakdown of which microbes were associated with flossing and which ones were associated with the other procedures tested (periodontal surgery and use of Stimudents) and so it is not possible to discuss what types of organisms were specifically associated with flossing-induced bacteraemia.

Ramadan et al. (1975) evaluated the incidence of bacteraemia after the use of interdental stimulators and ‘dental floss silk’ in 50 patients with ‘marginal periodontitis’ with no previous prophylaxis and reported a bacteraemia rate of 18%. The subjects comprised 19 males and 31 females, ranging in age from 15 to 50. Marginal periodontitis was defined as pocket depth of more than 4mm. It was not

clear when exactly the periodontal indices were assessed; i.e. whether it was a week prior the flossing procedure or whether it was five minutes prior to it. The detailing of exclusion criteria was also somewhat lacking. Recent antibiotic administration was an exclusion criterion but the authors did not detail the period of time they considered as recent. It was also unclear from the methodology whether any of the patients had received previous periodontal therapy.

Blood samples were taken prior to the procedure being performed and exactly ten minutes after the use of either dental floss or interdental stimulators. The time spent flossing or using the interdental stimulators was not mentioned nor was the technique used described. Further, it was not evident exactly how many subjects had their teeth flossed and how many had the interdental stimulators used on them. It also was not obvious whether the flossing was performed by the researchers or by the subjects. Blood samples were incubated aerobically and anaerobically and monitored for five days before being discarded. Positive broths were subcultured for microbial identification. Although the authors stated that there was no difference in the incidence of bacteraemia between floss and interdental stimulators, no data was presented to support these claims. They did not state how many of the positive bacteraemia samples were in subjects who had their teeth flossed and how many were in subjects who had interdental stimulators used on them. Indeed, it was also unclear whether the same numbers of subjects actually had their teeth flossed or engaged by the interdental stimulator. Further, the authors included four samples that tested positive for *S. aureus*, *S. albus* and Corynebacteria spp in their positive results. These are generally considered to be skin contaminants and are not regarded as normal commensals of the oral cavity. However, the authors considered them to be oral

cavity commensals and stated that there was no reason why they could not have entered the circulation through the oral cavity. A total of 11 strains were isolated, one of which was strictly anaerobic, the other ten being facultative anaerobes. The authors also performed a colony count and deduced that the intensity of the bacteraemia was less than one colony per mL in six of the positive cases and 6, 8 and 11 CFU/mL in the other three positive cases.

Ramadan et al. (1975) concluded by stating that the routine use of dental floss or interdental stimulators may be risky in dental patients at risk of infective endocarditis. Whilst this may well be the case, it should be remembered that the results of their study only apply to periodontitis patients, a fact not mentioned in their conclusion. The inclusion of a periodontally healthy group would have been a useful addition to this study as it would have facilitated a comparison in the rates of bacteraemia between the two groups.

Wank et al. (1976) studied the frequency of bacteraemia following use of unwaxed floss, as part of a study of flossing, brushing and use of a Perio-aid, in 21 healthy individuals with varying severities of untreated periodontal disease. The 11 male and 10 female subjects were aged between 31 and 49. The experimental procedure was carried out before and after periodontal treatment was performed and an overall bacteraemia rate of 22% was reported. Prior to periodontal treatment the rate of flossing induced bacteraemia was 29% whereas after periodontal treatment and a five to six week maintenance program the rate of flossing-induced bacteraemia was 14%. However, this difference was not statistically significant.

Periodontal probing was used to grade the severity of the periodontal disease but this was only performed at the mesio-buccal line angle. Exactly when the probing assessment was performed was not stated by the authors. The flossing component of the study involved flossing of all interproximal areas by the subjects after instruction in the procedure was provided, although the exact nature of the action and time taken for flossing was not detailed. A blood sample was taken immediately prior to the procedure and a second sample was taken within 90 seconds after completion of the flossing exercise. It was not evident if the same cannula was used for both samples, although it is difficult to envision that this was the case considering the subjects flossed their own teeth and the blood samples were taken from the antecubital fossa.

Wank et al. (1976) did not provide a specific analysis of the microbes identified in positive bacteraemias after flossing only. However, a cumulative analysis of the microbes identified in positive bacteraemias after all three procedures (brushing, flossing and use of a perio-aid) revealed that 15 obligate anaerobes and 13 facultative anaerobes were isolated. The authors concluded that despite a significant improvement in periodontal conditions and oral hygiene, the frequency of bacteraemia was not significantly different before and after periodontal therapy and placement on a plaque control program. This was the case for both flossing considered individually and all three oral hygiene measures considered together. However, they conceded that this may have been due to the small number of patients and the low incidence of bacteraemia encountered.

Carroll & Sebor (1980) evaluated flossing and bacteraemia after use of unwaxed floss on only four individuals. The subjects comprised one male and three females who

were aged between 23 and 29. No exclusion criteria were cited although the authors mentioned that all four subjects were in medical health. Two of the subjects exhibited periodontal health and the other two had marginal gingivitis but no criteria were stated to justify these classifications. A total of 16 flossing episodes were performed on the subjects over the course of the experiment but these were not evenly distributed between the recruited subjects. One of the subjects underwent ten flossing episodes whereas two of the subjects only underwent one flossing episode each. The subjects flossed their own teeth for three minutes but the flossing technique used was not defined. The two most frequently flossed subjects varied their home flossing regimen throughout the course of the experiment so that the blood samples that were taken from them after flossing were classified as 'on-floss' blood cultures when the subjects flossed daily and 'off-floss' blood cultures when the subject went two or more days without flossing.

A control blood sample was taken immediately prior to flossing and a second blood sample was taken exactly three minutes after the start of flossing. The site of venepuncture was not mentioned but it is difficult to envision that the antecubital fossa was used. This is because the subjects flossed their own teeth and the blood was drawn as soon as the subjects finished flossing for three minutes. The flossing procedure would have been impossible to perform with the cannula left in situ in the antecubital fossa after the drawing of the control blood sample. The occurrence of gingival bleeding, defined as visibly bloody floss during flossing, was recorded and correlated with any bacteraemia.

Nothing was mentioned about the microbiological culturing methods used in this study except for a solitary statement saying that the broth used was vented to 'room air'. This raises several questions regarding the laboratory procedures in this study with regards to whether anaerobic or aerobic (or both) conditions were utilised, the time that the cultures were monitored for and the nature of the subculturing method used. Indeed, it appears that there may not have been any subculturing performed at all and so it may well be that all the bacteraemias detected in this study may represent skin contaminants.

The results obtained demonstrated that no bacteraemias were observed in the 'on-floss' blood cultures when the subjects flossed daily. However, six out of seven 'off-floss' cultures (86%) resulted in bacteraemia being detected. Carroll & Sebor (1980) reported this as being statistically significant. The presence of gingival bleeding was not observed to be a requirement for bacteraemia with three out of the six bacteraemias detected associated with bleeding. The authors concluded that flossing alone, when performed every second day was highly likely to induce a bacteraemia. However, if flossing was performed daily then no bacteraemia was found. Thus, daily flossing in the same subject helped reduce the rate of bacteraemia. The authors also stated that due to the risk of bacteraemia during initiation of a flossing program, antibiotic prophylaxis may be indicated in patients for who are at high risk of infective endocarditis.

All of these studies had too few subjects in them and so were underpowered to demonstrate any results of statistical significance with regards to bacteraemia following flossing. The flossing technique was also not defined by all four studies and

the time taken for flossing was not stated or considered by some in any analysis (Ramadan et al. 1975, Wank et al. 1976). It was also unclear exactly how many subjects in the study actually had their teeth flossed (Ramadan et al. 1975) or whether it was the researchers or the subjects who did the flossing (Lineberger & De Marco 1973). When the flossing procedure was performed by the subjects themselves (Wank et al. 1976, Carroll & Sebor 1980), it was not indicated if the aspirating cannula was changed in between phlebotomies or left in situ. If the latter was the case, then it was unclear how the patients performed the flossing procedure with the cannula in the antecubital fossa.

As discussed earlier, it is likely that the state of periodontal health may be important as a risk factor for bacteraemia caused by oral hygiene procedures such as flossing. Some studies used inadequate definitions of periodontitis when categorising their subjects. Ramadan et al. (1975) defined marginal periodontitis as having pocket depths of more than 4mm. No requirements were detailed regarding the extent of the disease and clinical attachment levels were not considered. Thus, the presence of one 5mm pocket in the whole mouth would have been sufficient to categorise one of their subjects as possessing marginal periodontitis. Similarly, Lineberger & De Marco (1973) defined periodontitis as having pocket depths greater than 3mm in all quadrants. The presence of one 4mm pocket in each quadrant would have been enough for a subject to be categorised as having chronic periodontitis. Some studies also made use of partial recording measures when determining the degree of periodontal disease present in their subjects. Wank et al. (1976) only measured the mesio-facial periodontal pocket and the degree of gingival inflammation present, and therefore may have underestimated the extent of periodontitis in their subjects.

Nevertheless, the collected data was not reported in their paper. There was also a time gap of a week or more between measuring various indices and the actual performing of the flossing exercise and consequently any correlations drawn between these indices and any bacteraemia obtained could be inaccurate (Wank et al. 1976, Carroll & Sebor 1980). On the other hand, some authors did not mention at what point they performed their periodontal data collection and so it is not possible to determine the strength of any correlations between bacteraemia and the periodontal condition of the subjects (Lineberger & De Marco 1973, Ramadan et al. 1975). Only one study assessed bleeding on flossing but this was done as a dichotomous score on a whole mouth basis and by visual assessment of whether the floss was bloody or not (Carroll & Sebor 1980). Consequently, no study assessed the number of papillae that bled on flossing.

Some studies included bacteria commonly regarded as skin contaminants as positive results in their bacteraemia analysis (Lineberger & De Marco 1973, Ramadan et al. 1975, Wank et al. 1976) or did not report the type of microbes detected (Carroll & Sebor 1980) so that skin contaminants from the venepuncture procedure may have been present.

None of the four studies adequately addressed the issue of comparing the incidence of bacteraemia following flossing in a periodontally healthy group versus a periodontitis group. Wank et al. (1976) compared bacteraemia following flossing in the same group of patients before and after periodontal therapy was instituted but did not assess or state whether the treatment had eliminated or improved their periodontal disease status and so it is unclear whether the comparison was between a periodontally

healthy and a non-healthy group. Lineberger & De Marco (1973) did have a healthy control group but did not evaluate bacteraemia following flossing in this group because they felt that it was unnecessary to do so, given the low rate of bacteraemia following flossing that they obtained in the periodontitis group. The study designs of Ramadan et al. (1975) and Carroll & Sebor (1980) precluded comparison between a periodontitis group and a periodontally healthy group.

Carroll & Sebor (1980) stated that the risk of flossing-induced bacteraemia increased with a decreased frequency of flossing. However, as stated earlier, one of the subjects had ten flossing episodes performed on him whereas two others had only one flossing episode performed on them each. The seven off-floss cultures, of which six were positive, were only performed on two subjects. The fact that their study involved only four individuals invalidates the authors' conclusions, and does not support their claim that there is a need for antibiotic prophylaxis at the initiation of an oral hygiene program for patients in whom a bacteraemia poses a special hazard. Nevertheless, the claim does warrant further investigating. The findings in their study are also not relevant to patients with periodontitis as only subjects with periodontal health or gingivitis were used. It is unlikely that daily flossing alone would eliminate the risk of bacteraemia in a subject with periodontitis.

A quantitative analysis of bacteraemia was undertaken by only one group after culturing of blood samples (Ramadan et al. 1975). They obtained bacteraemia intensities ranging from <1 CFU/mL to 11 CFU/mL. However, they did not state the methodology used to arrive at these quantitative measurements. Further, since they

considered the presence of skin contaminants as a positive result, these results cannot be used for comparison with other studies.

No significant correlation between bacteraemia and gender, age, previous periodontal treatment or degree of flossing trauma was evident in any of these four previous underpowered studies dealing with flossing and bacteraemia.

Given the paucity of data on bacteraemia caused by flossing, there is a need for further investigations to clarify whether patients with untreated or treated periodontitis are more at risk of oral bacteraemia due to flossing than are patients with a healthy periodontium. If so, then it may be necessary to instruct patients to avoid flossing until their periodontal disease has been treated, such that their gingival tissues are healthy. This would require information as to how gingival factors such as inflammation and plaque scores might act as predictive factors for the occurrence of bacteraemia due to flossing. If it can be demonstrated that the incidence of bacteraemia after flossing is increased in individuals with periodontitis then, somewhat paradoxically, it may be possible to reduce this rate of flossing-induced bacteraemia by encouraging good oral hygiene practices, of which daily flossing would form an integral part. It should be noted that most individuals do not floss as an isolated procedure. Instead, flossing is generally performed in conjunction with toothbrushing. However, studies analysing the incidence of bacteraemia after flossing can estimate how much of the bacteraemia risk is contributed by the action of flossing.

18. Prevalence of flossing in the community

Most individuals are conscious of the need for regular toothbrushing. However, despite recommendations by dental professionals rates of flossing among individuals are consistently lower than those of toothbrushing (Frandsen 1985, Tedesco et al. 1991). The prevalence of dental flossing may be related to factors such as ethnicity, socioeconomic status, age and gender, (Davidson et al. 1997, Ronis et al. 1998, Segelnick 2004). Reported daily flossing rates range from 8% in English adolescents (Macgregor et al. 1997) to 22% in Canadian urban dwellers (Payne & Locker 1996). A survey of 708 affluent American families showed that daily flossing was practised by only 20% of wives, 11% of husbands and 6% of children (Chen & Rubinson 1982). Another study, which combined the results of five separate surveys, involving 5575 American adults reported a 40% rate of flossing at least once daily among the respondents (Bakdash 1995).

A survey of 186 Finnish university students revealed that 40% of females and 25% of males reported using dental floss but that only 2% of all students flossed daily (Murtomaa et al. 1984). Another survey of about 40,000 11-, 13- and 15-year olds in 11 European countries demonstrated that use of dental floss among this age group was a rare habit (Honkala et al. 1990). However, in a Norwegian population, it was found that 27% of schoolchildren flossed (Rise et al. 1991). In a study examining the association between periodontal treatment utilisation and tooth loss involving 1497 American subjects seeking periodontal treatment, 37% of individuals reported daily flossing, 10% reported flossing four to five times a week, 27% reported flossing two to three times a week and 26% reported flossing less than once a week (Hujoel et al.

2006). The results of a recent survey estimated that 21.6% of Australians used interproximal cleaning aids at least once a day, 32% cleaned interproximally between one and six times per week and that 47.4% did not clean interproximally at all (Slade 2007). It should be noted that the results of this last study reflect the use of all interproximal aids and are not exclusive to flossing only.

Flossing frequency was significantly associated with age, gender, Body Mass Index (BMI) and smoking status in a cross-sectional study of 1497 patients visiting a periodontist (Hujoel et al. 2006). Never smokers were 30% more likely to floss daily when compared to former smokers and 89% more likely to floss daily than current smokers. Individuals who had been to a periodontist previously were 62% more likely to be daily flossers than those who had not. Interestingly, the likelihood for daily flossing decreased with increasing BMI in a dose-dependant manner. Females were 71% more likely to floss daily when compared to males. This uneven ratio of females to males when it comes to flossing was also confirmed by another study involving 1090 American adults who reported that 71% of flossers were females and 29% were males (Stoltenberg et al. 1993).

The prevalence of flossing has been related to lifestyle factors such as alcohol consumption (Toneatto & Binik 1990), preventive dental maintenance visits (Ojima et al. 2005), preventive medical care (Toneatto & Binik 1990) and diabetes (Moore et al. 2001, Siudikiene et al. 2005). Further, unlike toothbrushing, the frequency of floss use by adolescents showed only a weak association with social group (Macgregor et al. 1998). Eighty three percent of Finnish university students had been given individual instruction on flossing by dental personnel but teaching had no effect on reported

floss use (Murtomaa et al. 1984). However, in all studies, it should be remembered that the frequency of flossing that is self-reported by individuals is most likely over-estimated in order to appear to conform to a socially agreeable habit.

19. Flossing technique

There is no evidence to suggest any flossing action is superior to another and most recommendations on flossing technique are based on opinion and observations. It has been stated that there is no need to force the floss as deeply into the pocket as possible as there is no plaque there to be removed (Waerhaug 1981).

The American Dental Association has recommended the following flossing technique on its website (American Dental Association 2008). A piece of floss about 18 inches long should be cut and most of it wrapped around the middle finger of one hand with the rest wrapped around the middle finger of the opposite hand. The floss should then be held between the thumb and forefinger of both hands or stretched tightly between both forefingers so that the fingers controlling the floss are no more than one half inch apart. It should then be inserted between the teeth using a back and forth motion without forcing or snapping it into place. The floss should then be curved into a C-shape against one tooth side and guided to the gum line. It should then be slid into the space between the gum and the tooth until light resistance is felt. The floss should then be moved up and down one side of the tooth using both hands. This action should then be repeated on the proximal surface of the adjacent tooth. All proximal surfaces of all teeth including the distal surface of the posterior tooth in each quadrant should be similarly cleaned. As the floss becomes frayed or soiled a fresh section should be brought up via a turn from one middle finger to the other. A similar action has also been proposed by Perry (1996).

In a survey of 622 individuals the average number of times each subject reported flossing with an up and down motion per site was 2.6 (Segelnick 2004). Those subjects who used a "proper" flossing technique flossed significantly more times up and down than those who did not (2.8 vs 2.3). In another study evaluating the effects of flossing on gingival bleeding, flossing was professionally performed (Carter et al. 1975). The floss was wrapped around each opposing interproximal tooth surface, carried slightly subgingivally and moved inciso-gingivally over that surface for three double strokes. A similar action was used in a study comparing the plaque removal abilities of Super Floss with unwaxed dental floss (Abelson et al. 1981). Unwaxed dental floss was used with three vertical strokes on each proximal surface. The researchers in another study instructed their subjects to pull the floss back and forth five times against each of the interproximal surfaces (Gjermo & Flotra 1970). In a study evaluating the plaque removing abilities of different types of floss, the researchers had the subjects floss their own teeth (Bergenholtz and Brithon 1980). A flossing technique was used whereby the floss was moved carefully from the facial to the lingual surface. Plaque removal was performed by pressing the floss firmly against the tooth surface, mesial and distal respectively, and scrubbed up and down but not in a 'shoe-shining' fashion. The number of times this action was to be carried out was not specified.

When evaluating the effect of flossing on bacteraemia, it should be noted that flossing may be more of a traumatic procedure than toothbrushing. The act of flossing involves scraping the approximal bacterial accumulations subgingivally and then repeating this action one or more times so that a displacement of the subgingival bacteria into the submucosal tissues may occur. There is also a disruption of the

junctional epithelial hemi-desmosome attachment to the tooth surface (Waerhaug 1981). Further, the floss is in contact with non-keratinised sulcular epithelium whereas other oral hygiene activities such as toothbrushing involve contact with a keratinised epithelium. These effects of flossing may have more of a potential for introducing bacteria into the circulation than other oral hygiene procedures such as toothbrushing. The total surface area of the gingival tissues that is disrupted is also likely to be larger during flossing than toothbrushing but no studies have investigated this aspect.

20. Definition of periodontitis

Investigation of any association between bacteraemia of oral origin and the presence of periodontitis will depend on the definition of periodontitis. However, the literature contains different definitions of periodontitis. Severe periodontitis has been defined as four or more sites with PPD ≥ 4mm (Robertson et al. 1987) or as eight or more teeth with attachment loss of ≥ 5mm (Moore et al. 1982). Established periodontitis has been defined as the presence of CAL ≥ 6mm in two or more teeth and one or more sites with PPD ≥ 5mm. This definition is considered to minimise the number of false positives or cases inaccurately classified as having periodontitis (Machtei et al. 1992).

A recent case-control study involving 1357 individuals analysed the association between periodontitis and risk of myocardial infarctions using four different definitions of periodontitis (Andriankaja et al. 2006). The four definitions used were: -

a) The CAL-3 definition: Individuals with mean CAL ≥ 3mm

b) The CAL-tertile definition: Individuals with ≥ 35.79% (third tertile) of tooth sites with CAL ≥ 3mm from all teeth examined

c) The CAL-PD tertile definition: Individuals with ≥7.14% (third tertile) of tooth sites with CAL ≥ 3mm and PPD ≥ 4mm (at the same site).

d) The PD-4 definition: Individuals with at least one tooth site with PD ≥4 mm.

Regardless of the definition used, periodontitis was found to be significantly associated with increased risk of developing myocardial infarctions. However, the odds ratio for developing myocardial infarctions changed according to the definition used (3.03 for the CAL-3 definition; 4.37 for the CAL-tertile definition; 2.84 for the CAL-PD tertile definition; and 1.94 for the PD definition). The investigators found

that the stricter the definition of periodontitis, the higher the odds ratio for there being a statistically significant correlation with myocardial infarctions.

Another recent study involving 1296 pregnant women tested for an association between periodontitis and pre-term birth or low birth weight using 14 different definitions of periodontitis (Manau et al. 2008). In contrast to the findings of Andriankaja et al. (2006), the authors of this study reported that the presence of a significant association between periodontitis and pre-term birth or low birth weight depended entirely on the definition of periodontitis used, with six of the 14 periodontitis definitions used in this study showing a statistically significant association and the other eight showing no association. However, like the study of Andriankaja et al. (2006), the strength any association when it was present varied according to the definition used.

Radiographic evidence of interproximal alveolar bone loss on OPGs has been shown to correlate well with the clinical presence of periodontitis (Jenkins & Mason 1984, Walsh et al. 1997). One of the criteria suggested for periodontitis is the loss of ≥4mm bone from the cemento-enamel junction to the alveolar bone crest (Persson et al. 1998, Persson et al. 2003). This definition was used in a study examining the relationship between periodontitis and myocardial infarction (Renvert et al. 2004). Study subjects were grouped based on presence of proximal bone loss of at least 4mm in 10%, 20%, 30%, 40%, 50% and 60% of periodontal sites. The odds ratio of having episodes of myocardial infarction was found to increase with the proportion of sites that had ≥4mm bone loss.

A consensus statement from the Fifth European Workshop in Periodontology suggested that a two-tiered definition of periodontitis be utilised for studies involving risk factors for periodontitis (Tonetti & Claffey 2005). Low-threshold periodontitis was defined as the presence of proximal attachment loss of ≥3mm in two or more non-adjacent teeth and high-threshold periodontitis as the presence of proximal attachment loss of ≥5mm in ≥30% of teeth present. The low-threshold definition of periodontitis is a sensitive one whereas the high-threshold definition is a specific one. Proximal areas and non-adjacent teeth were specified by the authors in order to minimise the chance of including sites with attachment loss for reasons other than periodontitis. These include areas such as gingival recession caused by toothbrush abrasion, recession caused by a subgingival restoration or recession on the distal of a second molar caused by extraction of a third molar. The 3mm threshold was based upon the error of the recording method which was surmised to be 2.5mm. The authors suggested that odds ratios should be calculated and recommended that both definitions of periodontitis be utilised when evaluating risk factors.

Recently, new case definitions have been proposed for defining periodontitis in epidemiological studies in an attempt to reduce the error in disease prevalence by minimising the numbers of false positives (Page & Eke 2007). Severe periodontitis was defined as two or more interproximal sites (not on the same tooth) showing ≥6mm CAL and one or more interproximal site with PPD ≥5mm. Moderate periodontitis was defined as two or more interproximal sites (not on the same tooth) showing ≥4mm CAL or two or more interproximal sites (not on the same tooth) with ≥5mm PPD. Mild periodontitis was defined as one or more teeth with interproximal sites showing ≥4mm CAL and ≥4mm PPD. The definition for severe periodontitis

was intended to be a very specific one in order to ensure that patients identified by the definition did have the disease whereas the moderate periodontitis definition was intended to detect patients who had less severe periodontitis and those who had been excluded incorrectly from the severe category (Page & Eke 2007). Both CAL and PPD measurements were incorporated into the definitions because the use of CAL alone could have included periodontally healthy patients exhibiting non-inflammatory gingival recession or those who had been treated successfully but still had attachment loss whereas the use of PPD alone would have resulted in an underestimation of prevalence, especially in older persons. The CAL threshold for severe periodontitis was set at ≥6mm because a threshold value of <6mm would probably have included some healthy sites due to measurement error. The rationale for stipulating that at least two interproximal sites on non-adjacent teeth meet the set criteria was identical to that stated by the aforementioned consensus statement of Tonetti & Claffey (2005).

The thresholds for extent and severity of periodontitis vary according to the definition used. There is, however, currently no consensus on what accurately defines periodontitis. When associating periodontitis with the presence or absence of a certain condition it is arguably more important to have specificity so that we can be sure that the disease truly exists when making the association. Incorporating involvement of multiple interproximal sites, incorporating both PPD and CAL, on a number of teeth, into a definition of periodontitis would seem to be essential in obtaining this specificity.

21. Bleeding on flossing

Bleeding on stimulation may be a more accurate sign of early gingival inflammation than changes in colour, contour or texture (Muhlemann & Son 1971). This is because the bleeding from interproximal tissue is more likely to originate from inflamed connective tissue in mid-interproximal areas where visibility is obscured (Meitner et al. 1979, Abrams et al. 1984, Amato et al. 1986). Bleeding points in the gingivae do not correlate directly with clinical inflammation but rather precede apparent inflammation (Lenox & Kopczyk 1973).

A modified Gingival Bleeding Index incorporating the use of floss was suggested by Carter & Barnes (1974). This index treats each papilla as a single unit and bleeding on flossing at any given papilla is determined by sliding unwaxed floss interproximally on both sides of the interdental papilla into the sulcus. The floss is then moved in an inciso-gingival motion to the bottom of the sulcus for one double stroke. Care is taken not to cause laceration of the papillae and a new length of clean floss is used for each interproximal unit. The presence or absence of bleeding on flossing is noted 30 seconds later and evidence of bleeding is scored separately for each side of the papilla. Although the interdental papilla is treated as one unit, a clinician may choose to score each sulcus individually. Areas involving third molars are not assessed because of variations in arch position, access and vision. Other areas may be classified as non-scoreable when tooth position, diastemas or other factors compromise the desirable interproximal relationships. The total number of units that bleed is then compared to the total number of susceptible areas at risk to arrive at a Gingival Bleeding Score. The magnitude of the gingival bleeding, if present, is not

assessed. This modified Gingival Bleeding Index was subsequently used in a study assessing sulcular bleeding after the use of various types of dental floss (Carter et al. 1975) and in two clinical trials evaluating the use of waxed versus unwaxed floss (Finkelstein & Grossman 1979, Wunderlich et al. 1982).

Carter et al. (1975) and Finkelstein & Grossman (1979) treated every papilla as a separate unit whereas Wunderlich et al. (1982) treated each side (mesial and distal) of every papilla as a separate unit for the purposes of the index. At baseline in the latter study, presence of bleeding from the distal of the papilla was significantly higher than presence of bleeding from the mesial. However, this relationship disappeared as the study progressed and the subjects' gingival condition improved so that there were only minor differences between the mesial and distal surfaces of the papillae in terms of bleeding. Finkelstein & Grossman (1979) also compared the use of the modified Gingival Bleeding Index with that of the GI of Löe & Silness (1963) - modified so that the papillae and the marginal gingivae were scored separately for both the buccal and lingual surfaces - and found that both indices were suitable for use and provided the same kind of information. However, the GI allowed for an assessment in the degree of reduction of gingival inflammation and enabled comparisons between the buccal and lingual papillary surfaces, something that the modified Gingival Bleeding Index was unable to do. A further failing of the modified Gingival Bleeding Index is that it does not take into account the magnitude of the bleeding caused by flossing nor does it account for the time taken for the bleeding to occur within 30 seconds. The index may also be prone to similar errors associated with bleeding on probing such as variability in force, pressure, depth and lateral movement.

The presence of bleeding on flossing, when a constant, standardised force is used, may indicate increased levels of inflammation and an ulcerated periodontal pocket epithelium which may lead to an increased rate of bacteraemia. The number of oral micro-organisms isolated from blood samples when bleeding after a dental procedure was present were statistically greater than when there was no bleeding (Roberts 1999). However, the presence of gingival bleeding during various oral hygiene procedures was not predictive of the rate of bacteraemia associated with oral commensal bacteria (Carroll & Sebor 1980, Roberts 1999). Significant bacteraemia occurred in the absence of clinically discernable bleeding. Further, as stated by Cherry et al. (2007), bleeding due to a dental procedure can only be assessed once the treatment has actually been performed. When assessing the risk of bacteraemia in patients it is preferable if this risk can be assessed prior to this dental procedure being carried out. Thus, any correlation of bleeding on flossing with bacteraemia may not necessarily provide clinically useful information.

22. Microbial culturing

The techniques used for microbial identification can determine the level of bacteraemia detected (Coulter et al. 1990, Heimdahl et al. 1990). Lysis centrifugation is considered the most sensitive quantitative culture technique used for identification of microbes (Lockhart 2000) and is more sensitive than the BACTEC method of bacterial identification and culturing. It involves the lysis and density gradient centrifugation of blood in a single tube to concentrate microbes prior to plating on agar. This has the advantage of separating out the serum inhibitory agents present in blood. However, its use is cumbersome and there is a high rate of contamination due to increased handling (Dorn et al. 1976).

Use of the PCR technique may be much more sensitive but also categorises the presence of non-viable bacteria as a positive result and does not allow for a quantitative analysis of bacteria. A recent development is that of real-time PCR which allows for quantification of bacteraemia. This is a modified PCR technique whereby the intensity of a fluorescent signal generated during every PCR amplification cycle is compared to a standard curve of fluorescence values generated by amplification of a known quantity of DNA. The larger the amount of DNA present in the sample, the earlier the amplification cycle in which the fluorescent signal is detected, which can then indicate the quantity of initial DNA. Real-time PCR is also faster and has a reduced rate of contamination than conventional PCR (Peters et al. 2004). A positive PCR but a negative BACTEC result can also be caused by contaminants during venepuncture or laboratory processing and lack of specificity of a primer if looking for a particular organism (Victor et al. 1993, Peters et al. 2004).

If a culturing method is used, then sensitivity may also be improved by adding an inhibitor such as sodium polyanetholsulfonate or cationic and polymeric adsorbent resins to neutralise the antibacterial action of blood as well as any antibiotics in the blood (Spaargaren et al. 1998). The BACTEC system contains these two ingredients in its broth. Using more than one culture medium for blood cultures, utilising an aerobic as well as an anaerobic medium for blood culturing and withdrawing a greater volume of blood during the sampling procedure may also improve the sensitivity (Bender et al. 1961).

The BACTEC system of microbiological processing involves inoculating blood samples into BACTEC Plus Aerobic/F (enriched Soybean-Casein Digest broth carbon dioxide) and BACTEC Lytic/10 Anaerobic/F (prereduced enriched Soybean-Casein Digest broth carbon dioxide) culture bottles. Each of these culture bottles contains specially formulated nutrients in order to encourage growth of micro-organisms. The bottles are then placed in an automated instrument which operates by detecting increased carbon dioxide levels in the BACTEC culture bottles. Increased carbon dioxide levels are caused by multiplying bacteria metabolising the various nutrients present in the culture bottles. The increased carbon dioxide levels then trigger a reaction with a chemical in the culture bottles that causes increased levels of fluorescence and this increased fluorescence is then detected by the automated instrument. The automated instrument checks for increased fluorescence levels every ten minutes. If the automated instrument signals a positive result then this indicates the presumptive presence of viable micro-organisms in the bottle. This is then confirmed by subculturing using appropriate methods.

The BACTEC method of microbial identification is currently accepted to be the gold standard for studies involving bacteraemia because it has good sensitivity and specificity (Lucas et al. 2002, Cockerill et al. 2004, Peters et al. 2004). However, it may not be possible to subculture some bacteria that initially test positive for the BACTEC system as they may have exhausted all the nutrients in the BACTEC medium and die out before subculturing is carried out. High levels of white blood cells can also give a false positive result with BACTEC bottles. Dental bacteria are relatively slow growing. Thus, it has been advocated that an incubation time of at least 14 days is necessary in order to maximize chances of detecting any bacteria present (Kara et al. 2004). However, it should be noted that several species of bacteria such as Campylobacter species and the slow-growing *Mycobacterium tuberculosis* are not supported by BACTEC (Hutchinson et al. 1992, Peters et al. 2004). PCR may be beneficial in the detection of such species. The BACTEC system may also not detect certain organisms, such as Bartonella species, that do not produce enough carbon dioxide to trigger the alerting mechanism, resulting in false negatives (Tierno et al. 1995). The volume of blood inoculated into the BACTEC bottles is also of importance with one study obtaining a 29.8% greater yield of microorganisms from 20mL blood samples than from 10mL blood samples (Cockerill et al. 2004). Another disadvantage of the BACTEC microbial culturing method is that although it provides a sensitive means for recovering microbes from blood, it is not possible to obtain a quantitative measure of the level of bacteraemia.

The BACTEC system also does not allow for optimal detection of polymicrobial bacteraemia. This is because organisms in the BACTEC system compete for the same nutrients in the bottles and this competitive inhibition may reduce the ability of the

BACTEC system to detect polymicrobial infections. The predominance of one type of organism may interfere with isolation of other types (Archibald et al. 2000). Further, incubation of the BACTEC bottles in the automated instrument is ceased whenever a bottle signals positive. A sample of the broth is then Gram stained and the positive bottles are subcultured. However, this may not allow enough time for the growth of other organisms in the BACTEC bottles that may have signaled positive if the bottles had been incubated for longer prior to subculturing. This is particularly the case with fastidious organisms such as the HACEK group of micro-organisms which are common Gram negative bacilli that inhabit the oropharynx. They are a common cause of oral bacteraemia and include organisms such as *Haemophilus parainfluenzae*, *A. actinomycetemcomitans*, and *Eikenella corrodens*. These organisms can take much longer to incubate than other bacteria (Brouqui & Raoult 2001) and often require specific media and conditions to multiply. Thus, broth based systems such as the BACTEC system probably underestimate the incidence of polymicrobial infections compared to culturing on solid media. Blood cultures on solid media are also less likely to demonstrate overgrowth of one type of organism and morphological differences between organisms are more easily recognised using this method (Henry et al. 1983). In one study, 14 out of 15 instances of polymicrobial bacteraemia were detected by the lysis centrifugation method whereas only three out of 15 episodes of bacteraemia were detected by a broth culture method (Kelly et al. 1983).

A fallibility in the sensitivity of broth-based systems is that even though bacteria cannot sometimes be detected immediately following an experimental procedure, they can be isolated from a blood sample taken a few minutes later (Lafaurie et al. 2007). Further, in the same subject, different microbes are often detected at different sample

timings following an experimental procedure (Lafaurie et al. 2007), once again demonstrating the limited sensitivity of broth-based systems in detecting polymicrobial bacteraemia.

There have been suggestions that blind subculturing should be performed on all bottles that fail to signal positive at the end of the incubation period. This would theoretically increase the rate of true positives that are detected, especially for polymicrobial infections (Hansen & Hetmanski 1983). However, blind terminal subculturing for broth based systems is currently not considered to be necessary (Araj et al. 1981) as most microbes will signal positive within seven days if they are present in the broth (Murray 1985). Blind terminal culturing should only be performed if a certain micro-organism is strongly suspected but a blood culture turns up negative.

23. Summary of literature review

Recent guideline changes have seen a reduction in the requirement for antibiotic prophylaxis prior to invasive dental procedures in patients at risk of infective endocarditis**.** One of the main factors contributing to these changes was that 'everyday bacteraemia' caused by oral hygiene procedures may be of more importance in the aetiology of infective endocarditis than bacteraemia caused by dental procedures. Several studies have evaluated bacteraemia caused by toothbrushing. However, only four studies have evaluated flossing-induced bacteraemia and there are several methodological deficiencies associated with these studies. Given the paucity of data on bacteraemia caused by flossing, there is a need for further investigation of flossing-induced bacteraemia and a need to clarify whether patients with periodontal disease are more at risk of oral bacteraemia due to flossing than are patients with a healthy periodontium.

24. References

Abelson, D. C., Barton, J. E., Maietti, G. M. & Cowherd, M. G. (1981). Evaluation of interproximal cleaning by two types of dental floss. *Clinical Preventive Dentistry*, **3**, 19-21.

Abrams, K., Caton, J. G. & Polson, A. M. (1984). Histologic comparisons of interproximal gingival tissues related to the presence or absence of bleeding. *Journal of Periodontology*, **55**, 629-632.

Amato, R., Caton, J. G., Polson, A. M. & Espeland, M. (1986). Interproximal gingival inflammation related to the conversion of a bleeding to a non-bleeding state. *Journal of Periodontology*, **57**, 63-68.

American Dental Association (2008). Oral Health topics A-Z. Cleaning your teeth and gums (oral hygiene). How do I floss my teeth? [WWW document]. URL http://www.ada.org/public/topics/cleaning_faq.asp#4 [accessed on 06 Jul 2008].

American Dental Association Council On Scientific Affairs (2004). Combating antibiotic resistance. *Journal of the American Dental Association*, **135**, 484-487.

Andriankaja, O. M., Genco, R. J., Dorn, J., Dmochowski, J., Hovey, K., Falkner, K. L., Scannapieco, F. & Trevisan, M. (2006). The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. *Journal of Periodontology*, **77**, 1067-1073.

Araj, G. F., Hopfer, R. L., Wenglar, M. & Fainstein, V. (1981). Value of terminal subcultures from negative blood culture bottles. *Journal of Clinical Microbiology*, **14**, 589-590.

Archibald, L. K., McDonald, L. C., Addison, R. M., McKnight, C., Byrne, T., Dobbie, H., Nwanyanwu, O., Kazembe, P., Reller, L. B. & Jarvis, W. R. (2000). Comparison of BACTEC Myco/F Lytic and Wampole Isolator 10 (lysis-centrifugation) systems for detection of bacteremia, mycobacteremia, and fungemia in a developing country. *Journal of Clinical Microbiology*, **38**, 2994-2997.

Axelsson, P. (1998). Needs-related plaque control measures based on risk prediction. In Proceedings of the European Workshop on Mechanical Plaque Control, eds. Lang, N., Attstrom, R. & Löe, H., pp. 190-247. Chicago: Quintessence.

Bahn, S. L., Goveia, G., Bitterman, P. & Bahn, A. N. (1978). Experimental endocarditis induced by dental manipulation and oral streptococci. *Oral Surgery*, **45**, 549-550.

Bakdash, B. (1995). Current patterns of oral hygiene product use and practices. *Periodontology 2000*, **8**, 11-14.

Baltch, A. L., Pressman, H. L., Hammer, M. C., Sutphen, N. C., Smith, R. P. & Shayegani, M. (1982). Bacteremia following dental extractions in patients with and without penicillin prophylaxis. *American Journal of Medical Science*, **283**, 129-140.

Baltch, A. L., Pressman, H. L., Schaffer, C., Smith, R. P., Hammer, M. C., Shayegani, M. & Michelsen, P. (1988). Bacteremia in patients undergoing oral procedures: study following parenteral antimicrobial prophylaxis as

recommended by the American Heart Association, 1977. *Archives of Internal Medicine*, **148**, 1084-1088.

Barco, C. T. (1991). Prevention of infective endocarditis: a review of the medical and dental literature. *Journal of Periodontology*, **62**, 510-523.

Barnes, J. M. & Trueta, J. (1941). Absorption of bacteria, toxins and snake venoms from the tissues. *The Lancet*, **1**, 623-626.

Barnes, P. F., Arevalo, C., Chan, L. S., Wong, S. F. & Reynolds, T. B. (1988). A prospective evaluation of bacteremic patients with chronic liver disease. *Hepatology*, **8**, 1099-1103.

Baumgartner, J. C., Heggers, J. P. & Harrison, J. W. (1976). The incidence of bacteremias related to endodontic procedures. Nonsurgical endodontics. *Journal of Endodontics*, **2**, 135-140.

Bayliss, R., Clarke, C., Oakley, C., Somerville, W., Whitfield, A. G. W. & Young, S. E. J. (1983). The microbiology and pathogenesis of infective endocarditis. *British Heart Journal*, **50**, 513-519.

Beeson, P. B., Brannon, E. S. & Warren, J. V. (1945). Observations on the sites of removal of bacteria from the blood in patients with bacterial endocarditis. *Journal of Experimental Medicine*, **81**, 9-23.

Bender, I. B., Seltzer, S., Meloff, G. & Pressman, R. S. (1961). Conditions affecting sensitivity of techniques for detection of bacteremia. *Journal of Dental Research*, **40**, 951-959.

Bergenholtz, A. & Brithon, J. (1980). Plaque removal by dental floss or toothpicks. An intra-individual comparative study. *Journal of Clinical Periodontology*, **7**, 516-524.

Berger, S. A., Weitzman, S., Edberg, S. C. & Casey, J. I. (1974). Bacteremia after the use of an oral irrigation device. A controlled study in subjects with normal-appearing gingiva: comparison with use of toothbrush. *Annals of Internal Medicine*, **80**, 510-511.

Berry, F. A., Jr., Blankenbaker, W. L. & Ball, C. G. (1973). A comparison of bacteremia occurring with nasotracheal and orotracheal intubation. *Anesthesia and Analgesia*, **52**, 873-876.

Bhanji, S., Williams, B., Sheller, B., Elwood, T. & Mancl, L. (2002). Transient bacteremia induced by toothbrushing: A comparison of the Sonicare toothbrush with a conventional toothbrush. *Pediatric Dentistry*, **24**, 295-299.

Bohnsack, J. F. & Brown, E. J. (1986). The role of the spleen in resistance to infection. *Annual Review of Medicine*, **37**, 49-59.

Bor, D. H. & Himmelstein, D. U. (1984). Endocarditis prophylaxis for patients with mitral valve prolapse. A quantitative analysis. *American Journal of Medicine*, **76**, 711-717.

Brouqui, P. & Raoult, D. (2001). Endocarditis due to rare and fastidious bacteria. *Clinical Microbiology Reviews*, **14**, 177-207.

Buckingham, J. K., Gould, I. M., Tervitt, G. & Williams, S. (1992). Prevention of endocarditis: communication between doctors and dentists. *British Dental Journal*, **172**, 414-415.

Burket, L. W. & Burn, C. G. (1937). Bacteremias following dental extraction. Demonstration of source of bacteria by means of a non-pathogen (Serratia Marcesens). *Journal of Dental Research*, **16**, 521-530.

Carroll, G. C. & Sebor, R. J. (1980). Dental flossing and its relationship to transient bacteremia. *Journal of Periodontology*, **51**, 691-692.

Carter, H. G. & Barnes, G. P. (1974). The Gingival Bleeding Index. *Journal of Periodontology*, **45**, 801-805.

Carter, H. G., Barnes, G. P., Radentz, W. H., Levin, M. P. & Bhaskar, S. N. (1975). Effects of using various types of dental floss on gingival sulcular bleeding. *Virginia Dental Journal*, **52**, 18-32.

Cerny, A., Neftel, K. A., Fux, C. & Hoigne, R. (2000). Beta-lactams: penicillins. In Meyler's side effects of drugs. 14th Edition, eds. Dukes, M.N.G. & Aronson, J.K., p. 81. Amsterdam: Elsevier Science.

Chen, M. S. & Rubinson, L. (1982). Preventive dental behavior in families: a national survey. *Journal of the American Dental Association*, **105**, 43-46.

Cherry, M., Daly, C. G., Mitchell, D. & Highfield, J. (2007). Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. *Journal of Clinical Periodontology*, **34**, 148-155.

Chung, A., Kudlick, E. M., Gregory, J. E., Royal, G. C. & Reindorf, C. A. (1986). Toothbrushing and transient bacteremia in patients undergoing orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, **90**, 181-186.

Clemens, J. D. & Ransohoff, D. F. (1984). A quantitative assessment of pre-dental antibiotic prophylaxis for patients with mitral-valve prolapse. *Journal of Chronic Diseases*, **37**, 531-544.

Cobe, H. (1954). Transitory bacteremia. *Journal of Oral Surgery*, **7**, 609-615.

Cockerill, F., Hughes, J., Vetter, E., Mueller, R., Weaver, A., Ilstrup, D., Rosenblatt, J. & Wilson, W. (1997). Analysis of 281,797 consecutive blood cultures performed over an eight-year period: trends in microorganisms isolated and

the value of anaerobic culture of blood. *Clinical Infectious Diseases*, **24**, 403-418.

Cockerill, F. R., Wilson, J. W., Vetter, E. A., Goodman, K. M., Torgerson, C. A., Harmsen, W. S., Schleck, C. D., Ilstrup, D. M., Washington, J. A., II & Wilson, W. R. (2004). Optimal testing parameters for blood cultures. *Clinical Infectious Diseases*, **38**, 1724-1730.

Coulter, W. A., Coffey, A., Saunders, I. D. & Emmerson, A. M. (1990). Bacteremia in children following dental extraction. *Journal of Dental Research*, **69**, 1691-1695.

Croxson, M. S., Altmann, M. M. & K.P., O. B. (1971). Dental status and recurrence of streptococcus viridans endocarditis. *The Lancet*, **1**, 1205.

Dajani, A. S., Taubert, K. A., Wilson, W., Bolger, A. F., Bayer, A., Ferrieri, P., Gewitz, M. H., Shulman, S. T., Nouri, S., Newburger, J. W., Hutto, C., Pallasch, T. J., Gage, T. W., Levison, M. E., Peter, G. & Zuccaro, G., Jr. (1997). Prevention of bacterial endocarditis. Recommendations by the American Heart Association. *Journal of the American Dental Association*, **277**, 1794-1801.

Daly, C. G. (1995). Antibiotic prophylaxis for the prevention of infective endocarditis: Protecting the patient or the dentist? *Periodontology*, **16**, 32-36.

Daly, C., Mitchell, D., Grossberg, D., Highfield, J. & Stewart, D. (1997). Bacteraemia caused by periodontal probing. *Australian Dental Journal*, **42**, 77-80.

Daly, C. G., Mitchell, D. H., Highfield, J. E., Grossberg, D. E. & Stewart, D. (2001). Bacteremia due to periodontal probing: a clinical and microbiological investigation. *Journal of Periodontology*, **72**, 210-214.

Davidson, P. L., Rams, T. E. & Andersen, R. M. (1997). Socio-behavioral determinants of oral hygiene practices among USA ethnic and age groups. *Advances in Dental Research*, **11**, 245-253.

Delahaye, F., Goulet, V., Lacassfn, F., Ecochard, R., Selton-Suty, C., Hoen, B., Etienne, J., Briancon, S. & Leport, C. (1995). Characteristics of infective endocarditis in France in 1991: A 1-year survey. *European Heart Journal*, **16**, 394-401.

Dinner, M., Tjeuw, M. & Artusio Jr, J. F. (1987). Bacteremia as a complication of nasotracheal intubation. *Anaesthetics and Analgesia*, **66**, 460-462.

Doern, G. V., Ferraro, M. J., Brueggemann, A. B. & Ruoff, K. L. (1996). Emergence of high rates of antimicrobial resistance among viridans group streptococci in the United States. *Antimicrobial Agents and Chemotherapy*, **40**, 891-894.

Dorn, G. L., Burson, G. G. & Haynes, J. R. (1976). Blood culture technique based on centrifugation: clinical evaluation. *Journal of Clinical Microbiology*, **3**, 258-263.

Dorn, G. L., Lemeshev, Y. & Hill, J. M. (1981). Quantitative blood cultures - their value with laboratory contaminants. *Journal of Clinical Hematology and Oncology*, **11**, 85-91.

Drangsholt, M. T. (1998). A new causal model of dental diseases associated with endocarditis. *Annals of Periodontology*, **3**, 184-196.

Durack, D. T. (1995). Prevention of infective endocarditis. *New England Journal of Medicine*, **332**, 38-44.

Durack, D. T. (2000). Prophylaxis of infective endocarditis. In Principles and Practice of Infectious Diseases. 5th Edition. Volume 1, eds. Mandell, G.L., Bennett, J.L. & Dolin, R., pp. 917-925. Philadelphia: Churchill Livingston.

Durack, D. T. & Beeson, P. B. (1972). Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. *British Journal of Experimental Pathology*, **53**, 44-49.

Durack, D. T., Kaplan, E. L. & Bisno, A. (1983). Apparent failures of endocarditis prophylaxis. Analysis of 52 cases submitted to a national registry. *Journal of the American Medical Association*, **250**, 2318-2322.

Durack, D. T., Lukes, A. S. & Bright, D. K. (1994). New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. *American Journal of Medicine*, **96**, 200-209.

Duval, X., Alla, F., Hoen, B., Danielou, F., Larrieu, S., Delahaye, F., Leport, C. & Briancon, S. (2006). Estimated risk of endocarditis in adults with predisposing cardiac conditions undergoing dental procedures with or without antibiotic prophylaxis. *Clinical Infectious Diseases*, **42**, e102-107.

Duval, X. & Leport, C. (2008). Prophylaxis of infective endocarditis: current tendencies, continuing controversies. *Lancet Infectious Diseases*, **8**, 225-232.

Dwyer, D., Chen, S., Wright, E., Crimmins, D., Collignon, P. & Sorrell, T. (1994). Hospital practices influence the pattern of infective endocarditis. *The Medical Journal of Australia*, **160**, 709-714.

Egelberg, J. & Claffey, N. (1998). Role of mechanical dental plaque removal in prevention and therapy of caries and periodontal diseases. Consensus Report of Group B. In Proceedings of the European Workshop on Mechanical Plaque Control, eds. Lang, N., Attstrom, R. & Löe, H., pp. 169-172. London: Quintessence.

Epstein, J. B., Chong, S. & Le, N. D. (2000). A survey of antibiotic use in dentistry. *Journal of the American Dental Association*, **131**, 1600-1609.

Everett, E. D. & Hirschmann, J. V. (1977). Transient bacteremia and endocarditis prophylaxis. A review. *Medicine*, **56**, 61-77.

Felix, J. E., Rosen, S. & App, G. R. (1971). Detection of bacteremia after the use of an oral irrigation device in subjects with periodontitis. *Journal of Periodontology*, **42**, 785-787.

Fiehn, N. E., Gutschik, E., Larsen, T. & Bangsborg, J. M. (1995). Identity of streptococcal blood isolates and oral isolates from two patients with infective endocarditis. *Journal of Clinical Microbiology*, **33**, 1399-1401.

Filice, G. A., Darby, C. P. & Fraser, D. W. (1980). Pneumococcal bacteremia in Charleston County, South Carolina. *American Journal of Epidemiology*, **112**, 828-835.

Finkelstein, P. & Grossman, E. (1979). The effectiveness of dental floss in reducing gingival inflammation. *Journal of Dental Research*, **58**, 1034-1039.

Forner, L., Larsen, T., Kilian, M. & Holmstrup, P. (2006). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *Journal of Clinical Periodontology*, **33**, 401-407.

Fowler, V. G., Scheld, W. M. & Bayer, A. S. (2005). Endocarditis and intravascular infections. In Principles and Practices of Infectious Diseases, eds. Mandell, G.L., Bennett, J.E. & Dolin, R., pp. 975-1021. Philadelphia: Elsevier Churchill Livingstone.

Frandsen, A. (1985). Changing patterns of attitudes and oral health behaviour. *International Dental Journal*, **35**, 284-290.

Freedman, L. R. (1987). The pathogenesis of infective endocarditis. *Journal of Antimicrobial Chemotherapy*, **20 (Suppl)**, 1-6.

Gjermo, P. & Flotra, L. (1970). The effect of different methods of interdental cleaning. *Journal of Periodontal Research*, **5**, 230-236.

Glass, R. T. & Lare, M. M. (1986). Toothbrush contamination: a potential health risk? *Quintessence International*, **17**, 39-42.

Glauser, M. P. & Francioli, P. (1987). Relevance of animal models to the prophylaxis of infective endocarditis. *Journal of Antimicrobial Chemotherapy*, **20 (Suppl A)**, 87-98.

Gould, F. K., Elliott, T. S. J., Foweraker, J., Fulford, M., Perry, J. D., Roberts, G. J., Sandoe, J. A. T. & Watkin, R. W. (2006a). Guidelines for the prevention of endocarditis: report of the Working Party of the British Society for Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy*, **57**, 1035-1042.

Gould, F. K., Elliott, T. S. J., Foweraker, J., Fulford, M., Perry, J. D., Roberts, G. J., Sandoe, J. A. T. & Watkin, R. W. (2006b). Guidelines for the prevention of endocarditis: report of the Working Party of the British Society for Antimicrobial Chemotherapy - authors' response. *Journal of Antimicrobial Chemotherapy*, **58**, 896-898.

Greenberg, M. S. (1990). Prechemotherapy dental treatment to prevent bacteremia. *NCI Monographs*, **9**, 49-50.

Guntheroth, W. G. (1984). How important are dental procedures as a cause of infective endocarditis? *The American Journal of Cardiology*, **54**, 797-801.

Hall, G., Hedstrom, S., Helmdahl, A. & Nord, C. E. (1993). Prophylactic administration of penicillins for endocarditis does not reduce the prevalence of post extraction bacteremias. *Clinical Infectious Diseases*, **17**, 188-194.

Hall, G., Heimdahl, A. & Nord, C. E. (1999). Bacteremia after oral surgery and antibiotic prophylaxis for endocarditis. *Clinical Infectious Diseases*, **29**, 1-8.

Hansen, C. P., Westh, H., Brok, K. E., Jensen, R. & Bertelsen, S. (1989). Bacteraemia following orotracheal intubation and oesophageal balloon dilatation. *Thorax*, **44**, 684-685.

Hansen, S. L. & Hetmanski, J. (1983). Enhanced detection of polymicrobic bacteremia by repeat subculture of previously positive blood cultures. *Journal of Clinical Microbiology*, **18**, 208-210.

Hartzell, J. D., Torres, D., Kim, P. & Wortmann, G. (2005). Incidence of bacteremia after routine tooth brushing. *American Journal of the Medical Sciences*, **329**, 178-180.

Heimdahl, A., Hall, G., Hedberg, M., Sandberg, H., Soder, P. O., Tuner, K. & Nord, C. E. (1990). Detection and quantification by lysis-filtration of bacteremia after different oral surgical procedures. *Journal of Clinical Microbiology*, **28**, 2205-2209.

Henry, N. K., McLimans, C. A., Wright, A. J., Thompson, R. L., Wilson, W. R. & Washington II, J. A. (1983). Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. *Journal of Clinical Microbiology*, **17**, 864-869.

Herzberg, M. & Meyer, M. (1996). Effects of oral flora on platelets: possible consequences in cardiovascular disease. *Journal of Periodontology*, **67**, 1138-1142.

Hockett, R. N., Loesche, W. J. & Sodeman, T. M. (1977). Bacteraemia in asymptomatic human subjects. *Archives of Oral Biology*, **22**, 91-98.

Honkala, E., Kannas, L. & Rise, J. (1990). Oral health habits of schoolchildren in 11 European countries. *International Dental Journal*, **40**, 211-217.

Horstkotte, D., Follath, F., Gutschik, E., Lengyel, M., Oto, A., Pavie, A., Soler-Soler, J., Thiene, G., von Graevenitz, A., Priori, S. G., Garcia, M. A. A., Blanc, J.-J., Budaj, A., Cowie, M., Dean, V., Deckers, J., Fernandez Burgos, E., Lekakis, J., Lindahl, B., Mazzotta, G., Morais, J., Oto, A., Smiseth, O. A., Lekakis, J., Vahanian, A., Delahaye, F., Parkhomenko, A., Filipatos, G., Aldershvile, J. & Vardas, P. (2004). Guidelines on prevention, diagnosis and treatment of infective endocarditis executive summary; the task force on infective endocarditis of the European Society of Cardiology. *European Heart Journal*, **25**, 267-276.

Huber, M. A. & Terezhalmy, G. T. (2005). The patient with a transient bacteremia. *General Dentistry*, **53**, 130-143.

Hujoel, P. P., Cunha-Cruz, J. & Kressin, N. R. (2006). Spurious associations in oral epidemiological research: the case of dental flossing and obesity. *Journal of Clinical Periodontology*, **33**, 520-523.

Hujoel, P. P., White, B. A., Garcia, R. I. & Listgarten, M. A. (2001). The dentogingival epithelial surface area revisited. *Journal of Periodontal Research*, **36**, 48-55.

Hutchinson, N. A., Thomas, F. D. & Shanson, D. C. (1992). The clinical comparison of Oxoid signal with Bactec blood culture systems for the detection of streptococcal and anaerobic bacteraemias. *Journal of Medical Microbiology*, **37**, 410-412.

Imperiale, T. F. & Horwitz, R. I. (1990). Does prophylaxis prevent postdental infective endocarditis? A controlled evaluation of protective efficacy. *The American Journal of Medicine*, **88**, 131-136.

Infective Endocarditis Prophylaxis Expert Group (2008). Prevention of Endocarditis. 2008 update from Therapeutic Guidelines: Antibiotic. Version 13. North Melbourne: Therapeutic Guidelines Ltd.

Jenkins, W. M. M. & Mason, W. N. (1984). Radiographic assessment of periodontitis. *British Dental Journal*, **156**, 170-177.

Jenkinson, H. F. & Lamont, R. J. (1997). Streptococcal adhesion and colonization. *Critical Reviews in Oral Biology and Medicine*, **8**, 175-200.

Jenney, A. W., Cherry, C. L., Davis, B. & Wesselingh, S. L. (2001). "Floss and (nearly) die": dental floss and endocarditis. *Medical Journal of Australia*, **174**, 107-108.

Kara, A., Kanra, G., Cengiz, A. B., Apis, M. & Gur, D. (2004). Pediatric blood culture: time to positivity. *Turkish Journal of Pediatrics*, **46**, 251-255.

Kaufman, D. W. & Kelly, J. P. (2003). Risk of anaphylaxis in a hospital population in relation to the use of various drugs: an international study. *Pharmacoepidemiology and Drug Safety*, **12**, 202.

Kelly, M. T., Fojtasek, M. F., Abbott, T. M., Hale, D. C., Dizikes, J. R., Boshard, R., Buck, G. E., Martin, W. J. & Matsen, J. M. (1983). Clinical evaluation of a lysis-centrifugation technique for the detection of septicaemia. *Journal of the American Medical Association*, **250**, 2185-2188.

Kennedy, H. F., Morrison, D., Tomlinson, D., Gibson, B. E. S., Bagg, J. & Gemmell, C. G. (2002). Gingivitis and toothbrushes: potential roles in viridans streptococcal bacteraemia. *Journal of Infection*, **46**, 67-70.

Kinane, D. F., Riggio, M. P., Walker, K. F., MacKenzie, D. & Shearer, B. (2005). Bacteraemia following periodontal procedures. *Journal of Clinical Periodontology*, **32**, 708-713.

Lacassin, F., Hoen, B., Leport, C., Selton-Suty, C., Delahaye, F., Goulet, V., Etienne, J. & Briancon, S. (1995). Procedures associated with infective endocarditis in adults. A case control study. *European Heart Journal*, **16**, 1968-1974.

Lafaurie, G. I., Mayorga-Fayad, I., Torres, M. F., Castillo, D. M., Aya, M. R., Baron, A. & Hurtado, P. A. (2007). Periodontopathic microorganisms in peripheric blood after scaling and root planing. *Journal of Clinical Periodontology*, **34**, 873-879.

Lazansky, J. P., Robinson, L. & Rodofsky, L. (1949). Factors influencing the incidence of bacteremias following surgical procedures in the oral cavity. *Journal of Dental Research*, **28**, 533.

Leinonen, M. & Saikku, P. (2002). Evidence for infectious agents in cardiovascular disease and atherosclerosis. *Lancet Infectious Diseases*, **2**, 11-17.

Lenox, J. A. & Kopczyk, R. A. (1973). A clinical system for scoring a patient's oral hygiene performance. *Journal of the American Dental Association*, **86**, 849-852.

Li, L., Messas, E., Batista, E. L., Jr., Levine, R. A. & Amar, S. (2002). Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. [erratum appears in Circulation 2002 Apr 2;105(13):1617]. *Circulation*, **105**, 861-867.

Lineberger, L. T. & De Marco, T. J. (1973). Evaluation of transient bacteremia following routine periodontal procedures. *Journal of Periodontology*, **44**, 757-762.

Lockhart, P. B. (1996). An analysis of bacteremias during dental extractions. A double-blind, placebo-controlled study of chlorhexidine. *Archives of Internal Medicine*, **156**, 513-520.

Lockhart, P. B. (2000). The risk for endocarditis in dental practice. *Periodontology 2000*, **23**, 127-135.

Lockhart, P. B., Brennan, M. T., Kent, M. L., Norton, H. J. & Weinrib, D. A. (2004). Impact of Amoxicillin prophylaxis on the incidence, nature and duration of bacteremia in children after intubation and dental procedures. *Circulation*, **109**, 2878-2884.

Lockhart, P. B. & Schmidtke, M. A. (1994). Antibiotic considerations in medically compromised patients. *Dental Clinics of North America*, **38**, 381-402.

Löe, H. & Silness, J. (1963). Periodontal disease in pregnancy - I. Prevalence and severity. *Acta Odontologica Scandinavica*, **21**, 533-551.

Lofthus, J. E., Waki, M. Y., Jolkovsky, D. L., Otomo-Corgel, J., Newman, M. G., Flemmig, T. & Nachnani, S. (1991). Bacteremia following subgingival irrigation and scaling and root planing. *Journal of Periodontology*, **62**, 602-607.

Lucas, V., Lytra, V., Hassan, T., Tatham, H., Wilson, M. & Roberts, G. (2002). Comparison of lysis filtration and an automated blood culture system (BACTEC) for detection, quantification and identification of odontogenic bacteremia in children. *Journal of Clinical Microbiology*, **40**, 3416-3420.

Lucas, V. & Roberts, G. J. (2000). Odontogenic bacteremia following tooth cleaning procedures in children. *Pediatric Dentistry*, **22**, 96-100.

Macgregor, I. D., Regis, D. & Balding, J. (1997). Self-concept and dental health behaviours in adolescents. *Journal of Clinical Periodontology*, **24**, 335-339.

Macgregor, I. D. M., Balding, J. W. & Regis, D. (1998). Flossing behaviour in English adolescents. *Journal of Clinical Periodontology*, **25**, 291-296.

Machtei, E. E., Christersson, L. A., Grossi, S. G., Dunford, R., Zambon, J. J. & Genco, R. J. (1992). Clinical criteria for the definition of "established periodontitis". *Journal of Periodontology*, **63**, 206-214.

Madsen, K. L. (1974). Effect of chlorhexidine mouthrinse and periodontal treatment upon bacteremia produced by oral hygiene procedures. *Scandinavian Journal of Dental Research*, **82**, 1-7.

Manau, C., Echeverria, A., Agueda, A., Guerrero, A. & Echeverria, J. J. (2008). Periodontal disease definition may determine the association between periodontitis and pregnancy outcomes. *Journal of Clinical Periodontology*, **35**, 385-397.

Manning, J. E., Hume, E. B., Hunter, N. & Knox, K. W. (1994). An appraisal of the virulence factors associated with streptococcal endocarditis. *Journal of Medical Microbiology*, **40**, 110-114.

Martin, M. (2003). Is there a link between tooth brushing and infective endocarditis? *International Dental Journal*, **53 (Suppl 3)**, 187-190.

Martin, M. V., Longman, L. P., Forde, M. P. & Butterworth, M. L. (2007). Infective endocarditis and dentistry: the legal basis for an association. *British Dental Journal*, **203**, E1.

McGowan, D. A. & Hardie, J. M. (1974). Production of bacterial endocarditis in prepared rabbits by oral manipulation. *British Dental Journal*, **137**, 129-131.

McShane, A. J. & Hone, R. (1986). Prevention of bacterial endocarditis: does nasal intubation warrant prophylaxis? *British Medical Journal*, **292**, 26-27.

Meitner, S. W., Zander, H. A., Iber, H. P. & Polson, A. M. (1979). Identification of inflamed gingival surfaces. *Journal of Clinical Periodontology*, **6**, 93-97.

Messini, M., Skourti, I., Markopulos, E., Koutsia-Carouzou, C., Kyriakopoulou, E., Kostaki, S., Lambraki, D. & Georgopoulos, A. (1999). Bacteremia after dental treatment in mentally handicapped people. *Journal of Clinical Periodontology*, **26**, 469-473.

Moore, P. A., Weyant, R. J., Etzel, K. R., Guggenheimer, J., Mongelluzzo, M. B., Myers, D. E., Rossie, K., Hubar, H., Block, H. M. & Orchard, T. (2001). Type 1 diabetes mellitus and oral health: assessment of coronal and root caries. *Community Dentistry and Oral Epidemiology*, **29**, 183-194.

Moore, W. E. C., Holdeman, L. V., Smibert, R. M., Hash, D. E., Burmeister, J. A. & Ranney, R. R. (1982). Bacteriology of severe periodontitis in young adult humans. *Infection and Immunity*, **38**.

Moreillon, P., Francioli, P., Overholser, D., Meylan, P. & Glauser, M. P. (1986). Mechanism of successful amoxicillin prophylaxis of experimental endocarditis due to streptococcus intermedius. *Journal of Infectious Diseases*, **154**, 801-807.

Moreillon, P. & Que, Y.-A. (2004). Infective endocarditis. *The Lancet*, **363**, 139-149.

Morris, A. M. (2007). Coming clean with antibiotic prophylaxis for infective endocarditis. *Archives of Internal Medicine*, **167**, 330-332.

Moss, R. & Munt, B. (2003). Injection drug use and right sided endocarditis. *Heart*, **89**, 577-581.

Muhlemann, H. R. & Son, S. (1971). Gingival sulcus bleeding--a leading symptom in initial gingivitis. *Helvetica Odontologica Acta*, **15**, 107-113.

Murdoch, F. E., Sammons, R. L. & Chapple, I. L. C. (2004). Isolation and characterization of subgingival staphylococci from periodontitis patients and controls. *Oral Diseases*, **10**, 155-162.

Murphy, A. M., Daly, C. G., Mitchell, D. H., Stewart, D. & Curtis, B. H. (2006). Chewing fails to induce oral bacteraemia in patients with periodontal disease. *Journal of Clinical Periodontology*, **33**, 730-736.

Murray, P. R. (1985). Determination of the optimum incubation period of blood culture broths for the detection of clinically significant septicemia. *Journal of Clinical Microbiology*, **21**, 481-485.

Murtomaa, H., Turtola, L. & Rytoumaa, I. (1984). Use of dental floss by Finnish students. *Journal of Clinical Periodontology*, **11**, 443-447.

Nasi, J. H. (1994). Background to, and implementation of, the Periodontal Screening and Recording (PSR) procedure in the USA. *International Dental Journal*, **44 (5 Suppl 1)**, 585-588.

National Institute for Health and Clinical Excellence Guideline Development Group (2008). Prophylaxis against infective endocarditis: antimicrobial prophylaxis against infective endocarditis in adults and children undergoing interventional procedures. [WWW document]. URL http://www.nice.org.uk/CG064 [accessed on 22 Mar 2008].

Nelson, D. B. (2003a). Infection control during gastrointestinal endoscopy. *Journal of Laboratory and Clinical Medicine*, **141**, 159-167.

Nelson, D. B. (2003b). Infectious disease complications of Gl endoscopy: Part I, endogenous infections. *Gastrointestinal Endoscopy*, **57**, 546-556.

Netzer, R. O., Zollinger, E., Seiler, C. & Cerny, A. (2000). Infective endocarditis: clinical spectrum, presentation and outcome. An analysis of 212 cases 1980-1995. *Heart*, **84**, 25-30.

Nissen, H., Nielsen, P. F., Frederiksen, M., Helleberg, C. & Nielsen, J. S. (1992). Native valve infective endocarditis in the general population: A 10-year survey of the clinical picture during the 1980s. *European Heart Journal*, **13**, 872-877.

Offenbacher, S., Beck, J. D., Lieff, S. & Slade, G. (1998). Role of periodontitis in systemic health: spontaneous preterm birth. *Journal of Dental Education*, **62**, 852-858.

Ojima, M., Kanagawa, H., Nishida, N., Nagata, H., Hanioka, T. & Shizukuishi, S. (2005). Relationship between attitudes toward oral health at initial office visit and compliance with supportive periodontal treatment. *Journal of Clinical Periodontology*, **32**, 364-368.

Okell, C. C. & Elliott, S. D. (1935). Bacteraemia and oral sepsis with special reference to the aetiology of subacute endocarditis. *The Lancet*, **226**, 869-872.

Oliver, R., Roberts, G. J. & Hooper, L. (2006). Penicillins for the prophylaxis of bacterial endocarditis in dentistry. *Cochrane Database of Systematic Reviews*.

Overholser, C. D., Moreillon, P. & Glauser, M. P. (1987). Experimental bacterial endocarditis after dental extractions in rats with periodontitis. *Journal of Infectious Diseases*, **155**, 107-112.

Overholser, C. D., Peterson, D. E., Williams, L. T. & Schimpff, S. C. (1982). Periodontal infection in patients with acute nonlymphocyte leukemia.

Prevalence of acute exacerbations. *Archives of Internal Medicine*, **142**, 551-554.

Page, R. C. & Eke, P. I. (2007). Case definitions for use in population-based surveillance of periodontitis. *Journal of Periodontology*, **78**, 1387-1399.

Pallasch, T. J. (1989). A critical appraisal of antibiotic prophylaxis. *International Dental Journal*, **39**, 183-196.

Pallasch, T. J. (2003). Antibiotic prophylaxis: problems in paradise. *Dental Clinics of North America*, **47**, 665-679.

Pallasch, T. J. & Slots, J. (1996). Antibiotic prophylaxis and the medically compromised patient. *Periodontology 2000*, **10**, 107-138.

Payne, B. J. & Locker, D. (1996). Relationship between dental and general health behaviors in a Canadian population. *Journal of Public Health Dentistry*, **56**, 198-204.

Pelletier, L. L., Durack, D. T. & Petersdorf, R. G. (1975). Chemotherapy of experimental streptococcal endocarditis. IV. Further observations of prophylaxis. *Journal of Clinical Investigations*, **56**, 319.

Perry, D. A. (1996). Plaque control for the periodontal patient. In Carranza's Clinical Periodontology, 9th edition, eds. Newman, M.G., Takei, H.H. & Carranza F.A., pp. 660-661. Philadelphia: WB Saunders Co.

Persson, G. R., Attstrom, R., Lang, N. P. & Page, R. C. (2003). Perceived risk of deteriorating periodontal conditions. *Journal of Clinical Periodontology*, **30**, 982-989.

Persson, R. E., Hollender, L. G. & Persson, G. R. (1998). Assessment of alveolar bone levels from intraoral radiographs in subjects between ages 15 and 94 years seeking dental care. *Journal of Clinical Periodontology*, **25**, 647-654.

Peters, R. P. H., van Agtmael, M. A., Danner, S. A., Savelkoul, P. H. M. & Vandenbroucke-Grauls, C. M. J. E. (2004). New developments in the diagnosis of bloodstream infections. *Lancet Infectious Diseases*, **4**, 751-760.

Peterson, D. E. (1990). Pretreatment strategies for infection prevention in chemotherapy patients. *NCI Monographs*, **9**, 61-71.

Peterson, D. E. & Overholser, C. D. (1981). Increased morbidity associated with oral infection in patients with acute nonlymphocytic leukemia. *Oral Surgery, Oral Medicine and Oral Pathology*, **51**, 390-393.

Pierce, G. & Murray, P. R. (1986). Current controversies in the detection of septicemia. *European Journal of Clinical Microbiology*, **5**, 487-491.

Prabhu, R. M., Piper, K. E., Baddour, L. M., Steckelberg, J. M., Wilson, W. R. & Patel, R. (2004). Antimicrobial susceptibility patterns among viridans group streptococcal isolates from infective endocarditis patients from 1971 to 1986 and 1994 to 2002. *Antimicrobial Agents and Chemotherapy*, **48**, 4463-4465.

Rajasuo, A., Perkki, K., Nyfors, S., Jousimies-Somer, H. & Meurman, J. H. (2004). Bacteremia following surgical dental extraction with an emphasis on anaerobic strains. *Journal of Dental Research*, **83**, 170-174.

Ramadan, A. E., Zaki, S. A. & Nour, Z. M. (1975). A study of transient bacteremia following the use of dental floss silk and interdental stimulators. *Egyptian Dental Journal*, **21**, 19-28.

Ramsdale, D. R. (2007). Response to draft document by NICE on "Antimicrobial prophylaxis against infective endocarditis in adults and children undergoing interventional procedures" [WWW document]. URL http://www.bcs.com/pages/news_full.asp?NewsID=18369177 [accessed on 26 January 2008].

Ramsdale, D. R., Turner-Stokes, L., Advisory Group of the British Cardiac Society Clinical Practice, C. & Unit, R. C. E. a. E. (2004). Prophylaxis and treatment of infective endocarditis in adults: a concise guide. *Clinical Medicine*, **4**, 545-550.

Reinhardt, R. A., Bolton, R. W. & Hlava, G. (1982). Effect of nonsterile versus sterile water irrigation with ultrasonic scaling on postoperative bacteremias. *Journal of Periodontology*, **53**, 96-100.

Renvert, S., Ohlsson, O., Persson, S., Lang, N. P. & Persson, G. R. (2004). Analysis of periodontal risk profiles in adults with or without a history of myocardial infarction. *Journal of Clinical Periodontology*, **31**, 19-24.

Rimola, A., Soto, R., Bory, F., Arroyo, V., Piera, C. & Rodes, J. (1984). Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology*, **4**, 53-58.

Rise, E., Smith, J. F. & Bell, J. (1969). Reduction of bacteremia after oral manipulations. *Archives of Otolaryngology*, **90**, 106-201.

Rise, J., Haugejorden, O., Wold, B. & Aaro, L. E. (1991). Distribution of dental health behaviors in Nordic schoolchildren. *Community Dentistry and Oral Epidemiology*, **19**, 9-13.

Roberts, G. J. (1999). Dentists are innocent! "Everyday" bacteremia is the real culprit: A review and assessment of the evidence that dental surgical procedures are a principal cause of bacterial endocarditis in children. *Pediatric Cardiology*, **20**, 317-325.

Roberts, G. J., Gardner, P., Longhurst, P., Black, A. E. & Lucas, V. S. (2000). Intensity of bacteraemia associated with conservative dental procedures in children. *British Dental Journal*, **188**, 95-98.

Roberts, G. J., Gardner, P. & Simmons, N. A. (1992). Optimum sampling time for detection of dental bacteraemia in children. *International Journal of Cardiology*, **35**, 311-315.

Roberts, G. J., Holzel, H. S., Sury, M. R., Simmons, N. A., Gardner, P. & Longhurst, P. (1997). Dental bacteremia in children. *Pediatric Cardiology*, **18**, 24-27.

Roberts, G. J., Jaffray, E. C., Spratt, D. A., Petrie, A., Greville, C., Wilson, M. & Lucas, V. S. (2006). Duration, prevalence and intensity of bacteraemia after dental extractions in children. *Heart*, **92**, 1274-1277.

Roberts, G. J., Radford, P. & Holt, R. (1987). Prophylaxis of dental bacteraemia with oral amoxycillin in children. *British Dental Journal*, **162**, 179-182.

Robertson, P. B., Buchanan, S. A., Armitage, G. C., Newbrun, E., Taggart, E. J. & Hoover, C. I. (1987). Evaluation of clinical and microbiological measures to predict treatment response in severe periodontitis. *Journal of Periodontal Research*, **22**, 230-232.

Romans, A. R. & App, G. R. (1971). Bacteremia, a result from oral irrigation in subjects with gingivitis. *Journal of Periodontology*, **42**, 757-760.

Ronis, D. L., Lang, W. P., Antonakos, C. I. & Borgnakke, W. S. (1998). Preventive oral health behaviors among African-Americans and whites in Detroit. *Journal of Public Health Dentistry*, **58**, 234-240.

Saito, I., Watanabe, O., Kawahara, H., Igarashi, Y., Yamamura, T. & Shimono, M. (1981). Intercellular junctions and the permeability barrier in the junctional epithelium. *Journal of Periodontal Research*, **16**, 467-480.

Sandre, R. M. & Shafran, S. D. (1996). Infective endocarditis: review of 135 cases over 9 years. *Clinical Infectious Diseases*, **22**, 276-286.

Scannapieco, F. (1998). Position paper of the American Academy of Periodontology: periodontal disease as a potential risk factor for systemic diseases. *Journal of Periodontology*, **69**, 841-850.

Schiff, T. & Borden, L. (1994). The effect of a new experimental prebrushing dental rinse on plaque removal. *Journal of Clinical Dentistry*, **4**, 107-110.

Schlein, R. A., Kudlick, E. M., Reindorf, C. A., Gregory, J. & Royal, G. C. (1991). Toothbrushing and transient bacteremia in patients undergoing orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, **99**, 466-472.

Sconyers, J. R., Albers, D. D. & Kelly, R. (1979). Relationship of bacteremia to toothbrushing in clinically healthy patients. *General Dentistry*, **27**, 51-52.

Sconyers, J. R., Crawford, J. J. & Moriarty, J. D. (1973). Relationship of bacteremia to toothbrushing in patients with periodontitis. *Journal of the American Dental Association*, **87**, 616-622.

Scott, J. F., Morgan, D., Avent, M., Graves, S. & Goss, A. N. (2005). Patients with artificial joints: do they need antibiotic cover for dental treatment? *Australian Dental Journal*, **50**, S45-53.

Scott, T. A. (1996). Concise Encyclopedia Biology, p. 277. Berlin, New York: Walter de Gruyter

Segelnick, S. L. (2004). A survey of floss frequency, habit and technique in a hospital dental clinic and private periodontal practice. *New York State Dental Journal*, **70**, 28-33.

Seto, T. B., Kwiat, D., Taira, D. A., Douglas, P. S. & Manning, W. J. (2000). Physicians' recommendations to patients for use of antibiotic prophylaxis to

prevent endocarditis. *Journal of the American Medical Association*, **284**, 68-71.

Seymour, G., Savage, N. & Walsh, L. (1995). Immunology: An introduction for the health sciences, pp. 1-41. New York: McGraw Hill.

Seymour, R. A., Lowry, R., Whitworth, J. M. & Martin, M. V. (2000). Infective endocarditis, dentistry and antibiotic prophylaxis; time for a rethink? *British Dental Journal*, **189**, 610-616.

Silness, J. & Löe, H. (1964). Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica*, **22**, 121-135.

Silver, J. G., Martin, A. W. & McBride, B. C. (1977). Experimental transient bacteraemias in human subjects with varying degrees of plaque accumulation and gingival inflammation. *Journal of Clinical Periodontology*, **4**, 92-99.

Silver, J. G., Martin, A. W. & McBride, B. C. (1979). Experimental transient bacteraemias in human subjects with clinically healthy gingivae. *Journal of Clinical Periodontology*, **6**, 33-36.

Silver, J. G., Martin, L. & McBride, B. C. (1975). Recovery and clearance rates of oral microorganisms following experimental bacteraemias in dogs. *Archives of Oral Biology*, **20**, 675-679.

Siudikiene, J., Maciulskiene, V. & Nedzelskiene, I. (2005). Dietary and oral hygiene habits in children with type I diabetes mellitus related to dental caries. *Stomatologija*, **7**, 58-62.

Slade, G. D. (2007). Interim analysis of validity of periodontitis screening questions in the Australian population. *Journal of Periodontology*, **78**, 1463-1470.

Socransky, S. S. & Haffajee, A. D. (1997). Microbiology of periodontal diseases. In Clinical Periodontology and Implant Dentistry. 3rd edition, eds. Lindhe, J., Karring, T. & Lang, N.P., p. 138. Copenhagen: Munksgaard.

Spaargaren, J., van Boven, C. P. & Voor, G. P. (1998). Effectiveness of resins in neutralizing antibiotic activities in Bactec Plus Aerobic/F culture medium. *Journal of Clinical Microbiology*, **36**, 3731-3733.

Starkebaum, M., Durack, D. T. & Beeson, P. B. (1977). The incubation period of subacute bacterial endocarditis. *Yale Journal of Biology and Medicine*, **50**, 49-58.

Steckelberg, J. M. & Wilson, W. R. (1993). Risk factors for infective endocarditis. *Infectious Disease Clinics of North America*, **7**, 9-19.

Stoltenberg, J. L., Osborn, J. B. & Pihlstrom, B. L. (1993). Prevalence of periodontal disease in a health maintenance organisation and comparisons to the national survey of oral health. *Journal of Periodontology*, **64**, 853-858.

Strom, B. L., Abrutyn, E., Berlin, J. A., Kinman, J. L., Feldman, R. S., Stolley, P. D., Levison, M. E., Korzeniowski, O. M. & Kaye, D. (1998). Dental and cardiac risk factors for infective endocarditis. A population-based, case-control study. *Annals of Internal Medicine*, **129**, 761-769.

Strom, B. L., Abrutyn, E., Berlin, J. A., Kinman, J. L., Feldman, R. S., Stolley, P. D., Levison, M. E., Korzeniowski, O. M. & Kaye, D. (2000). Risk factors for infective endocarditis : Oral hygiene and nondental exposures. *Circulation*, **102**, 2842-2848.

Tamimi, H. A., Thomassen, P. R. & Moser, E. H., Jr. (1969). Bacteremia study using a water irrigation device. *Journal of Periodontology*, **40**, 4-6.

Tedesco, L. A., Keffer, M. A. & Fleck-Kandath, C. (1991). Self-efficacy, reasoned action and oral health behavior reports: A social cognitive approach to compliance. *Journal of Behavioral Medicine*, **14**, 341-355.

Tierno, P. M., Jr., Inglima, K. & Parisi, M. T. (1995). Detection of *Bartonella (Rochalimaea) henselae* bacteremia using BacT/Alert blood culture system. *American Journal of Clinical Pathology*, **104**, 530-536.

Tomas, I., Alvarez, M., Limeres, J. P., C., Medina, J. & Diz, P. (2007). Prevalence, duration and aetiology of bacteraemia following dental extractions. *Oral Diseases*, **13**, 56-62.

Tomas, I., Pereira, F., Llucian, R., Poveda, R., Diz, P. & Bagan, J. V. (2008). Prevalence of bacteraemia following third molar surgery. *Oral Diseases*, **14**, 89-94.

Toneatto, A. & Binik, Y. M. (1990). Internal structure of prevention and dental health behaviors. *Journal of Behavioral Medicine*, **13**, 481-487.

Tonetti, M. S. & Claffey, N. (2005). Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *Journal of Clinical Periodontology*, **32 (Suppl 6)**, 210-213.

Touroff, A. S. W. (1942). Blood cultures from pulmonary artery and aorta in patient with infected patent ductus arteriosus. *Proceedings of the Society for Experimental Biology and Medicine*, **49**, 568-569.

van der Meer, J. T., Thompson, J., Valkenburg, H. A. & Michel, M. F. (1992a). Epidemiology of bacterial endocarditis in The Netherlands. I. Patient characteristics. *Archives of Internal Medicine*, **152**, 1863-1868.

van der Meer, J. T., Thompson, J., Valkenburg, H. A. & Michel, M. F. (1992b). Epidemiology of bacterial endocarditis in The Netherlands. II. Antecedent procedures and use of prophylaxis. *Archives of Internal Medicine*, **152**, 1869-1873.

van der Meer, J. T., van Wijk, W., Thompson, J., Vandenbroucke, J. P., Valkenburg, H. A. & Michel, M. F. (1992c). Efficacy of antibiotic prophylaxis for prevention of native-valve endocarditis. *The Lancet,* **339**, 135-139.

Victor, T., Jordaan, A., du Toit, R. & van Helden, P. D. (1993). Laboratory experience and guidelines for avoiding false polymerase chain reaction results. *European Journal of Clinical Chemistry and Clinical Biochemistry*, **31**, 531-535.

Waerhaug, J. (1981). Healing of the dento-epithelial junction following the use of dental floss. *Journal of Clinical Periodontology*, **8**, 144-150.

Wahl, M. (1995). Myths of dental-induced prosthetic joint infections. *Clinical Infectious Diseases*, **20**, 1420-1425.

Wahlmann, U., Al-Nawas, B., Jutte, M. & Wagner, W. (1999). Clinical and microbiological efficacy of single dose cefuroxime prophylaxis for dental surgical procedures. *International Journal of Antimicrobial Agents*, **12**, 253-256.

Walsh, T. F., Al-Hokail, O. S. & Fosam, E. B. (1997). The relationship of bone loss observed on panoramic radiographs with clinical periodontal screening. *Journal of Clinical Periodontology*, **24**, 153-157.

Wank, H. A., Levison, M. E., Rose, L. F. & Cohen, D. W. (1976). A quantitative measurement of bacteremia and its relationship to plaque control. *Journal of Periodontology*, **47**, 683-686.

Warren, P. R. & Chater, B. V. (1996). An overview of established interdental cleaning methods. *Journal of Clinical Dentistry*, **7 (3 Spec No)**, 65-69.

Wilson, W., Taubert, K. A., Gewitz, M., Lockhart, P. B., Baddour, L. M., Levison, M., Bolger, A., Cabell, C. H., Takahashi, M., Baltimore, R. S., Newburger, J. W., Strom, B. L., Tani, L. Y., Gerber, M., Bonow, R. O., Pallasch, T., Shulman, S. T., Rowley, A. H., Burns, J. C., Ferrieri, P., Gardner, T., Goff, D. & Durack, D. T. (2007). Prevention of infective endocarditis: Guidelines from the American Heart Association: A guideline from the American Heart Association Rheumatic Fever, Endocarditis and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Journal of the American Dental Association*, **138**, 739-760.

Wright, A. J. & Wilson, W. R. (1982). Experimental animal endocarditis. *Mayo Clinical Procedures*, **57**, 10-14.

Wunderlich, R. C., Lamberts, D. M. & Caffesse, R. G. (1982). The effect of waxed and unwaxed dental floss on gingival health. Part II. Crevicular fluid flow and gingival bleeding. *Journal of Periodontology*, **53**, 397-400.

Yagupsky, P. & Nolte, F. S. (1990). Quantitative aspects of septicaemia. *Clinical Microbiology Reviews*, **3**, 269-279.

PART B: SCIENTIFIC PAPER

Bacteraemia due to dental flossing.

K. Crasta[1], D. Mitchell[2], B. Curtis[3], D. Stewart[4], L.J.A. Heitz-Mayfield[1] & C.G. Daly[1]

[1]Discipline of Periodontics, University of Sydney, [2]Institute of Clinical Pathology and Medical Research, Westmead Hospital, [3]Discipline of Epidemiology and Biostatistics, University of Sydney, [4]Conscious Sedation and Pain Control Unit, Westmead Centre for Oral Health, New South Wales, Australia.

Key words: - bacteraemia, flossing, oral hygiene, infective endocarditis, antibiotic prophylaxis

ABSTRACT

Objectives: The objectives of this study were to: 1. Investigate the incidence of bacteraemia following flossing in subjects with chronic periodontitis or periodontal health; 2. Identify the micro-organisms in detected bacteraemias; and 3. Identify any factors associated with such bacteraemia.

Materials and Methods: A baseline blood sample was obtained from 30 individuals with chronic periodontitis (17M:13F, 29-75years) and 30 with periodontal health (17M:13F, 28-71years) following a non-invasive examination**.** Each subject's teeth were then flossed utilizing a standardized flossing procedure. Bleeding on flossing and flossing time were recorded. Further blood samples were obtained 30s and 10min after flossing cessation. A full periodontal examination was then performed**.** Blood samples were inoculated into BACTEC bottles and cultured in an automated processor. Bottles which signaled positive were then subcultured for identification.

Results: 40.0% of the periodontitis subjects and 41.4 % of the periodontally healthy subjects tested positive for bacteraemia following flossing. Oral streptococci, which are commonly implicated in infective endocarditis (IE), were isolated from 18.6% of all positive subjects and accounted for 34.9% of all microbial species isolated. 20.3% of all subjects had a detectable bacteraemia at 10 minutes post-flossing. No factors assessed in this study were associated with post-flossing bacteraemia.

Conclusions: Dental flossing can produce bacteraemia in periodontally healthy as well as periodontally diseased individuals at a rate comparable with that caused by dental treatments. Flossing may therefore be implicated as a causative factor in IE.

INTRODUCTION

Bacteraemia of oral origin is considered to be important in the pathogenesis of infective endocarditis (IE) since oral streptococci account for 20% of cases of native valve IE and 26% of cases of late prosthetic valve endocarditis (Moreillon & Que 2004) and IE caused by viridans streptococci has been reported to have a mortality rate of 6-16% (Sandre & Shafran 1996, Netzer et al. 2000). Thus, European, American and Australian guidelines for prevention of IE recommend that antibiotic prophylaxis should be given to individuals in specified cardiac risk groups prior to having dental treatment likely to cause bacteraemia (Horstkotte et al. 2004, Wilson et al. 2007, Infective Endocarditis Prophylaxis [IEP] Expert Group 2008). Recent British guidelines are the exception and do not recommend antibiotic prophylaxis for dental procedures (National Institute for Health and Clinical Excellence [NICE] Guideline Development Group 2008). All of these guidelines have stressed that good oral hygiene and oral health are important factors in reducing the incidence of IE in susceptible individuals (Horstkotte et al. 2004, Wilson et al. 2007, NICE Guideline Development Group 2008, IEP Expert Group 2008) and therefore it is recommended that oral hygiene should be reinforced for patients at risk of IE and that they attend regular preventive dental check-ups (Duval & Leport 2008).

Paradoxically, the various national guidelines suggest that patient-performed oral hygiene procedures could be implicated in IE due to their potential to cause bacteraemia on a daily basis as compared with dental treatment which might occur infrequently (Daly et al. 2008). Indeed, the most recent American guidelines go so far as to state that the vast majority of cases of IE caused by oral microflora most likely

result from bacteraemias caused by routine daily activities including oral hygiene activities (Wilson et al. 2007). Similarly, the UK guidelines concluded that it was "biologically implausible" that a dental procedure would lead to a greater risk of IE than an everyday oral hygiene activity such as regular toothbrushing (NICE Guideline Development Group 2008). The question then arises as to which individuals may be most at risk of bacteraemia due to their oral hygiene activities. Whilst it has been reported that bacteraemia caused by toothbrushing is more common in the presence of periodontal inflammation than health (Silver et al. 1977), the situation as regards dental flossing is unclear. Although anecdotal reports exist that flossing may be implicated in causing specific cases of IE (Jenney et al. 2001), epidemiological studies have reported that subjects who floss daily appear to be less susceptible to IE (Strom et al. 2000).

Dental flossing is the most common method of interproximal cleaning recommended by dentists and the most common method of interproximal cleaning utilised by patients (Warren & Chater 1996). The risk of bacteraemia following flossing is not clear as there is little published evidence available. Only 4 studies have previously sought to address the issue of whether flossing causes bacteraemia (Lineberger & De Marco 1973, Ramadan et al. 1975, Wank et al. 1976, Carroll & Sebor 1980). A critical appraisal of these previous studies reveals a number of methodological shortcomings such as a lack of a periodontal diagnosis (Ramadan et al. 1975); the use of partial recording indices when assessing the amount of periodontal disease present (Wank et al. 1976); no indication as to when periodontal assessment was performed in relation to the flossing exercise (Lineberger & De Marco 1973); the presence of an interval of a week or more between measuring indices and the actual performance of

the flossing exercise (Wank et al. 1976, Carroll & Sebor 1980); and failure to record whether the subject or the investigator performed the flossing (Lineberger & De Marco 1973). Some studies included commonly regarded skin contaminants as positive results in their bacteraemia analysis (Ramadan et al. 1975, Wank et al. 1976) or did not identify the bacteria present in positive blood cultures to ensure they were of likely oral origin (Carroll & Sebor 1980).

From the previous studies, it is not possible to determine whether individuals with periodontal disease are more at risk of bacteraemia from flossing than those who are periodontally healthy. Although one study reported a drop in the incidence of bacteraemia due to flossing from 29% to 14% in the same group of patients before and after non-surgical periodontal treatment, no data were presented on the degree of gingival inflammation, plaque accumulation, bleeding on probing or probing depths which could assist in identifying individuals at risk for bacteraemia (Wank et al. 1976). One study sought to investigate whether daily flossing resulted in a lower incidence of bacteraemia as compared with flossing every 2 to 3 days (Carroll & Sebor 1980). However, as the study involved only 4 subjects (2 gingivitis; 2 healthy), no reliable conclusions can be drawn as to the impact of periodontal status or plaque levels on bacteraemia caused by flossing. Another study which investigated bacteraemia due to dental flossing, use of stimudents or periodontal surgery did contain a periodontally healthy control group (Lineberger & De Marco 1973). Unfortunately, the investigators did not subject the control group to flossing on the basis that the incidence of bacteraemia was only 20% in the untreated, periodontitis group.

Given the paucity of data on bacteraemia caused by flossing, there is a need for further investigation to clarify whether patients with periodontal disease are more at risk of oral bacteraemia due to flossing than are patients with a healthy periodontium. Thus, the aims of this study were:

1 To determine if there is an increased incidence of flossing-induced bacteremia in patients with periodontitis as compared with periodontal health.
2 To identify any factors which might be associated with such bacteraemia.
3 To identify the micro-organisms present within positive blood cultures.

MATERIALS AND METHODS

Study Design

This study was a cohort investigation in which 30 individuals with chronic periodontitis and 30 with periodontal health attended for a single visit during which the experimental flossing protocol was performed, blood samples collected and periodontal data gathered.

Ethics approval

The study was approved by the Human Research Ethics Committees of the University of Sydney and the Sydney West Area Health Service. Subjects were given written and verbal advice about the experiment and were required to sign a witnessed consent

form. Research was conducted in accordance with the World Medical Association Declaration of Helsinki (version VI, 2002 www.wma.net/e/policy/b3.htm).

Population screening

Thirty volunteers with chronic periodontitis were sought by telephone interview from patients on the periodontal waiting list of the Westmead Centre for Oral Health (WCOH) and in person from patients presenting to clinics at the same institution, for whom clinical and radiographic records were available. The experimental procedures and purposes of the study were explained to all potential subjects and their medical and dental histories were reviewed to assess inclusion and exclusion criteria and suitable patients then invited to attend for the clinical procedure. Thirty subjects with periodontal health were recruited from the clinics and from staff and dental students at the WCOH, all of whom were contacted in person by KC. Subjects in the periodontitis group were recruited first and then subjects in the periodontally healthy group were recruited on the basis of age and gender matching. Subject recruitment occurred between 05 Mar 2007 and 13 Jul 2008.

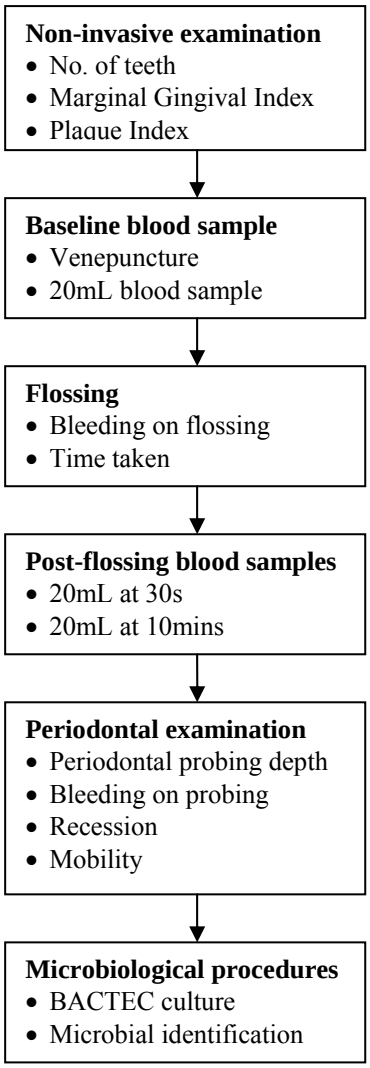
Selection criteria

Subjects in the periodontitis group were selected initially on the basis of radiographic evidence of interproximal bone loss when viewed on an orthopantomogram (OPG) (Walsh et al. 1997). They all had at least 4mm of bone loss at ≥2 proximal sites on non-adjacent teeth and not on the same tooth when measured on the OPG. They were

required to have had no history of periodontal treatment in the last 12 months. Subjects in the periodontally healthy group were selected on the basis of having no evidence of chronic periodontitis as defined by the American Academy of Periodontology (2000a, b) nor of having generalised plaque-induced gingivitis (Mariotti 1999).

All subjects were required to have at least 10 teeth. Volunteers with significant medical problems (e.g. diabetes), cardiac defects or other conditions requiring prophylactic antibiotic cover, immune defects, haematological disorders, pregnancy, upper respiratory tract infections, mouth ulceration, pulpal/periapical infections or those taking immunosuppressive or corticosteroid medication or those who had taken antibiotics in the past 3 months were excluded from the study. Subjects were instructed not to brush or floss their teeth, chew any food or perform any intra-oral manipulations for at least an hour prior to the experimental protocol.

***Fig. 1*. Experimental design**



Data collection

The experimental design is shown in Fig. 1. All experimental procedures and clinical data gathering were performed at the dental clinics at WCOH between 10 Mar 2007 and 17 Jul 2008. All data collection was performed by KC during a single visit. All invasive data-gathering procedures were performed immediately after the flossing and blood collection procedures because periodontal probing can cause bacteraemia (Daly

et al. 2001, Kinane et al. 2005). Data were gathered in three stages; pre-flossing, during flossing and post-flossing.

Pre-flossing data collection involved the use of a patient questionnaire assessing medical history, personal interproximal cleaning regimen, age, gender and smoking status (never, former or current). The number of teeth were then counted. Following this, the Marginal Gingival Index (MGI; Lobene et al. 1986) was utilised on the vestibular and oral surfaces at all gingival papillae to assess gingival inflammation. The gingival tissue adjacent to a tooth bounding a saddle area was also considered a papilla. Finally, the Plaque Index (PI; Silness & Löe 1964) was utilised at each approximal surface on facial and lingual/palatal aspects. A 1% erythrosine disclosing dye (Plaque Disclose, Professional Dental Supplies, Bayswater, VIC, Australia) was used to disclose plaque. No probing was performed as part of the PI.

Data collection during flossing comprised of noting papillary bleeding on flossing (Carter & Barnes 1974). Only the presence or absence of bleeding was noted and each papilla was treated as a single unit, regardless of whether bleeding was noted on the vestibular and/or oral aspect of the papilla or on the mesial and/or distal aspect of the papilla. Presence of papillary bleeding was checked for up to 30s after flossing. The time taken to floss was also recorded.

Post-flossing data collection involved recording of periodontal probing depth (PPD) and recession (REC) to the nearest mm using a PCP-11 periodontal probe (Hu-Friedy, Chicago, IL, USA) at six sites per tooth (mesial, mid and distal on vestibular and oral aspects), bleeding on probing (BOP) assessed as Yes/No at six sites per tooth and tooth mobility assessed as Y/N and graded as Grade I, II or III (Miller 1938).

Flossing technique

Flossing was performed by KC to ensure standardisation of technique and because the blood sampling procedure precluded subjects being able to use their own hands for flossing. A 150 cm strand of waxed dental floss (DentaFloss, Caredent, Hornsby, NSW, Australia) was pre-measured and then wrapped around the index fingers of each hand. The floss was gently moved through the contact area with a back and forth action using a combination of the thumbs and/or middle fingers of both hands. A recommended flossing action was then utilised (Perry 1996, American Dental Association 2008). The floss was shaped into a "C" configuration and moved apically along the tooth surface from the contact area to a position under the gingival papilla where it could not penetrate any further and then back again to the contact area. A standardised, calibrated force of 50g (0.5N) was used when flossing (Smith et al. 1986). This flossing action was repeated 3 times on the tooth surface before the adjacent tooth surface on the other side of the gingival papilla was similarly flossed (Carter et al. 1975). The floss was then unravelled slightly and moved between the fingers before progressing from one interproximal area to the next so that there was no transfer of blood or plaque from one area to another. The mesial and distal surfaces of all teeth were each flossed in this manner.

Calibration and reproducibility

Calibration scoring of the PI, MGI, mobility, PPD and REC was undertaken prior to the study. This was achieved by comparing the scores of KC to those of an experienced clinician, considered to be the referent standard. Intra-examiner reproducibility was calculated during the study by randomly re-assessing six selected sites within each subject for each of the indices, and calculating the percentage agreement within those scores. The force used when flossing was calibrated at 50g (0.5N) by performing repeated measurements with the floss on an electronic weighing scale (Tronic S, Maul, Bad Konig, Germany) prior to the study.

Blood sampling

Three blood samples (total volume of 60mL) were obtained from each subject by a registered nurse under the direct supervision of the investigator. The skin was initially wiped with a sterile 70% isopropyl alcohol wipe (Medind® Alcohol Prep, Medical Industries Australia, Sydney, NSW, Australia) and venepuncture performed with an intravenous 25mm/22 gauge cannula (Protectiv® Plus, Cincinnati, OH, USA) inserted into the median cubital vein after application of a cuff tourniquet. The cannula was fitted with a one-way valve (RV1000NC Safsite®, Braun Medical Inc., Bethlehem, PA, USA) and a minimum volume extension set (Tuta Healthcare, Lane Cove, NSW, Australia). A 20mL baseline blood sample was then taken with a 20mL syringe (Becton Dickinson, Singapore) after removal of the cuff tourniquet. After taking the baseline sample, the cannula was taped and secured with a sterile dressing (OPSITE

IV3000™ 10x14 cm, Smith & Nephew, Hull, UK) to prevent contamination of the venepuncture site. The cannula was then left in place in the subject's outstretched and supported arm whilst the flossing procedure was performed.

Subsequent collections of 20mL blood samples were initiated at 30s and again at 10 mins after cessation of flossing. Immediately prior to these samples being obtained, 4mL of 0.9% saline (Sodium Chloride Injection BP, Pfizer Australia Pty Ltd, West Ryde, NSW, Australia) was flushed into the vein with a 5mL disposable syringe (Becton Dickinson, Singapore) and the first 4mL of blood was aspirated and discarded in order to avoid any contamination of the blood sample with saline. From each 20 mL blood sample, 10 mL was immediately inoculated into a BACTEC™ Plus Aerobic/F* 9240 media culture bottle (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) and 10 mL into an BACTEC™ Lytic/10 Anaerobic/F* 9240 media culture bottle (Becton Dickinson Diagnostic Systems).

Microbiological procedures

All microbiological procedures were carried out in the microbiology laboratory of the Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, which is a tertiary referral centre and an anaerobic reference laboratory. All bottles were transported to the laboratory within 30 mins of collection. The blood culture bottles were then incubated in the BACTEC 9240 automated processor (Becton Dickinson Diagnostic Systems). The staff at the microbiology laboratory were blinded as to whether the blood samples

belonged to a subject with periodontitis or to one with periodontal health. Bottles were monitored continuously for 14 days before being discarded if they failed to signal positive.

Bottles that signaled positive were Gram-stained and subcultured onto Horse Blood Agar and Chocolate Agar plates (BioMedia Laboratories, Singapore), which were incubated at 5% CO_2 and 35°C; onto MacConkey agar plates (Biomedia Laboratories) that were incubated aerobically at 35°C; and Brain Heart Infusion agar plates supplemented with vitamin K (BioMedia Laboratories), which were incubated anaerobically at 35°C. Any isolated bacteria were identified to at least genus level using conventional microbiological techniques (Murray et al. 2003) including colonial morphology, Gram stain appearance, catalase and oxidase reactions, and where appropriate, reactions in kit systems, such as API (Analytab products, Plainview, NY, USA).

Interpretation of results and statistical analysis

The primary outcome measure for this study was the incidence of bacteraemia following flossing. A priori, sample size estimation was undertaken based on an expected difference in the incidence of post flossing bacteraemia levels of 25% between subjects with chronic periodontitis and healthy controls. Based on that difference, a sample size of 28 subjects per group was calculated necessary to detect a significant difference (α =0.05) with 80% power and therefore 30 subjects were recruited in each group.

A correlation matrix was used to examine associations between subject and clinical variables. The Spearman (non-parametric) analysis was used in order to account for the relatively small sample size and high variance in the data. Mean scores for clinical measures that demonstrated normality of distribution are presented, otherwise median values are represented.

Appropriate statistical methods such as chi-squared tests for proportions, t-test for continuous outcomes and logistic and linear regression for predictors of outcome were used to explore differences for binary and continuous outcomes. In all cases α of <0.05 was considered to be statistically significant. All data analysis was undertaken using SPSS® Version 14.0 (SPSS Inc., Chicago, IL, USA) and SAS® Version 8.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Population screening

A total of 106 periodontitis subjects were screened of whom 58 did not meet the inclusion criteria, 9 could not be contacted and 7 declined to participate. A total of 53 subjects with periodontal health were approached of whom 4 did not meet the inclusion criteria and 18 declined to participate.

Intra-observer reproducibility

Analysis of the Pearson correlation co-efficient in each case revealed substantial intra-observer reliability: 0.91 for the PI; 0.92 for the GI; 0.97 for mobility; 0.89 for PPD; and 0.95 for REC. A score of ≥0.75 for the Pearson statistic is considered substantial (Thompson & Walter 1988).

Data for subject groups

The age and clinical data for the periodontitis group and the periodontally healthy group are shown in Table 1. All subjects in the periodontitis group were found to fulfil the diagnosis of severe chronic periodontitis as defined by Page & Eke (2007). They all had at least two interproximal sites, on non-adjacent teeth and not on the same tooth, with clinical attachment loss (CAL) ≥6mm with at least one of these sites exhibiting concomitant PPD of ≥5mm.

The periodontitis and periodontally healthy groups were age and gender matched. Both groups consisted of 17 males and 13 females with a mean age of 49.4 (±12.0) years in the periodontitis group and 48.6 (±12.1) years in the periodontally healthy group (p=0.9) as shown in Table 1. There were 5 current smokers, 8 former smokers and 17 never smokers in the periodontitis group with the corresponding numbers in the periodontally healthy group being 2, 7 and 21. This difference was not statistically significant (p=0.4). There were 6 daily, 3 less than daily and 21 never flossers in the periodontitis group, the corresponding figures being 15, 9 and 6 in the periodontally healthy group. This difference was statistically significant (p=0.001).

The median GI (p<0.001), median PI (p<0.001), mean PPD (p<0.001), mean number of sites with PPD≥5mm (p<0.001), mean CAL (p<0.001), percentage of sites with BOP (p<0.001) and number of teeth with mobility grade II/III (p<0.001) were significantly greater for the periodontitis group compared to the periodontally healthy group as evident in Table 1. There was no statistically significant difference in the number of teeth (p=0.3) between both groups.

Table 1 - Clinical and patient data for the periodontitis and periodontally healthy groups

Variable	Mean +/- SD		p value
	Periodontitis	Periodontal health	
Age (years)	49.4 (12.0)	48.6 (12.1)	0.9*
Teeth (n)	26.2 (4.7)	26.9 (3.6)	0.3*
Gingival Index (median score per site)	1.9 (0.4)	0.5 (0.6)	<0.001^
Plaque Index (median score per site)	2.0 (0.6)	0.9 (0.9)	<0.001^
Periodontal probing depth per site (mm)	3.4 (0.9)	2.3 (0.2)	<0.001*
Mean no. of periodontal probing depth sites≥5mm	28.9 (27.6)	0	<0.001*
Clinical attachment level per site (mm)	3.9 (1.0)	2.5 (0.4)	<0.001^
Bleeding on probing (% of sites)	41.1 (19.4)	5.9 (3.8)	<0.001^
Mean no. teeth with mobility Grade II/III	0.9 (1.4)	0	<0.001*
Bleeding on flossing (% of sites)	30 (16.5)	8 (6.9)	<0.001^
Bleeding on flossing (mean no. of sites/subject)	9.2 (5.3)	2.5 (2.1)	<0.001*

BOP = bleeding on probing, PI = Plaque Index, GI = Gingival Index
Independent t-test*
Mann-Whitney U test^

Flossing

It was possible to floss all interproximal areas of all teeth and no discomfort or adverse sequelae were reported by any of the subjects during or after flossing. The mean time taken to floss the subjects' teeth was 280.1 (±52.3)s in the periodontitis group and 258.7 (±50.2)s in the periodontally healthy group. This difference was not significant (p=0.8). The occurrence of bleeding on flossing is shown in Table 1. The percentage of papillae which bled on flossing was higher in the periodontitis group (30%) when compared to the periodontally healthy group (8%). This difference was statistically significant (p<0.001). The mean number of papillae which bled on

flossing was higher in the periodontitis group (9.2±5.3) than in the periodontally healthy group (2.5±2.1) and this difference was statistically significant (p<0.001).

Bacteraemia

One subject with periodontal health tested positive for bacteraemia of oral origin at baseline. The organism isolated prior to flossing in this subject was *Actinomyces odontolyticus.* The subject confirmed that he had avoided any brushing, flossing or chewing prior to the experimental procedure and there was no evidence of any periapical pathology on his OPG. This positive result at baseline necessitated that the subject's results be excluded from further analysis.

Four blood samples tested positive for bacteraemia of non-oral origin. A Staphylococcus spp. was isolated from one subject with periodontal health and Propionibacterium spp. were isolated from 1 subject with periodontitis and 2 subjects with periodontal health following flossing. These isolates were considered skin contaminants from the blood sampling procedure and were excluded from analysis (Cockerill et al. 1997, McBryde et al. 2005).

Table 2 summarises the microbiological findings results for those subjects who demonstrated a positive oral bacteraemia at 30s and/or 10 mins post-flossing. In the periodontitis group, 12/30 (40.0%) of the subjects were positive for bacteraemia of oral origin following flossing whereas in the periodontally healthy group 12/29 (41.4%) of the subjects were positive for flossing-induced bacteraemia of oral origin. This difference was not statistically significant (p=0.8). Ten minutes following flossing, 8/30 of the periodontitis group (26.7%) and 4/29 of the periodontally healthy

group (13.8%) tested positive for bacteraemia of oral origin (20.3% of all subjects). This difference, however, was not significant (p=0.2). Those subjects who tested positive for bacteraemia at 10 mins post-flossing did not necessarily test positive at 30s post-flossing. In those subjects who exhibited a bacteraemia at both 30s and 10 mins, the microbial isolates were often different at the two time points.

Table 2 - Microbiological findings of those subjects with positive oral bacteraemia post-flossing

	Periodontitis group			Periodontally healthy group*	
Subject	30 seconds	10 minutes	Subject	30 seconds	10 minutes
7	Neisseria subflava		1	Actinomyces odontolyticus	
10	Streptococcus mitis, Neisseria subflava		25	Prevotella nigrescens	
11	Streptococcus spp, Neisseria spp	Eikenella corrodens	29		Streptococcus bovis
14	Capnocytophaga sputigena		30	Gemella morbillorum	
16	Streptococcus anginosus, Corynebacterium spp	Corynebacterium spp	38	Streptococcus milleri	
17	Streptococcus viridans x 2, Corynebacterium spp	Corynebacterium spp, Streptococcus viridans	41	Streptococcus bovis	
18	Aggregatibacter actinomycetemcomitans		45	Diphtheroids, Streptococcus intermedius	
20		Corynebacterium spp	47	Streptococcus milleri	
21	Streptococcus spp, Micrococcus spp, Eikenella spp	Streptococcus intermedius, Peptostreptococcus anaerobius	50		Neisseria mucosa
23	Streptococcus milleri	Peptostreptococcus anaerobius	51	Gemella haemolysans	
26	Streptococcus milleri	Peptostreptococcus anaerobius	52	Actinomyces gerencseriae	Micromonas micros
35	Neisseria subflava	Selemonas spp	54		Streptococcus gordonii, Diphtheroids

*1 subject not included due to presence of positive oral bacteraemia at baseline

Microbiological findings

A wide variety of oral micro-organisms was identified in the isolates. Viridans streptococci were identified in positive cultures from 23.3% (7/30) of the periodontitis group and 13.8% (4/29) of the periodontally healthy group. This difference was not statistically significant (p=0.3). Overall, viridans streptococci were found in 18.6% of

all positive subjects, and accounted for 34.9% (15/43) of all microbes isolated. Eight blood samples from 7 subjects with periodontitis had more than one species isolated in culture while only 2 samples from 2 subjects had more than one species isolated from their blood samples following culturing.

Data for bacteraemia subjects

No significant correlations were found between any of the subject or clinical indices assessed and the occurrence of bacteraemia following flossing. Neither the presence of periodontitis vs periodontal health (p=0.2), GI (p=0.09), PI (p=0.6), number of sites bleeding on flossing (p=0.2), percentage of sites bleeding on flossing (p=0.2), percentage of sites bleeding on probing (p=0.2), time spent flossing (p=0.8), smoking status (p=0.7), age (p=0.2), gender (p=0.5), PPD (p=0.5), CAL (p=0.6) or self-reported daily flossing (p=0.4) were found to be significantly associated with the occurrence of bacteraemia.

DISCUSSION

This study found that the incidence of bacteraemia caused by dental flossing was not significantly different in subjects with periodontitis (40.0%) when compared to those with periodontal health (41.4%). This incidence is comparable to the rate of bacteraemia occurring with periodontal treatment procedures in adults such as periodontal probing (Daly et al. 2001), ultrasonic scaling (Reinhardt et al. 1982, Cherry et al. 2007), subgingival irrigation (Lofthus et al. 1991) and scaling and prophylaxis (Baltch et al. 1982).

When compared to previous investigations of bacteraemia caused by flossing, the incidence found in the present study, is similar to that reported by Carroll & Sebor (1980) (38%), but higher than those reported by Lineberger & De Marco (1973) (20%), Ramadan et al. (1975) (18%) and Wank et al. (1976) (22%). Comparison with these previous studies is made difficult by the fact that none of the studies described either the technique of flossing which was used nor was there any indication of the force utilised for flossing. Flossing of the subjects' teeth was performed by several investigators in 1 study (Ramadan et al. 1975), by the subjects themselves in 2 studies (Wank et al. 1976, Carroll & Sebor 1980) and the remaining study did not describe whether flossing was performed by the investigator or the subject (Lineberger & De Marco 1973). One of the studies (Carroll & Sebor 1980) involved only 4 subjects which means that it was grossly underpowered. Whereas previous studies used broths or agar plates for bacterial culture, the present study used the automated, continuous culture BACTEC system which has been shown to be effective in detecting oral micro-organisms in bacteraemias caused by oral procedures (Daly et al. 2001, Lucas et al. 2002, Tomas et al. 2004, Kinane et al. 2005). The BACTEC system can detect odontogenic bacteraemia in 24-30h (Pauli et al. 1999, Lucas et al. 2002) and thus the 14 day continuous culture used in our study would have permitted ample time for the detection of bacteria present within the blood samples.

The finding of a 41.4% incidence of bacteraemia in the periodontally healthy group was unexpected. However, with the exception of Carroll & Sebor's (1980) investigation of just 2 subjects, no previous study has investigated bacteraemia due to flossing in a periodontally healthy group. Our findings of a 41.4% incidence of bacteraemia in the periodontally healthy group differs from previous studies of

toothbrushing in adult subjects with periodontal health in which the incidence of bacteraemia has been found to be low (8%; Silver at al. 1979) or zero (Sconyers et al. 1979, Hartzell et al. 2005, Forner at al. 2006) regardless of whether toothbrushing was performed by the investigator (Silver at al. 1979, Sconyers et al. 1979) or by the subject (Hartzell et al. 2005, Forner at al. 2006). The 40% incidence of bacteraemia we found for flossing in a periodontitis group was similar to the 43% incidence reported for adult subjects with periodontal disease who underwent investigator-brushing (Silver et al. 1977) but higher than that reported in other studies of toothbrushing (17%; Sconyers et al. 1973, 3%; Kinane et al. 2005, 2%; Forner at al. 2006).

The generally higher incidence of bacteraemia found for flossing as compared with toothbrushing may indicate that flossing may not be as benign a procedure as toothbrushing. The act of flossing involves mechanically disrupting the approximal and interproximal bacterial biofilm in such a manner that displacement of bacteria subgingivally into the tissue may occur. To compound this, disruption of the junctional epithelium can also occur as the floss slides 2-3mm below the interproximal gingival margin (Waerhaug 1981). In contrast, toothbrush bristles cannot reach the interproximal regions of teeth and are restricted to the approximal tooth surfaces (Egelberg & Claffey 1998).

Thus, flossing may have a greater potential to introduce bacteria into the circulation than toothbrushing. A similar observation has been made in relation to rubber dam placement in children in which an incidence of 31.4% has been found (Roberts et al. 2000). The investigators in that study ascribed this unexpectedly high incidence to the

technique of rubber dam placement, which involves "interproximal rubber being forced down between the teeth and at the same time pushing the dental plaque ahead of it and then squashing it onto the inflamed gingivae". The act of flossing involves a similar action to rubber dam placement and thus tissue trauma and bacterial displacement caused by flossing may be factors accounting for a higher rate of bacteraemia than might be caused by toothbrushing. Use of interdental woodsticks for interproximal cleaning may also cause trauma and bacterial displacement and may account for the 30% incidence of bacteraemia found following the use of interdental woodsticks in subjects with periodontitis (Lineberger & De Marco 1973).

The incidence of flossing-induced bacteraemia found in the present study is similar to that following investigator-performed manual toothbrushing in children (39%; Roberts et al. 1997, 46%; Bhanji et al. 2002) but less than that for investigator-performed powered toothbrushing (78%; Bhanji et al. 2002). However, it should be noted that children may demonstrate higher rates of bacteraemia than adults due to their smaller blood volume which means that bacteraemia can be of a higher concentration than in adults and thus more easily detectable. Children also have an immature immune system such that there may also be a difference in the rate of bacteraemia clearance as compared with adults (Yagupsky & Nolte 1990). Thus, it is not possible to directly compare the findings of the present investigation of adults with studies involving children.

In previous studies of bacteraemia due to toothbrushing, the toothbrushing has sometimes been performed by the one investigator in order to standardize the brushing action amongst the subjects (Silver at al. 1979, Sconyers et al. 1979). In this

study, the flossing was performed by the one investigator (KC) which ensured that a standardised, recommended technique was used in all subjects (Perry 1996, American Dental Association 2008) and that a standardised flossing force of 50g was utilised (Smith et al. 1986). The average time taken to floss all the teeth was 4.5 mins which is similar to the average of 4 mins reported in a previous study of whole mouth, subject-performed flossing (Gjermo & Flotra 1970). In addition, because each subject had an intravenous cannula present in one of their arms for the entire experimental visit, it was physically impossible for them to perform flossing without the cannula being removed and then re-inserted after the flossing exercise. However, it has been shown that bacteraemia peaks at 30s following a dental procedure and so blood samples should be taken then in order to maximise chances of bacteraemia detection (Roberts et al. 1992). If subjects carried out their own flossing, the time taken to perform the venepuncture procedure and then obtain the blood sample would be too long, thus lowering the chances of detecting bacteraemia.

No clinical variables, including gingival inflammation, plaque levels or bleeding on flossing, were found to be predictive of bacteraemia in this study. In contrast, the incidence of bacteraemia caused by toothbrushing has been reported to increase significantly when gingival inflammation increased (Silver et al. 1977) and a positive but weak correlation between gingival inflammation and the incidence of bacteraemia due to scaling has also been reported (Forner et al. 2006). However, the role of gingival inflammation and plaque control in bacteraemia is contentious and it has been stated that it cannot be assumed that a healthy mouth reduces the risk of odontogenic bacteraemia (Wilson et al. 2007, NICE Guideline Development Group 2008). No associations have been found between bacteraemia and gingival

inflammation for scaling (Cherry et al. 2007) nor for scaling and root planing (Lafaurie et al. 2007) and no associations between plaque levels and bacteraemia have been found for toothbrushing (Silver et al. 1977), periodontal probing (Daly et al. 2001), ultrasonic scaling (Cherry et al. 2007) or extractions (Wahlmann et al. 1999, Tomas et al. 2008). The finding that the presence of bleeding on flossing was not associated with an increased risk of bacteraemia is in agreement with the findings of Roberts (1999) that gingival bleeding caused by various oral hygiene and dental procedures is not predictive of odontogenic bacteraemia.

None of the periodontitis group had a positive bacteraemia of oral origin in the baseline blood sample but one of the periodontally healthy subjects did (*A. odontolyticus*) giving a baseline bacteraemia rate of 1.6%. Previous studies of flossing have not found any baseline, pre-flossing oral bacteraemia (Lineberger & De Marco 1973, Ramadan et al. 1975, Wank et al. 1976, Carroll & Sebor 1980) nor have any toothbrushing studies in adults (Sconyers et al. 1973, Silver at al. 1977, Sconyers et al. 1979, Silver at al. 1979, Hartzell et al. 2005, Forner at al. 2006). A baseline bacteraemia is usually absent (Heimdahl et al. 1990, Okabe et al. 1995, Cherry et al. 2007) or low (5%; Kinane et al. 2005, 2.5%; Lafaurie et al. 2007, 2%; Tomas et al. 2008). The rate of spontaneous oral bacteraemia has been reported to range from <1% in adults (Everett & Hirschmann 1977) to 9.3% for children (Roberts et al. 2000). However, in this latter study, the child subjects had undergone nasotracheal intubation for general anaesthesia which can cause bacteraemia, most likely due to trauma of the pharyngeal and tracheal mucosa (McShane & Hone 1986, Dinner et al. 1987) and so the high rate of baseline bacteraemia should be interpreted with caution. It is not currently known what causes a baseline bacteraemia of oral origin but it may be

speculated that a continual low-grade bacteraemia in the absence of an intra-oral procedures may be present in all individuals and that this may occasionally be detected by sampling procedures.

The finding that viridans streptococci accounted for the majority (34.9%) of isolates in the post-flossing samples is important since this group of bacteria is a principal pathogen in IE (Netzer et al. 2000). The high incidence of bacteraemia detected following dental flossing in this study raises the question as to whether individuals in risk groups for IE should avoid flossing, even if they have a healthy periodontium, so as to limit their exposure to streptococcal bacteraemia. This proposal would be at odds with an epidemiological study which found a trend for a reduced risk of IE in those who flossed daily and which recommended that patients with cardiac valvular abnormalities should be vigilant about oral hygiene (Strom et al. 2000). As well as the incidence of bacteraemia, its magnitude may also be important in the development of IE. Although the incidence of flossing-induced bacteraemia was the same between the periodontitis and periodontally healthy groups, it is possible that the magnitude of bacteraemia may have been higher in the periodontitis group. As the BACTEC system does not allow for quantification of bacteraemia, the magnitude of any bacteraemia was beyond the scope of this study. However, the finding that the duration of bacteraemia was significantly higher in the periodontitis group may have been due to a higher magnitude of bacteraemia which would require a longer period of time for clearance from the circulation. As such, daily flossing to maintain good oral health may still be important to reduce the quantity of bacteria that enter the bloodstream although there are no data to demonstrate that a greater magnitude of bacteraemia is more likely to cause IE in humans (Wilson et al. 2007).

The findings of the present study are important in light of recent changes to guidelines regarding antibiotic prophylaxis for IE which have reduced the need for antibiotic prophylaxis, primarily because bacteraemia caused by 'everyday procedures', such as daily oral hygiene, may be of more importance in the aetiology of IE than bacteraemia caused by dental procedures (Wilson et al. 2007, NICE Guideline Development Group 2008, IEP Expert Group 2008). As stated by Wilson et al. (2007), there may not be a clinically significant difference in the frequency, nature, magnitude and duration of bacteraemia associated with a dental procedure compared with that resulting from routine daily activities and so it is inconsistent to recommend prophylaxis for dental procedures but not for these same patients during routine daily procedures. The present study supports this view by documenting that the incidence of bacteraemia due to dental flossing is comparable to that caused by dental procedures, regardless of whether individuals have severe, untreated chronic periodontitis or are periodontally healthy.

In conclusion, given the experimental constraints of this study, it would appear that dental flossing can produce bacteraemia in periodontally healthy as well as periodontally diseased individuals and that there are no patient or clinical factors which are predictive of such bacteraemia occurring. In addition, the high percentage of viridans streptococci in positive blood samples suggests that flossing has the potential to be implicated as a causative factor in cases of streptococcal IE. Studies investigating the magnitude of bacteraemia due to flossing are required to further clarify the role of flossing in contributing to the daily cumulative exposure of an individual to bacteraemia.

ACKNOWLEDGMENTS

This study was funded by the Dental Board of New South Wales and the Australian Periodontology Research Foundation.

REFERENCES

American Academy of Periodontology (2000a). Parameter on chronic periodontitis with advanced loss of periodontal support. *Journal of Periodontology*, **71**, 856-858.

American Academy of Periodontology. (2000b) Parameter on chronic periodontitis with slight to moderate loss of periodontal support. *Journal of Periodontology*, **71**, 853-855.

American Dental Association (2008). Oral Health topics A-Z. Cleaning your teeth and gums (oral hygiene). How do I floss my teeth? [WWW document]. URL http://www.ada.org/public/topics/cleaning_faq.asp#4 [accessed on 06 Jul 2008].

Baltch, A.L, Schaffer, C., Hammer, M.C., Sutphen, N.T., Smith, R.P., Conroy, J., & Shayegani, M. (1982). Bacteremia following dental cleaning in patients with and without penicillin prophylaxis. *American Heart Journal*, **104**, 1335-1339.

Beeson, P. B., Brannon, E. S. & Warren, J. V. (1945). Observations on the sites of removal of bacteria from the blood in patients with bacterial endocarditis. *Journal of Experimental Medicine*, **81**, 9-23.

Carroll, G. C. & Sebor, R. J. (1980). Dental flossing and its relationship to transient bacteremia. *Journal of Periodontology*, **51**, 691-692.

Carter, H. G. & Barnes, G. P. (1974). The Gingival Bleeding Index. *Journal of Periodontology*, **45**, 801-805.

Carter, H. G., Barnes, G. P., Radentz, W. H., Levin, M. P. & Bhaskar, S. N. (1975). Effects of using various types of dental floss on gingival sulcular bleeding. *Virginia Dental Journal*, **52**, 18-32.

Cherry, M., Daly, C. G., Mitchell, D. & Highfield, J. (2007). Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. *Journal of Clinical Periodontology*, **34**, 148-155.

Cockerill, F., Hughes, J., Vetter, E., Mueller, R., Weaver, A., Ilstrup, D., Rosenblatt, J. & Wilson, W. (1997) Analysis of 281,797 consecutive blood cultures performed over an eight-year period: Trends in microorganisms isolated and the value of anaerobic culture of blood. *Clinical Infectious Diseases* **24**, 403-418.

Daly, C.G., Currie, B.J., Jeyasingham, M.S., Moulds, R.F., Smith, J.A., Strathmore, N.F., Street, A.C. & Goss, A.N. (2008). A change of heart: the new infective endocarditis prophylaxis guidelines. *Australian Dental Journal* (In press).

Daly, C., Mitchell, D., Grossberg, D., Highfield, J. & Stewart, D. (1997). Bacteraemia caused by periodontal probing. *Australian Dental Journal*, **42**, 77-80.

Daly, C. G., Mitchell, D. H., Highfield, J. E., Grossberg, D. E. & Stewart, D. (2001). Bacteremia due to periodontal probing: a clinical and microbiological investigation. *Journal of Periodontology*, **72**, 210-214.

Dinner, M., Tjeuw, M. & Artusio Jr, J. F. (1987). Bacteremia as a complication of nasotracheal intubation. *Anaesthetics and Analgesia*, **66**, 460-462.

Duval, X. & Leport, C. (2008). Prophylaxis of infective endocarditis: current tendencies, continuing controversies. *Lancet Infectious Diseases*, **8**, 225-232.

Egelberg, J. & Claffey, N. (1998). Role of mechanical dental plaque removal in prevention and therapy of caries and periodontal diseases. Consensus Report of Group B. In Proceedings of the European Workshop on Mechanical Plaque Control, eds. Lang, N., Attstrom, R. & Löe, H., pp. 169-172. London: Quintessence.

Everett, E. D. & Hirschmann, J. V. (1977). Transient bacteremia and endocarditis prophylaxis. A review. *Medicine*, **56**, 61-77.

Felix, J. E., Rosen, S. & App, G. R. (1971). Detection of bacteremia after the use of an oral irrigation device in subjects with periodontitis. *Journal of Periodontology*, **42**, 785-787.

Forner, L., Larsen, T., Kilian, M. & Holmstrup, P. (2006). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *Journal of Clinical Periodontology*, **33**, 401-407.

Gjermo, P. & Flotra, L. (1970). The effect of different methods of interdental cleaning. *Journal of Periodontal Research*, **5**, 230-236.

Horstkotte, D., Follath, F., Gutschik, E., Lengyel, M., Oto, A., Pavie, A., Soler-Soler, J., Thiene, G., von Graevenitz, A., Priori, S. G., Garcia, M. A. A., Blanc, J.-J., Budaj, A., Cowie, M., Dean, V., Deckers, J., Fernandez Burgos, E., Lekakis, J., Lindahl, B., Mazzotta, G., Morais, J., Oto, A., Smiseth, O. A., Lekakis, J., Vahanian, A., Delahaye, F., Parkhomenko, A., Filipatos, G., Aldershvile, J. & Vardas, P. (2004). Guidelines on prevention, diagnosis and treatment of infective endocarditis executive summary; the task force on infective endocarditis of the European society of cardiology. *European Heart Journal*, **25**, 267-276.

Infective Endocarditis Prophylaxis Expert Group (2008). Prevention of Endocarditis. 2008 update from Therapeutic Guidelines: Antibiotic. Version 13. North Melbourne: Therapeutic Guidelines Ltd.

Jenney, A. W., Cherry, C. L., Davis, B. & Wesselingh, S. L. (2001). "Floss and (nearly) die": dental floss and endocarditis. *Medical Journal of Australia*, **174**, 107-108.

Kinane, D. F., Riggio, M. P., Walker, K. F., MacKenzie, D. & Shearer, B. (2005). Bacteraemia following periodontal procedures. *Journal of Clinical Periodontology*, **32**, 708-713.

Lafaurie, G. I., Mayorga-Fayad, I., Torres, M. F., Castillo, D. M., Aya, M. R., Baron, A. & Hurtado, P. A. (2007). Periodontopathic microorganisms in peripheric blood after scaling and root planing. *Journal of Clinical Periodontology*, **34**, 873-879.

Lineberger, L. T. & De Marco, T. J. (1973). Evaluation of transient bacteremia following routine periodontal procedures. *Journal of Periodontology*, **44**, 757-762.

Lobene, R. R., Weatherford, T., Ross, N. M., Lamm, R. A. & Menaker, L. (1986). A modified gingival index for use in clinical trials. *Clinical Preventive Dentistry*, **8**, 3-6.

Lofthus, J. E., Waki, M. Y., Jolkovsky, D. L., Otomo-Corgel, J., Newman, M. G., Flemmig, T. & Nachnani, S. (1991). Bacteremia following subgingival irrigation and scaling and root planing. *Journal of Periodontology*, **62**, 602-607.

Lucas, V., Lytra, V., Hassan, T., Tatham, H., Wilson, M. & Roberts, G. (2002). Comparison of lysis filtration and an automated blood culture system

(BACTEC) for detection, quantification and identification of odontogenic bacteremia in children. *Journal of Clinical Microbiology*, **40**, 3416-3420.

Mariotti, A. (1999). Dental plaque-induced gingival diseases. *Annals of Periodontology*, **4**, 7-19.

McBryde, E., Tilse, M. & McCormack, J. (2005) Comparison of contamination rates of catheter-drawn and peripheral blood cultures. *Journal of Hospital Infection* **60**, 118-121.

McShane, A. J. & Hone, R. (1986). Prevention of bacterial endocarditis: does nasal intubation warrant prophylaxis? *British Medical Journal*, **292**, 26-27.

Miller, S. (1938). In: Textbook of periodontia, 1st edition. Philadelphia: Blakiston.

Moreillon, P. & Que, Y.-A. (2004). Infective endocarditis. *Lancet*, **363**, 139-149.

Murray, P., Baron, E. & Pfallar, M. (2003). In: Manual of Clinical Microbiology, 8th edition. Washington: American Society for Microbiology.

National Institute for Health and Clinical Excellence Guideline Development Group (2008). Prophylaxis against infective endocarditis: antimicrobial prophylaxis against infective endocarditis in adults and children undergoing interventional procedures. [WWW document]. URL http://www.nice.org.uk/CG064 [accessed on 22 Mar 2008].

Netzer, R. O., Zollinger, E., Seiler, C. & Cerny, A. (2000). Infective endocarditis: clinical spectrum, presentation and outcome. An analysis of 212 cases 1980-1995. *Heart*, **84**, 25-30.

Okabe, K., Nakagawa, K. & Yamamoto, E. (1995). Factors affecting the occurrence of bacteremia associated with tooth extraction. *International Journal of Oral and Maxillofacial Surgery*, **24**, 239-242.

Page, R. C. & Eke, P. I. (2007). Case definitions for use in population-based surveillance of periodontitis. *Journal of Periodontology*, **78**, 1387-1399.

Pauli, I., Shekhawat, P., Kehl, S. & Sasidharan, P. (1999). Early detection of bacteremia in the neonatal intensive care unit using the new BACTEC system. *Journal of Perinatology*, **19**, 127-131.

Perry, D. A. (1996). Plaque control for the periodontal patient. In Carranza's Clinical Periodontology, 9th edition, eds. Newman, M.G., Takei, H.H. & Carranza F.A., pp. 660-661. Philadelphia: WB Saunders Co.

Ramadan, A. E., Zaki, S. A. & Nour, Z. M. (1975). A study of transient bacteremia following the use of dental floss silk and interdental stimulators. *Egyptian Dental Journal*, **21**, 19-28.

Reinhardt, R. A., Bolton, R. W. & Hlava, G. (1982). Effect of nonsterile versus sterile water irrigation with ultrasonic scaling on postoperative bacteremias. *Journal of Periodontology*, **53**, 96-100.

Roberts, G. J. (1999). Dentists Are Innocent! "Everyday" Bacteremia Is the Real Culprit: A Review and Assessment of the Evidence That Dental Surgical Procedures Are a Principal Cause of Bacterial Endocarditis in Children. *Pediatric Cardiology*, **20**, 317-325.

Roberts, G. J., Gardner, P., Longhurst, P., Black, A. E. & Lucas, V. S. (2000). Intensity of bacteraemia associated with conservative dental procedures in children. *British Dental Journal*, **188**, 95-98.

Roberts, G. J., Gardner, P. & Simmons, N. A. (1992). Optimum sampling time for detection of dental bacteraemia in children. *International Journal of Cardiology*, **35**, 311-315.

Roberts, G. J., Holzel, H. S., Sury, M. R., Simmons, N. A., Gardner, P. & Longhurst, P. (1997). Dental bacteremia in children. *Pediatric Cardiology*, **18**, 24-27.

Sandre, R. M. & Shafran, S. D. (1996). Infective endocarditis: review of 135 cases over 9 years. *Clinical Infectious Diseases*, **22**, 276-286.

Sconyers, J. R., Albers, D. D. & Kelly, R. (1979). Relationship of bacteremia to toothbrushing in clinically healthy patients. *General Dentistry*, **27**, 51-52.

Silness, J. & Löe, H. (1964). Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavia*, **22**, 121-135.

Silver, J. G., Martin, A. W. & McBride, B. C. (1977). Experimental transient bacteraemias in human subjects with varying degrees of plaque accumulation and gingival inflammation. *Journal of Clinical Periodontology*, **4**, 92-99.

Smith, B. A., Collier, C. M. & Caffesse, R. G. (1986). In vitro effectiveness of dental floss in plaque removal. *Journal of Clinical Periodontology*, **13**, 211-216.

Strom, B. L., Abrutyn, E., Berlin, J. A., Kinman, J. L., Feldman, R. S., Stolley, P. D., Levison, M. E., Korzeniowski, O. M. & Kaye, D. (2000). Risk factors for infective endocarditis : Oral hygiene and nondental exposures. *Circulation*, **102**, 2842-2848.

Tomas, I., Alvarez, M., Limeres, J., Otero, J., Saavedra, E., Lopez-Melendez, C. & Diz, P. (2004). In vitro activity of monifloxacin compared to other antimicrobials against streptococci isolated from iatrogenic oral bacteremia in Spain. *Oral Microbiology and Immunology*, **19**, 331-335.

Tomas, I., Pereira, F., Llucian, R., Poveda, R., Diz, P. & Bagan, J. V. (2008). Prevalence of bacteraemia following third molar surgery. *Oral Diseases*, **14**, 89-94.

Thompson, W. & Walter, S. (1988). Kappa and the concept of independent errors. *Journal of Clinical Epidemiology*, **41**, 969-970.

Waerhaug, J. (1981). Healing of the dento-epithelial junction following the use of dental floss. *Journal of Clinical Periodontology*, **8**, 144-150.

Wahlmann, U., Al-Nawas, B., Jutte, M. & Wagner, W. (1999). Clinical and microbiological efficacy of single dose cefuroxime prophylaxis for dental surgical procedures. *International Journal of Antimicrobial Agents*, **12**, 253-256.

Walsh, T. F., Al-Hokail, O. S. & Fosam, E. B. (1997). The relationship of bone loss observed on panoramic radiographs with clinical periodontal screening. *Journal of Clinical Periodontology*, **24**, 153-157.

Wank, H. A., Levison, M. E., Rose, L. F. & Cohen, D. W. (1976). A quantitative measurement of bacteremia and its relationship to plaque control. *Journal of Periodontology*, **47**, 683-686.

Warren, P. R. & Chater, B. V. (1996). An overview of established interdental cleaning methods. *Journal of Clinical Dentistry*, **7 (3 Spec No)**, 65-69.

Wilson, W., Taubert, K. A., Gewitz, M., Lockhart, P. B., Baddour, L. M., Levison, M., Bolger, A., Cabell, C. H., Takahashi, M., Baltimore, R. S., Newburger, J. W., Strom, B. L., Tani, L. Y., Gerber, M., Bonow, R. O., Pallasch, T., Shulman, S. T., Rowley, A. H., Burns, J. C., Ferrieri, P., Gardner, T., Goff, D. & Durack, D. T. (2007). Prevention of infective endocarditis: Guidelines from the American Heart Association: A guideline from the American Heart Association Rheumatic Fever, Endocarditis and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the

Quality of Care and Outcomes Research Interdisciplinary Working Group. *Journal of the American Dental Association*, **138**, 739-760.

Yagupsky, P. & Nolte, F. S. (1990). Quantitative aspects of septicaemia. *Clinical Microbiology Reviews*, **3**, 269-279.

Zhang, J. & Boos, D. D. (1997). Mantel-Haenszel test statistics for correlated data. *Biometrics,* **53**, 185-1198.