Hollow Fibre Membrane Bioreactors for Tissue Engineering Applications

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Abstract

Hollow fibre membrane bioreactors (HFB) provide a novel approach towards tissue engineering applications in the field of regenerative medicine. For adherent cell types HFBs offer an *in vivo*-like microenvironment as each fibre replicates a blood capillary and mass transfer rate across the wall is independent from the shear stresses experienced by the cell. HFB also possesses the highest surface area to volume ratio of all bioreactor configurations. In theory these factors enable a high quantity of the desired cellular product with less population variation, and favourable operating costs. Experimental analyses of different cell types and bioreactor designs show encouraging steps towards producing a clinically relevant device. This review discusses the basic HFB design for cell expansion and *in vitro* models; compares data produced on commercially available systems and addresses the operational differences between theory and practice. HFBs are showing some potential for mammalian cell culture but further work is needed to fully understand the complexities of cell culture in HFBs and how best to achieve the high theoretical cell yields.

KEYWORDS bioprocessing; bioreactor; cell therapies; hollow fibre membrane; in vitro; scale up; tissue engineering

Introduction

The requirement to culture mammalian cells in large quantities and in an *in vivo*-like environment has led to the application of a wide range of different bioreactor configurations based on traditional biochemical engineering designs. One bioreactor type that is showing promise is the hollow fibre membrane bioreactor (HFB); this configuration has been applied across all tissue engineering applications (TE-apps), i.e. cell expansion for regenerative medicine (Gundersen et al. 2010; Nold et al. 2013; Roberts et al. 2012), cell-scaffold constructs for regenerative medicine from injectable cell-gel composites (Seliktar 2012; Shin et al. 2013; Vermonden et al. 2012) and large bone defect augmentation (Niemeyer et al. 2010; Soardi et al. 2011; Torres et al. 2011), to cell delivery after myocardial infarction (Bernstein and Srivastava 2012; Roberts et al. 2012; Usuludin et al. 2012); and bioartificial organs (Oh et al. 2010; Oo et al. 2011); *in vitro*/toxicology models (Usuludin et al. 2012; Zhang et al. 2012), and most recently cultured meat, in the author's lab.

Relatively simple scale-up is theoretically possible with HFBs, compared to other bioreactor configurations, as scale-up can be based on Krogh cylinder modelling. The current trend for companies utilising TE-apps, when developing high quality cost effective bioprocesses, is to incorporate stirred tank bioreactors, which are traditionally and successfully used for the large-scale production of biopharmaceuticals. Despite the sensible lateral application of this configuration to TE-apps, the complexity of tissues and the sensitivity of mammalian cells to subtle changes to their environment has driven the design of a second generation of bioreactors to meet the biological, financial and regulatory requirements to enable mass-production to meet global demands.

Given the breadth of TE-apps where the final product from a cell expansion process will range from undifferentiated stem cells to a tissue-like cell-scaffold construct, it is unlikely there will be a single bioreactor type that exhibits broad utility, as the stirred tank has done for biopharmaceutics. Not only will the cell type(s) and required extent of differentiation vary, but so will the downstream processing step of removing the product, purifying it and packaging it for delivery and application in the clinic. As such HFBs are not suited to all tissues and all applications and the tissue engineer should choose the bioreactor configuration only once the product is fully understood. Herein HFBs are reviewed in

the context of the applications for which they are particularly suited – as devices for cell expansion and as *in vitro* models.

HFBs for Regenerative Medicine Applications

The versatility of the HFB is owed to the ease at which environmental changes can be applied to the system. For example if used as a device for cell expansion the researcher would focus on applying the optimum conditions to accelerate rates of cell growth and cell number. Other practical considerations include the removal and extraction of the cells from the bioreactor once the desired number of cells is met. For a regenerative medicine construct, multiple cell types may be used (particularly when modelling heterogeneous tissues), and the fibres may be used to deliver drugs or growth factors to improve endogenous tissue regeneration. Cells can be cultured on the internal or external surfaces of the hollow fibre, as well as be suspended in a gel in the extracapillary space (ECS) (Figure 1).

FIGURE 1 PLACED HERE

HFBs for cell expansion

Given their design, HFBs have the potential to expand a population of cells to a clinically significant number and enable the differentiation of stem cells along desired lineages. These properties make HFBs an attractive prospect for regenerative medicine. To support this statement, a variety of cell types have already been successfully expanded (Table 1) and differentiated (Table 2) using the HFB system.

TABLE 1 AND TABLE 2 PLACED HERE

Several methods are available for determining the efficacy of cellular expansion. These methods include (i) direct quantification of seeding and harvesting cell numbers or densities (ii) indirectly quantifying by comparing changes in DNA, glucose or lactate concentrations over time or (iii) qualitatively depicting changes through imaging. These methods are not entirely comparable,

78 particularly where the result has not been quantified. Therefore, it is not possible to directly determine
79 differences in efficacy between various experimental systems in the literature. A standardised direct
80 quantitative method of cell growth alongside supplementary methods of determining cellular
81 expansion would allow for efficient comparisons between HFB setups.

82

83 While the HFB system could potentially generate a renewable cell source at therapeutically significant
84 levels, problems with producing clinically useful cells include maintaining population heterogeneity
85 during the culture process (Williams et al. 2012) and the requirement of having to produce yields
86 approximating 10^5 to 10^{13} cells per dose (Simaria et al. 2014). Therefore, it is important to identify
87 how bioreactor design, culture conditions and scale-up can be improved to achieve the standards
88 required for therapeutic use.

89

90 ***HFBs for regenerative medicine constructs***

91 A three dimensional (3D) culture scaffold wherein the HFB contains a gel or cells cultured on hollow
92 fibres are assembled sufficiently close to allow bridging holds a number of advantages over traditional
93 two dimensional (2D) cultures grown in tissue culture flasks. 3D culture substrates are able to better
94 represent the *in vivo* environment such that cell phenotype, gene expression and function are
95 improved compared to the 2D environment; this is due to complex cellular interactions that occur
96 between cells and the extracellular matrix (ECM) (Sun et al. 2006; Zhou et al. 2008).

97 Cells can be cultured on the intraluminal wall of hollow fibre membranes. This has been demonstrated
98 with alveolar epithelial cells, where an air-liquid interface similar to that in lung tissue was
99 reproduced by passing air through the fibre lumen (Grek et al. 2009). Endothelial cells constitute the
100 inner surface of the vasculature. While they do not form tight junctions under 2D culture conditions,
101 when cultured on the lumen surface and exposed to the shear stresses of media flow through the
102 lumen, (similar to the flow of blood in vessels) they behave in a similar fashion to that seen *in vivo*
103 (Ott and Ballermann 1995). The issue with this approach is that the mass transfer rate to the cells in

104 the ECS is now coupled to the shear stress experienced by the endothelial cells which may limit the
105 feed flow rates used. Yamazoe and Iwata (2006) showed that culturing cells within the fibre lumen
106 could potentially protect the cells from rejection by the host immune system if they were implanted
107 into a patient, due to the ability to control the molecular weight cut off of the semipermeable
108 membrane.

109

110 HFBs for use as *in vitro* models

111 *In vitro* models allow for the study of tissue function, as well as drug discovery and toxicology
112 testing, and metabolism analysis without the need for using animal models; the same considerations
113 can be applied to bioartificial organs but these are not reviewed here in detail. The HFB setup has
114 been utilised in bioartificial organ design to replicate liver (Gautier et al. 2009) and kidney functions
115 (Oo et al. 2011) with the intent of utilisation as clinical therapy. In the case of drug and toxicology
116 testing, repeated dosage, acute and chronic effects may be examined depending on how long the
117 model is maintained (Zeilinger et al. 2011). However, there are certain considerations associated with
118 creating a system that is able to replicate the *in vivo* setting successfully, such as the interactions
119 between different cell types and culture conditions that exactly match those found *in vivo* so that
120 accurate extrapolation of data can be made. Furthermore, high cell growth rates may not be desirable
121 once the cells have formed the model construct, instead requiring conditions that facilitate the desired
122 cell number at a stationary phase but not their growth phase.

123

124 Physical control of the environment is relatively easy as the membrane characteristics, flow rates and
125 mass transfer rates can be prescribed, however the biological environment in terms of the culture
126 media is also of importance but is very complex. Basic culture medium conditions provide cells with
127 the nutrients and growth factors specific to their environment to thrive. The medium can be further
128 supplemented with growth factors and cytokines to aid proliferation and differentiation depending on

129 the requirements. pH may be controlled through the addition of an appropriate buffer or by careful
130 assessment in media containing the indicator phenol red. Foetal bovine serum can also be added to the
131 culture medium to allow deposition of surface proteins, aiding attachment of adherent cultures. The
132 addition of antibiotics and antifungals can reduce the likelihood of infection within the closed system
133 of the bioreactor. However, artificial culture media do not necessarily provide an exact replica of the
134 types of nutrients (and the appropriate concentration) and gas seen physiologically which could affect
135 cellular functions.

136 Whole blood has been utilised in HFB as the culture medium equivalent to *in vivo* conditions, to
137 provide both nutrients and oxygenation to the cells whilst removing waste products. This route has
138 drawbacks due to a lack of a supply of constant comparable blood, strict regulations for safe use,
139 fouling by blood cell attachment to the membrane, and clotting. A compromise is to separate the red
140 blood cells from whole blood and use them to supplement the chosen culture medium, thus preventing
141 immune responses (Gundersen et al. 2010; Sullivan et al. 2007) or to add an anticoagulant to the
142 system, either in the medium or coating the intraluminal surface to reduce thrombogenicity (Zhang et
143 al. 2012). A study by Chen and Palmer (2010) added bovine haemoglobin to the culture medium in a
144 HFB to act as an oxygen carrier. This showed a higher cell mass, improved efficiency of hepatocyte
145 metabolism and drug detoxification, and conservation of albumin synthesis , and ammonia
146 detoxifying functions compared to controls. Oxygen requirements vary between cell types, for
147 example hepatocytes have different functions depending on oxygen concentration based upon their
148 location on the portovenous axis, a phenomenon known as liver zonation (Burke and Tosh 2006;
149 Davidson et al. 2012). Mathematical modelling of oxygen transport, and application of the
150 mathematical models to the oxygen transport in laboratory settings (Davidson et al. 2012; Patzer
151 2004) as well as control of operating parameters (Shipley et al. 2011), is an important component of
152 HFB *in vitro* model design.

153

154 ## Common design aspects and basic operation of HFBs

HFB configuration and operation

The configuration of a HFB provides a greatly increased surface area for cellular attachment and proliferation, in comparison to other bioreactor configurations. HFBs only require a volume that is 0.1% the capacity of a T-flask, or 0.5% the size of a stirred tank to grow an equivalent number of cells (Table 3) (Ellis et al. 2005). The culture parameters within the HFB must also be considered to ensure that they produce large cell yields and high viability without loss of phenotype; it is therefore very important that the physical, chemical and biological environment within the bioreactor is as close as possible to the *in vivo* environment and the HFB should be tailored to each cell type or types; attempts have been made to do this based on oxygen requirements for different cell types using computational fluid dynamics (Shipley et al. 2011). The semipermeable nature of the hollow fibre membrane is conducive to selective diffusion between media flowing through the fibre lumen and cells in the ECS, and the flux of media across the membrane can be prescribed based on membrane and bioreactor physical properties (Shipley et al. 2010). By culturing the cells in the ECS, shear stresses are decoupled from the bulk media flow in the lumen thus preventing cell damage, detachment from the culture substrate and undesirable shear stress responses. It should be noted however that a second media stream can be passed through the ECS if desired. Figure 2 shows several different configurations of HFBs. The important parameters to be controlled are the temperature, the flow rate of the media through the lumen, pH, the pressure differentials across the bioreactor, and dissolved oxygen, nutrient, waste product and metabolite concentrations and residence times. Other properties intrinsic to the fibre itself are also important to consider. For example, when culturing a desired cell number a bundle of fibres of a suitable length and diameter are necessary to provide the correct surface area for growth.

TABLE 3 PLACED HERE

FIGURE 2 PLACED HERE

Hollow fibre membrane fabrication and properties

The macroarchitecture of the hollow fibre membrane provides a highly permeable, minimally resistant

barrier that acts as a scaffold for adherent cells. The hollow fibre membranes used in HFBs for TE-apps are usually made from a polymer, the fibre being fabricated using dry-wet or wet-wet spinning (Ellis and Chaudhuri 2007). This produces a porous fibre with a hollow core, the lumen, through which cell medium can flow. Structural, mechanical and topographical factors are dependent on the manufacture of the fibre itself, including the type of polymer used, the solvent and nonsolvent selection, the phase inversion process it has undergone, and any coating applied (Ellis and Chaudhuri 2007). For example, pore size can be modified by altering the initial casting dope solution (Ellis and Chaudhuri 2008) to allow the selective passage of specific cellular products through the porous polymer matrix based on their molecular weight. Careful selection of fibre fabrication conditions, alongside the selected operating conditions, ensures good mass transfer of nutrients and oxygen throughout the construct.

The hollow fibre biomaterial should also be biocompatible. Whether the surface is a suitable environment for cell adherence is further influenced by several factors; *in vivo* this is achieved through interaction between cell adhesion receptors such as integrins and the ECM. In the *in vitro* setting, these interactions can be affected by the surface energy and topography of the biomaterial surface (De Bartolo et al. 2002). A hydrophilic surface is more conducive to cell attachment due to the ability for proteins within culture media serum to adsorb on to the biomaterial. Modification of the biomaterial surface to mediate the biochemical signalling required for cell-matrix interactions can be achieved through surface treatment, such as plasma treatment for the addition of functional groups to increase hydrophilicity (Jacobs et al. 2012), or surface grafting of bioactive molecules, thus allowing interaction between the cells and the added molecules rather than the polymer surface (Bellis 2011). Alternatively, the ECS can also be filled with a gel to mimic the ECM. Immobilisation of cells in sodium alginate has previously shown increased induction of vasculogenesis of human embryoid bodies from human embryonic stem cells when compared to static or rotating bioreactor setups (Gerecht-Nir et al. 2004). In addition, the use of alginate to immobilise primary porcine pancreatic cells in a HFB setup demonstrated increased intracellular insulin compared to suspension cultures (Hoesli et al. 2009).

208 The exact setup and operation of the HFB clearly depends on the tissue and the application, however
209 there are a number of common considerations for their use. Figure 3 presents a generalised flow chart
210 for cell culture in HFBs.

211 **FIGURE 3 PLACED HERE**

212

213 The Design and Application of Commercially-Available HFBs

214 Due to the advantages of growing cells in 3D structures, various research groups have strived to
215 develop their own HFB systems. Others have developed HFBs into commercial brands, providing a
216 variety of products with dimensions, separation properties and material types to suit numerous
217 laboratory-testing applications. Some of the companies who sell bench scale HFBs are Fibercell
218 Systems, Spectrum Labs, Terumo BCT and Eurotechnologies. The equipment provided by these three
219 companies has been used in a small number of published articles for physical experimentation
220 (FiberCell) (Usuludin et al. 2012) and used as a base for mathematical modelling (Spectrum Labs)
221 (Chen and Palmer 2009).

222 Fibercell primarily produce hollow fibres made from polysulfone with the surface areas between 75
223 cm^2 and 2.5 m^2 with increasing ECS volume of 12 ml to 150 ml and a 50% packing density within a
224 reactor housing made from glass. The additional surface area allows a greater number of cells to grow
225 on and within the fibres, a maximum of $10^9 – 10^{11}$ over the range of HFB sizes. Molecular weight cut
226 off (MWCO) ranges from 5 kD and 0.1µm and flow rates up to 200 ml min^{-1} can be applied. This
227 system is advertised to produce monoclonal antibodies and secreted proteins in high concentrations, to
228 expand lymphocytes and endothelial cells, and to be used for *in vitro* toxicology tests (FiberCell
229 2014).

230 The HFBs sold by Spectrum Labs (brand name ‘Cellmax’) offer a wider range of materials:
231 polysulfone, polypropylene, polyethylene, and regenerated cellulose. It recommends the most suitable
232 polymer depending on the application: hollow fibres made from polysulfone if required to collect

secreted cell products, or made from either polypropylene or polyethylene for experimentation on cellular adhesion and gas diffusion (Spectrum Laboratories 2012). Pore sizes between 10 kD and 0.5 μm are available, and their HFBs allow a flow rate between 5-120 ml min^{-1} through the lumen of fibres with Reynolds numbers of less than 10 and velocities of 10^{-4} -10^{-2} ms^{-1} depending on membrane size. Side ports are also included in this system to allow for a secondary flow through the ECS if required.

It could be argued that the presence of market leaders in lab scale HFB systems could allow parallels to be drawn between different research groups who use the same commercially available equipment, providing some sort of standard by which data could be compared. However in the process of writing this review only a handful of publications have actually used commercial HFB products, with many groups opting to make their own hollow fibres. If it can be demonstrated that a commercial HFB system could be constructed from materials of a known standard, and built using automated manufacturing techniques, a commercial system may prove advantageous for larger collaborative research projects and clinical utility to ensure consistency.

Theoretical Promises versus Actual Success and Current Issues with HFBs

The use of the commercial systems outlined above has been documented in journal articles, allowing data to be generated to check the claims reported in the catalogues of their manufacturers. A HFB cartridge purchased from Fibercell Systems was used (model C2011) to grow a co-culture of stromal and erythroleukaemia cells, of which 4.4 x 10^8 were successfully harvested from the HFB (Usuludin et al. 2012). This is almost an order of magnitude less than the advertised maximum cell number of 10^9. The authors describe how a complete cell harvest was not achievable because some cells remained trapped between fibres, an issue which would arise in any tightly packed HFB. This harvesting issue of course is not a problem for hollow fibre membrane constructs designed for implantation.

The hollow fibre construct can also be a disadvantage because it does not allow the direct real time

visual inspection of cellular growth within the porous hollow fibre network, instead resorting to more indirect methods such as mass balances on gas exchange and nutrient uptake. One study comparing the performance of a commercially available HFB called the 'Quantum Cell Expansion System' (from Terumo BCT) with a static T-flask control for a modified human embryonic stem cell (hESC) line showed that despite achieving a larger cell count whilst utilising an equivalent of 15% of the T-flask growth medium, the HFB system achieved a lower cell viability (93-94%) and a lower cell density (18,000-34,000 cells cm^{-2}) than the control (99%, 190,000 cells cm^{-2}) (Roberts et al. 2012). The viability of the cells in the HFB was 93.5%, identical to that advertised by the manufacturer when growing Mesenchymal Stem Cells (MSC) (Nguyen et al. 2012). This level of viability was linked to longer exposure to lactate which was present in the HFB in higher concentrations at lower flow rates, causing moderate acidic conditions which has been shown to affect hESC growth (Chen and Palmer 2010). Increased flow rates through the reactor should mitigate stagnant conditions occurring. However this would need to be balanced with ensuring the subsequent high shear rates do not strip the cells from their scaffold (Titmarsh et al. 2011), or other modifications such as pore size and porosity.

In a more general sense, bioreactors typically fail to meet cell number and functional requirements because they provide unsuitable conditions for either the initial cell attachment or for the subsequent cell expansion. They are also much less sophisticated than the conditions found *in vivo,* with some aspects being much too elaborate for a bioreactor to control.

Conclusion

It is apparent from the studies reviewed here that the versatility of HFB design and their ability to expand a variety of cell types bodes well for their application in cell therapies, where mass production of cells is required for regular clinical use, and *in vitro* models, to reduce reliance upon animal experimentation. However, further study is required in both the understanding of the cellular interactions with the bioreactor and subsequent modification of the culture environment to ensure that there is homogeneity in the cell population. It is undisputable that HFBs provide the highest

theoretical culture efficiency based on surface area to volume ratio. HFBs are scalable and applicable to the culture of any cell type although are more suited to adherent cells and those from vascularised tissues. The product can be a population of cells removed by trypsin, or a single solid construct removed in its entirety from the module. A number of commercial HFBs are available although there is no standard operating procedure and optimal operation has not yet been achieved. The design and operation is specific to the cell type and end-use, and all aspects from hollow fibre material to pressure gradients and flow configuration need to be considered. HFBs have been successfully used for a range of tissues and this review suggests there is considerable interest and reason in continuing to explore and optimise their application for tissue engineering.

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Table 1 Cellular expansion using a HFB system

Cell	Type	Seeding		Expanded					References
		Number (cells)	Density (cells cm^{-2})	Number (cells)	Density (cells cm^{-2})	Fold increase	Visual depiction	Other analysis (e.g. biochemical)	
Lymphocyte	Human (primary tumour infiltrating)	1×10^9	-	1×10^{11} (dose reached)	-	-	-	Increased lactate (14d)	(Malone et al. 2001)
	Human (primary)	1.92×10^8	-	-	-	-	-	Increased glucose uptake and lactate (8d)	(Curcio et al. 2012)
Embryonic stem cell	Human (Shef3)	6×10^7	$\sim 3 \times 10^3$	3.83×10^9 (8d)	1.8×10^4	-	-	Increased lactate (52h)	(Roberts et al. 2012)
				7.08×10^9 (8d)	3.4×10^4	-	-		
Mesenchymal stem cell	Human (primary bone marrow)	2×10^6	-	1.87×10^7 (6d)	-	9.4	-	Increased glucose uptake and lactate (25d)	(Nold et al. 2013)
		3.5×10^6	-	5×10^7 (11d)	-	14.2	-		
		5×10^6	-	9.8×10^7 (13d)	-	20	-		
		7.6×10^6	-	1.72×10^7 (13d)	-	2.3	-		
		-	5×10^3	-	-	25 (DNA concentration)	-	Increased DNA concentration (120h, 168h)	(Morgan et al. 2007)
Embryonic liver cell	Rat (RLC-18)	-	1×10^4	-	-	1.97 (14d)	SEM (8d, 14d)	-	(Salerno et al. 2013)
Bone marrow stromal cell	Human (HS-5)	1×10^8	4.76×10^4	4.42×10^8 (28d)	2.11×10^5	-	-	Increased glucose uptake and protein concentration (28d)	(Usuludin et al. 2012)
Haematopoietic cell	Human (K562 co-culture with HS-5)	5×10^5	-	-	-	3130 (14d)	-	-	
Adipose stem cell	Human (primary)	-	1×10^5	-	-	-	Live-dead stain (3d, 7d)	Increased DNA concentration (1d, 3d, 7d)	(Diban et al. 2013)

Table 2 Cellular differentiation using a HFB system

Cell	Type	Differentiated Cell	References
Haematopoietic stem cell	Human	Neutrophil Erythrocyte Lymphocyte	(Housler et al. 2012)
Embryonic stem cell	Mouse	Dopaminergic neuron	(Yamazoe and Iwata 2006)
		Hepatocyte	(Amimoto et al. 2011)
Induced pluripotent stem cell	Mouse	Hepatocyte	(Amimoto et al. 2011)
Mesenchymal stem cell	Sheep	Osteoblast	(De Napoli et al. 2011)
Embryonic liver cell	Rat	Hepatocyte	(Salerno et al. 2013)

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439 **Table 3** Bioreactor design configurations and their capacities (Ellis et al. 2005).

Configuration	Media change	Mixing conditions	Tissue development	Culture dimensions	Size to grow an organ
Tissue culture flask	Batch	Poorly mixed No shear Diffusion	2D sheet	290 cm^2 L^{-1} 1 x 10^5 cells ml^{-1}	10-1000 L
Stirred tank	Batch or continuous	Well mixed Shear Convection	2D or 3D	2800 cm^2 L^{-1} 5 x 10^5 cells ml^{-1}	2-200 L
Packed beds	Continuous feed (perfusion)	Well mixed Shear Convection	3D	18,000 cm^2 L^{-1} 2.5 x 10^6 cells ml^{-1}	0.4-40L
Fluidised bed	Continuous feed (perfusion)	Well mixed Shear Convection	3D	25,000-70,000 cm^2 L^{-1} 5-6 x 10^6 cells ml^{-1}	0.2-20L
Membrane bioreactors	Continuous feed	Well mixed No Shear or Shear Convection and diffusion	3D	100,000-200,000 cm^2 L^{-1} 2 x 10^8 cells ml^{-1}	0.05-0.5L

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449 **FIGURE LEGENDS**

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451 Figure 1 – Cell attachment and growth in a hollow fibre membrane. Cells can be seeded and
452 consequently cultured on the lumen surface or the external surface of the membrane wall, or
453 encapsulated in a gel in the extracapillary space. Cells could be encapsulated within the fibre wall
454 itself, or allow migration if the pores are large enough, however such an approach is not covered in
455 this review. Typical fibre outer diameter ranges between 500 – 1000 μm with a wall thickness of 200
456 μm. Figure not to scale.

457 Figure 2 – The different operational configurations of a hollow fibre bioreactor. A-D have retentate
458 streams, the permeate flux can be prescribed by applying back pressure on the retentate stream; E-H
459 are 'dead-end' with the retentate stream shut off (H is of no use in practice but shown for
460 completeness); (I) Starling flow for which the extracapillary ports are shut off. A, C, E, G & I show
461 co-current configurations, and B, D, F & H show counter current configurations.

462 Figure 3 – Common steps for performing cell culture in a hollow fibre bioreactor.

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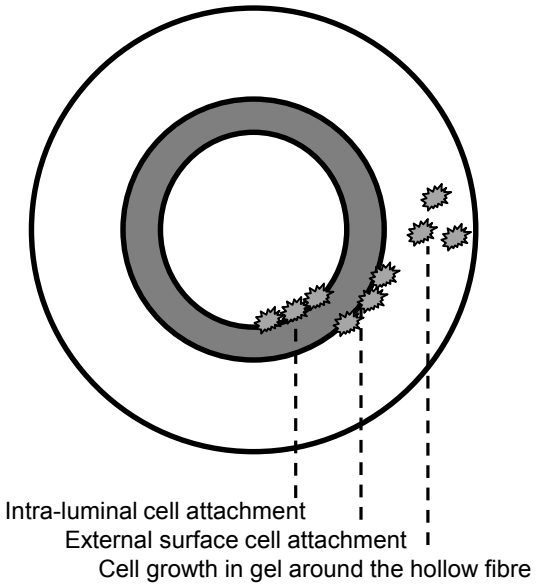
473 **Figure 1**



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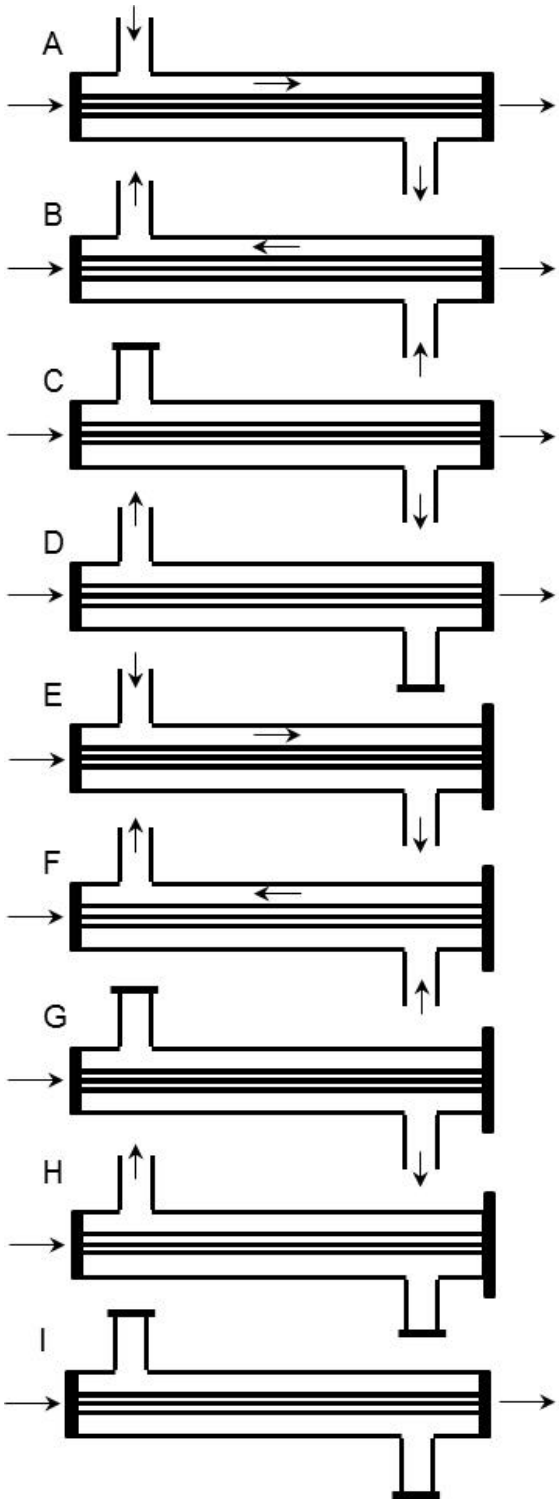
489 **Figure 2**



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494 **Figure 3**

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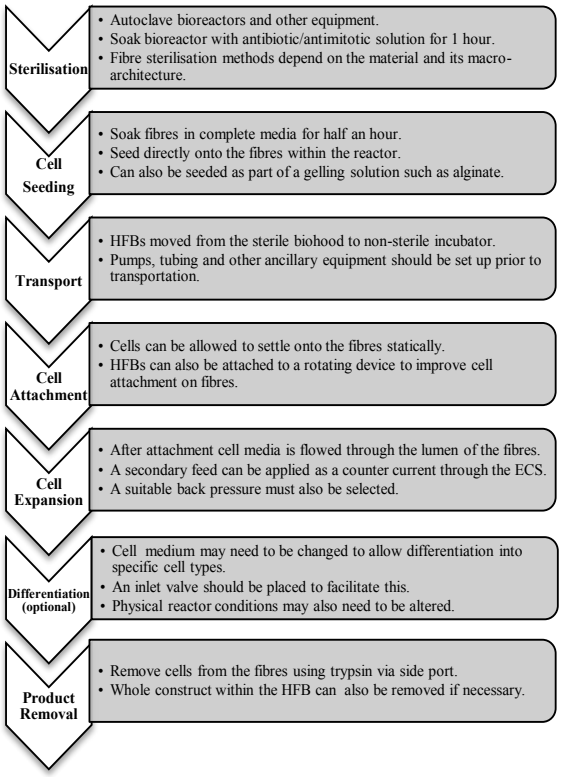

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