

PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination

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Abstract – Periodontal cells capable of proliferation were studied immunohistochemically on extracted human teeth after 2-min irrigation with saline or ozonized water and marking of Proliferating Cell Nuclear Antigen (PCNA). All specimens expressed PCNA. The labelling index (LI), i.e. the number of positive cells compared to the total number of cells, was 6.6% after irrigation with saline and 7.8% after irrigation with ozone. There was no difference in number and distribution of PCNA-positive cells from the coronal to the apical thirds of the roots. Irrigation with ozonized water showed higher labelling indices in comparison with saline, but this could not be statistically substantiated ($P = 0.24$). Ozonized water, not being isotonic, had no negative effect on periodontal cells remaining on the tooth surface after irrigation for 2 min.

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About 20–35% of adolescents in Europe are subjected to dental trauma of their permanent dentition before reaching 18 years of age (1–4). Avulsion, i.e. the complete separation of a tooth from the organism, is not a rare result of trauma. The incidence is reported to be 1–16% (5–7). While a necrotic pulp can be treated endodontically during or after replantation, necrosis in the periodontal ligament (PDL) adhering to the root surface results in the resorption of the root substances and finally the loss of the entire tooth. Avulsed teeth are often contaminated with dirt and microorganisms. Microorganisms may disturb the periodontal reintegration. Intensive irrigation of the avulsed tooth before replantation is recommended. The proposals range from water (irrigation for 1 min) to sterile isotonic solutions (6, 8).

Ozone is not only an outstanding disinfectant, but also has medically relevant properties. Its use in oral medicine has been intensively investigated in recent years, the main question being, if ozonized water was an alternative to water disinfection of dental treatment units (9–11). Practicability and toxicologic as-

pects were also scrutinized (12, 13). Apart from its use as a disinfectant, it could be shown that ozone has an accelerating influence on wound healing (14, 15). Under the influence of ozone, an activation of cellular metabolism (16), higher intracellular ATP concentrations (17) and higher cellular expression of cytokines relevant to wound healing – especially Transforming Growth Factor TGF- β 1 (18) – were observed. In a recent publication, it could be shown that ozonized water clearly accelerates the healing of the human oral mucosa (19). The osteotomy of third molars with ozonized water compared to sterile isotonic saline solution showed identical post-operative healing (swelling, pain), whereas wound infections were more rarely seen (20). Ozone has a broad spectrum of microbial activity in water. Ozonized water takes effect more quickly than chlorinated water (21). Microorganisms have different sensitivities when subjected to ozone, e.g. *Echerichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens*, as well as *Candida albicans* (ozone concentration necessary for elimination 0.05–0.7 $\mu\text{g ml}^{-1}$) are more susceptible than

staphylococcae ($3\text{--}5\ \mu\text{g ml}^{-1}$) (22). Ozone concentrations over $5\ \mu\text{g ml}^{-1}$ will eliminate all microorganisms (23) in a few seconds. The irrigation of avulsed teeth with ozonized water could, hence, not only decontaminate the root surface, but also may have a positive effect on the cementoblasts in the remaining PDL adhering to the root surface. The aim of this study was to investigate the effect of irrigation with ozonized water on the proliferation of cells in the periodontal ligament adhering to the roots of extracted teeth in humans.

Materials and methods

A total of 23 completely erupted third molars without antagonists were extracted for prophylactic reasons in patients between 20 and 35 years of age. This guaranteed the investigation of healthy tissues and periodontal structures. Exclusion criteria were gingivitis, marginal periodontitis as well as the presence of caries. The extractions were performed as carefully as possible. Immediately after extraction, the teeth were randomly treated by intensive irrigation with ozonized water for 2 min (ozone generator prototype Sirona M1, Sirona, Bensheim, Germany; ozone concentration in water $2.5\text{--}3.5\ \mu\text{g ml}^{-1}$) or irrigation with a sterile isotonic saline solution, serving as a control group. All the teeth were then fixated with formalin (4%) and were decalcified over a period of 12 weeks using EDTA. All the specimens underwent routine histologic processing (paraffin embedding), which produced $4\text{-}\mu\text{m}$ thick transversal serial cuts of the roots. A total of 10 consecutive cuts taken from the coronal, middle and apical planes of the roots were examined, resulting in a total of 690 specimens. The specimens were stained immunohistochemically. The visualization of proliferating cells was performed with the help of Proliferating Cell Nuclear Antigen (PCNA) (24, 25), because PCNA labels all phases of the cell cycle. Microscopy was performed with a light microscope at a magnification of 250. The PCNA-expression varies according to phases in the cell cycle. There is nearly no expression in the G₀-phase, a slight rise in the early G₁-phase and a clear rise of expression in the late G₁- and early S-phases. The maximum is reached in the late S-phase. G₂- and M-phases show a gradual reduction of expression reaching levels seen at the beginning of the cell cycle (26, 27). The immunohistochemical preparation was performed according to the APAAP-method (Dianova, Hamburg, Germany). The monoclonal PC10-antibody (Dianova, Hamburg, Germany), defining an epitope of PCNA, served as primary antibody. Development was performed with neofuchsin, which produces a dark red colour when reacting.

In every cut, the number of marked cells and the total number of cells were investigated. Quantifica-

tion was performed by defining the geometrical mean value and the percentual labelling index (LI). The first step was to multiply the number of counted cells of the 10 investigated specimens. In a second step, the geometrical mean value was calculated by the 10th root of the result. Finally, the relation between the number of marked cells to the total number of cells was calculated. The evaluation was performed for apical (region 1), middle (region 2) and coronal parts of the root (region 3) as well as the entire root (regions 1–3); the relating 95% confidence intervals were also calculated. The LI documents the amount of proliferation in a cell population showing the percentage of marked cells in relation to the total number of cells and a certain method (28). The percentual LI corresponds to the geometrical mean value multiplied by 100. The values for all teeth in the respective groups were calculated. The statistical evaluation of possible influences of the irrigation solutions and the different planes on the geometrical mean value was performed with double factor variance analysis being used. The statistical planning and evaluation of the study were carried out at the Institute of Statistics, University of Giessen, Germany.

Results

The PCNA-marked cells were either in direct vicinity to the cementum or within the remaining periodontal ligament. Marked cells were found solitary or in groups on the root surface. Often, positively marked cells were seen lining the hard structures of the root like a string of pearls (Figs. 1 and 2). These cells were all found to be lying directly on the cementum or in the immediate layer of ligament above the cementum. Cementoblasts within the cementum showed very weak reaction with the antibodies, and cementocytes in the deeper layers of the cementum did not show any reaction (Fig. 3). Positively reacting cells were also found in periodontal layers, further away from the

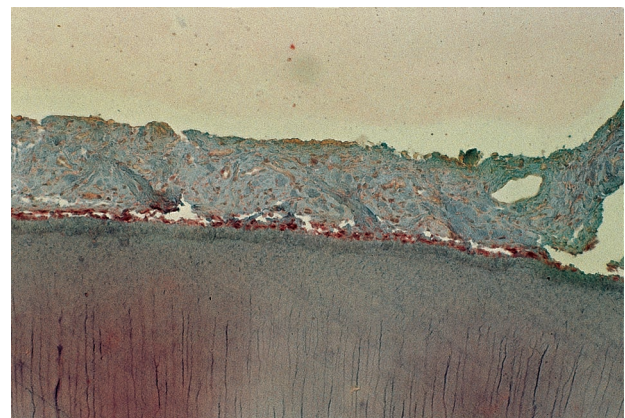


Fig. 1. PCNA-positive cells along the surface of cementum (PCNA; original magnification $\times 50$).

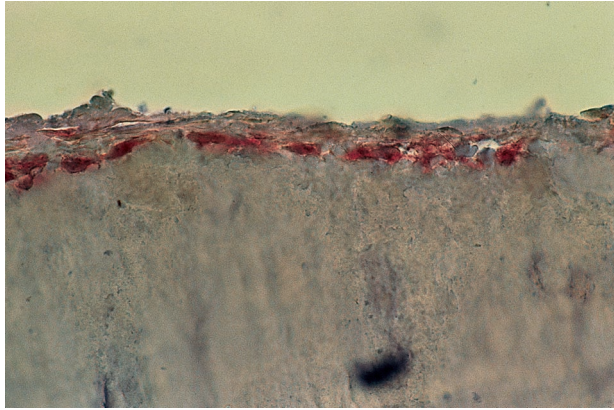


Fig. 2. Pearl-string arrangement of positively marked cells (PCNA; original magnification $\times 200$).

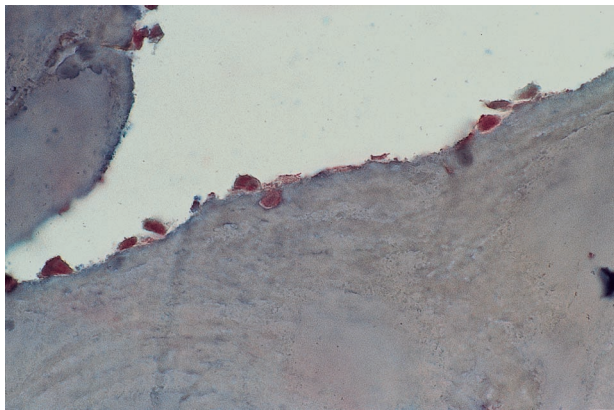


Fig. 3. Diminishing antigen reactivity of cementoblasts (PCNA; original magnification $\times 150$).

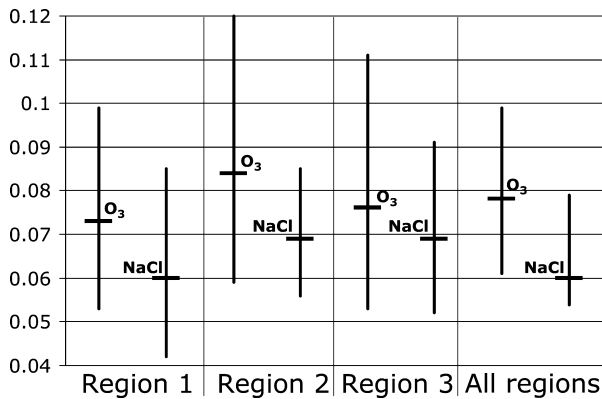


Fig. 4. Geometrical mean values and their corresponding 95% confidence intervals for respective regions and the entire root (region 1: apical; region 2: middle; region 3: coronal parts of the root; O₃: ozonized water; NaCl: saline).

root surface. They were less numerous and irregularly dispersed.

The geometrical mean values and their corresponding 95% confidence intervals are shown in Fig. 4. A

difference in the total number of cells between the two groups could not be found. The percentual LI was found to be 6.6% for all the teeth in the control group and 7.8% for the group treated with ozonized water. Although more marked cells were found in all the root regions under the influence of ozone, no difference could be found between ozone and saline when the double factor variance analysis was used. The variance analysis was performed on $n = 12$ subjects in the control group and $n = 11$ subjects in the group treated with ozone. There was no influence of root regions on the geometrical mean value and no relation between plane and substance used for irrigation ($P_{\text{region}} = 0.51$; $P_{\text{irrigation}} = 0.24$; $P_{\text{region-irrigation}} = 0.91$).

Discussion

Avulsion by trauma leads to rupture of the entire periodontal ligament. Periodontal tissue remainders are found on the root surface as well as in the alveolus. The vitality of cells remaining on the root surface is of great importance for the functioning of periodontal regeneration after replantation. Areas without cells and areas with necrotic cells undergo replacement resorption after replantation. The absence of cementoblasts leads to a loss of intercellular signals that keep osteoclasts at a distance: the tooth is incorporated in the physiologic remodelling of the bone. The root is replaced by bone; the survival time depends on the age of the patient when the trauma occurred. If microorganisms are present, infection-related root resorption will result and the tooth is lost within weeks to months.

Labelling indices for PCNA in different oral tissues in physiological and pathological condition have been published. The LI measured in this study for the periodontal tissue remaining on the root surface after irrigation with saline was 6.6%. The LI in the junctional epithelium of dogs is $35.70 \pm 14.02\%$, in human oral mucosa is 9.3% and in human parotid gland $3.83 \pm 0.61\%$ (29–31). The LI measured in this study lies between the latter two. It has already been shown that the number of proliferating cells in the periodontium is smaller than in the junctional epithelium (32). Periodontal cells show a higher proliferation rate than pulpal cells (33). Neoplastic tissue and tooth germs have a high LI, ranging up to $63.7 \pm 11.7\%$ (34).

The width of the periodontium varies from the coronal to the apical part of the root. It has been shown that osseous proliferation is the highest in the cervical and apical parts of the root, which are subjected to higher excursions of the root during articulation of the teeth (35). The expected higher rate of PCNA expression in the apical and coronal parts of the root could not be statistically verified in this study. An

explanation could lie in the missing functional stress on the third molars used in this study. Experimental studies show that periodontal fibroblasts subjected to mechanical stress react with a higher rate of DNA synthesis (36).

Ozone has microbiologic and metabolic properties that make it a useful disinfectant with a wide range of activity. The microbiological effect commences after only a few seconds of contact and leads to eradication of microorganisms very quickly according to their number and spectrum (19). The concentrations needed lie under $5 \mu\text{g ml}^{-1}$ (20, 21). The teeth irrigated with ozone descriptively show a higher cell marking rate in comparison to the control group, a fact that suggests enhancement of metabolism as shown in an earlier study on the oral mucosa (19); the difference cannot be statistically substantiated. There is evidence that ozonized water, not being an isotonic solution, does not have any negative effect on the vitality of the remaining cells on the root surface when used as irrigation for 2 min (8). It could be suggested that ozonized water leads not only to a mechanical cleansing, but also decontaminates the root surface. This has to be proved in further studies.

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