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Studies on Mechanical Factors Influencing Tissue Generation in Bioreactors :A Review

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ABSTRACT

Considering the actual techniques in cell culture, the stimulation of cellular proliferation and the formation of bi-dimensional tissues such as skin are widely performed in laboratories. The process becomes more complex for the formation of a cohesive three-dimensional tissue. In this case, a special environment, which is achieved and maintained in a specific bioreactor, is required. A bioreactor reproduces a pseudo-physiological environment favourable for tissue regeneration and specific to three-dimensional cell culture. Furthermore, bioreactors can be used for studying and understanding the mechanical factors influencing tissue regeneration. This review presents principal types of bioreactors, some of their applications and a comparison of main studies, dealing with the influence of mechanical stresses and strains during the culture period on the final properties of regenerated tissues.

Key Words :Tissue Generation,Bioreactor,in vivo studies,Biomaterial,perfusion system

INTRODUCTION

Tissue engineering is a new research field in rapid expansion. Its goal is to find a new solution to the current problem of organ shortage and biomaterial failures.It may provide an efficient solution to the problem of arterial failure which is usually treated by grafting of an inert prosthesis having only a five to ten year life time [1].Bioreactors have already improved the processing and the final results of skin and cartilage healing, the only two lab-grown products commercially available now days. Some *in vivo* studies are currently in progress in humans to test bioengineered corneas, bones, urethras and pancreatic cells [2]. Upto date, significant results were obtained in laboratory for these applications and their culture leaded to the growth of functional tissues with suitable dimension.Most of the regenerated tissues are actually tested *in vivo* in animals (blood vessels, muscles, heart valves, tracheas, ears, livers, kidneys, pancreas, bladders, intestines, salivary glands, etc. [3]). The experiments the closest at hand to actual human implantation seem to be on blood vessels, bladders and heart valves.Three major strategies are used to control the regeneration of three-dimensional tissues. The first is the implantation of an acellular matrix to encourage the formation of a new tissue [4]. *In vivo* studies have shown that it is difficult to encourage cell migration into the scaffold, resulting in poor tissue formation [5].The second is to encourage the self assembly of cells [4]. Although much effort and several studies have been made, no functional tissue has yet been regenerated with this method because of a lack of cohesion between cells, dedifferentiation and an inadequate resulting tissue shape. In fact, external guides and signals, such as mechanical stress and strain, are essential to make cells grow into functional three-dimensional implantable organs [6], and these guides are difficult to apply on non-supported cells. Finally, the use of a scaffold offers the possibility to tailor the initial properties of the construct and allows an easier application of mechanical conditions on the young and fragile construct at the beginning of the regeneration. Eventually, these scaffolds, if biodegradable, will disappear, thus leading to a highly coherent, totally biological and functional tissue. Already, resulting regenerated tissues have been successfully implanted *in vivo* [3, 4, 7, 8].

Bioreactors

A bioreactor can be defined as any apparatus that attempts to mimic physiological conditions in order to maintain and encourage tissue regeneration. Culture parameters such as temperature, pH, biochemical gradients and mechanical stresses are permanently controlled. Every culture condition can be modified to study their influence on the growth of different tissues.In the case of a perfusion bioreactor, the culture medium has to be continually renewed to supply gas and nutrients to cells and to remove metabolites and catabolites. Thus, the required perfusion system is usually composed of an oxygenator, a pump and a medium culture reservoir as shown in the figure 1. All, or a portion, of the culture medium can be recirculated with or without a supply of fresh medium.

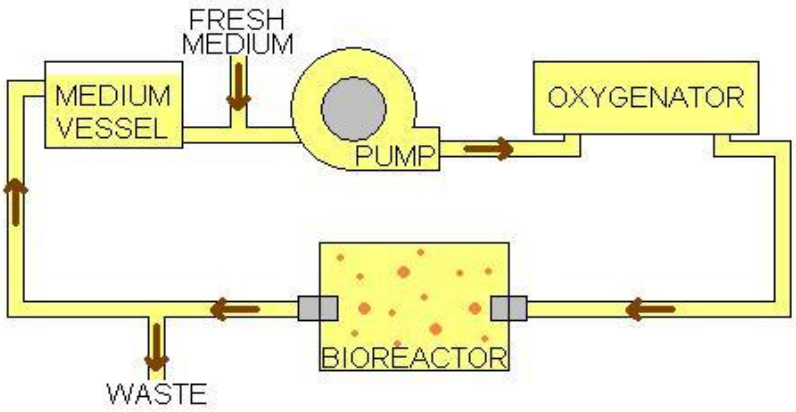

Figure 1 : Simplified perfusion system

Specific bioreactors are essential for the research in tissue engineering [7]. In the body, cells are always stimulated by mechanical, electrical and chemical signals that influence their behaviour. If these signals are inadequate or non-existent, cells dedifferentiate, become disorganised, and it can lead to cells death [6]. In fact, biological tissues adapt their structure and composition to surrounding specific and functional demands [9]. Current bioreactors can be divided into two main classes, rotating and non-rotating. Rotating bioreactors (figure 2) have a culture chamber permanently in rotation. It encourages the uniform growth of the tissues. Also, the rotation speed can be adjusted to produce a free-falling state. This protects fragile tissues because it decreases shear stresses and it avoids contact between cells and the walls of the bioreactor [10].

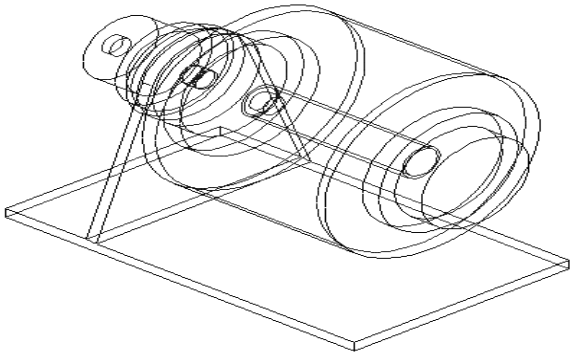

Figure 2 : Example of a rotating bioreactor

A non-rotating bioreactor has a motionless culture chamber which allows for the culture of complex tissues. Specific mechanical stresses can easily be applied on the cultivated tissues. The perfusion solution can flow through the culture chamber, and eventually through the tissues. An example is shown in figure 3. In this case, it is possible to apply shear stresses on cultivated cells from 0.02 to 1 Pa by increasing the pressure in the lower chamber which moves the silicon membrane [11].

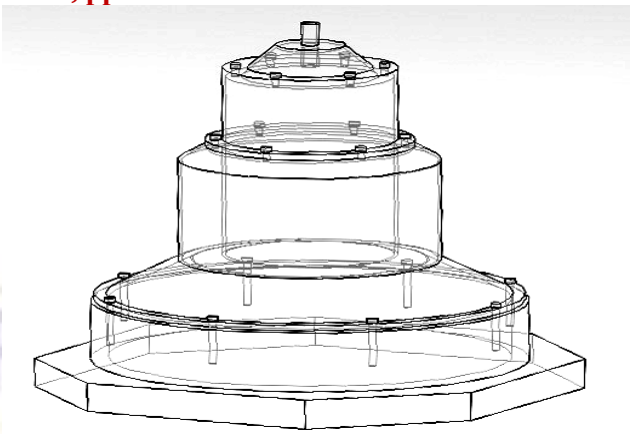

Figure 3 : Example of a non-rotating bioreactor

Applications of bioreactors

Even if the works that present the full design methodology used to elaborate the bioreactor are rare, it is possible to review their functions and to compare their specifications. The following are the main applications of bioreactors designed for the growth of cartilage, cardiac tissues, vascular tissues, cardiac valves and hepatic cells. Each section consists of a summary of the characteristics of the tissues and examples of cell culture using bioreactors.

Cartilage

Cartilage is a non-vascularized tissue made of chondrocytes and an extracellular matrix (ECM) composed of collagen and glycosaminoglycans (GAG) [12]. Chondrocytes are spherical cells located in little cavities in the ECM [13]. They are responsible for the synthesis and the degradation of this ECM. These cells compose 1% of cartilage volume while water composes 80% of cartilage weight [14]. Because this tissue is not vascularized, chondrocytes extract their nutrients from the synovial fluid.Cartilage is located on the articular surfaces of bones and also constitutes some parts of the skeleton [13].

Table 1 : Bioreactor applications for cartilage culture

	D.Pazzano et. al., 2000[15]	B.Obradovic et. al., 1999[16]	K.J.Gooch et. al., 2001[12]	T.Nishikori et. al. 2002[17]	L.Freed et. al., 1997[18]
Cell source	Bovin calf glenohumeral joint surfaces	Fermoropatellar grooves of 2-3 week old bovice calves	Knee joints of 2-4 week old bovine calves	Hip, mnee and shoulder joint of 10 week old rabbits	Fermoropatellar grooves of 2-3 week old bovine calves
Scaffold	PGA coated by PLLA	PGA	PGA	Collagen	PGA
Type of the bioreactor	Not-rotating	Rotating	Mixing	Non-rotating	Rotating
Flow	1 µm/s through tissues	None	Induced by mixing	None	Perfusion
Mechanical stresses	Induced by flow	Low stresses (free-falling conditions)	Shear stresses induced by mixing	Induced by ultrasound	Quasi inexistent (weightless environment)
Studied parameters	Flow and perfusion	Gas exchanges and replacement frequency of culture medium	Reynolds number	Ultrasounds	Weightless environment

D. Pazzano *et al.* [15] showed that a perfusion flow of 1 µm/s through the cartilaginous tissue is beneficial in regard to its expected properties. The perfused group consisted of more cells and a better quality ECM (cohesion, GAG content) than in the control group. Medium perfusion encourages cellular proliferation by ensuring efficient transport of nutrient, gas, catabolites and metabolites. Therefore, the quality of the ECM was improved because of the mechanical stresses induced by the flow and the better uniformity of the pH. K.J. Gooch *et al.* [12] studied the effect of mixing on cartilage regeneration.

Cardiac tissue

Cardiac tissue is a specialized tissue and, in opposition to striated muscles such as biceps and quadriceps, cardiac muscle is not able to repair itself. This is due to the restricted regeneration potential of cardiomyocytes [22] and the lack of myoblasts in this tissue, a cell type with the capacity to divide and differentiate to form new muscular tissues [23].Another particularity of heart tissue is its continuous and independent contractions. Even if the heart is isolated, it will continue to beat at its intrinsic rhythm as long as it is supplied with glucose and oxygen [24].

Studies about the *in vitro* culture of cardiac tissue are more complex, less advanced and rarer than those about cartilage.

Table 2 : Bioreactor applications for cardiac tissue culture

	R.L.Carrier et. al. 1999[26]	R.L.Carrier et. al., 2002[27]	M.Radisic et. al., 2003[28]	C.Fink et. al., 2000[29]
Cell source	Cardiac myocytes of young rats and chicks	Cardiac myocytes of neonatal rats	Cardiac myocytes of neonatal rats	Cardiac myocytes of embryonic chicks and neonatal rats
Scaffold	PGA	PGA	Ultrafoam® collagen hemostat	Cell/collagen master mix
Type of the bioreactor	One fixed, one agitated and one rotating	Mixed flast (control) and perfused vessels	Perfused fixed cartridges	Fixed
Flow	Depending on the bioreactor	Mixing or direct perfusion	Direct perfusion through the constructs	None
Mechanical stresses	Depending on the bioreactor	Shear stresses induced by mixing and flow	Shear stresses induced by flow	Strain
Studied parameters	Influence of the cultivation vessel	Effects of perfusion rate and partial pressure of oxygen	Effects of seeding methods (direct perfusion)	Effects of a uridirectional chronic stretch

R.L. Carrier *et al.* [26] found that a rotating bioreactor improves cell quantity, distribution and metabolism compared to fixed or agitated culture vessels. First, gas and nutrient transport approaches more closely intracorporeal conditions than in the other vessels. Also, the free-falling state in the rotating bioreactor is possible thanks to a rotation of 11 to 12 rpm. It allows cell flotation with low shear stresses. C. Fink *et al.* [29] studied the effects of strain (elongation) instead of shear stresses. Instead being deteriorated, the cardiac tissues responded to a 20% strain by hypertrophy.

Blood vessels

Blood vessels have the role of connecting tissues and organs together. More specifically, arteries transport blood from the heart to the organs [31]. The intima is responsible for the hemocompatibility of the artery [31-33].Normal blood pressures are around 80 to 120 mmHg (11 to 16 kPa) for most arteries, except for the pulmonary artery that has a pressure between 10 and 25 mmHg (1.3 and 3.3 kPa). Stresses on the arteries are pulsatile because of the pulsation of blood flow [34]. Abnormal pressures are often the cause or the effect of arterial disease.

Table 3 : Bioreactor applications for vascular tissue culture

	C.B. Weinberg et. Al., 1986[35]	**L.E. Niklason et. al., 1999[36]**	**L. Heureux et. al., 1998[37]**	**S.P.Hoerstrup et. al., 2001[38]**	**S.C.Muluk et. al. 1998[39]**
Cell source	Bovine aortic endothelial cells, smooth muscle cells and adventitial fibroblasts	Bovine aortic smooth muscle cells and endothelial cells	Human umbilical venin smooth muscle cells and endothelial cells and human skin fibroblasts	Myofibroblasts and endothelial cells from ovine carotid artery	Intact human saphenous vein and pig internal jugular vein
Scaffold	Collagen and Dacron mesh	PGA	None	PGA coated by P4HB	n/a
Type of the bioreactor	Fixed	Fixed	Fixed	Fixed	Fixed
Flow	None	Perfusion in the lumen	Perfusion in the lumen	Pulsatile perfusion in the human	Perfusion in the human
Mechanical stresses	None	Pulsatile radial stress	Shear stresses and pressure induced by the flow	Shear stresses and pressure induced by the flow	Axial stretching and twisting
Studied parameters	Collagen concentration and culture time	Effects of dynamical mechanical stresses	No use of a scaffold	Effect of pulsatile flow	Combination of mechanical stresses
Burst strength obtained	< 100 mmHg	2000 mmHg	2000 mmHg	300 mmHg	n/a

In their pioneering work published in 1986, C.B. Weinberg *et al.* [35] obtained a well differentiated artery structure by doing separate annular castings supported by a Dacron mesh. The burst strength of the regenerated arteries was around 90 mmHg, which is less than normal systolic blood pressures. Mechanical stresses during the culture period are essential to vascular tissues. Niklason *et al.* [36] seeded a biodegradable scaffold with smooth muscle cells and cultivated it in a bioreactor under a pulsatile radial stress of 165 beats/minute. After eight weeks, the arteries had a thickness twice that of non pulsed controls and their burst strengths were greater than 2000 mmHg instead of 300 mmHg.

Heart valves

The four heart valves ensure the unidirectionality of the blood flow in the heart [34]. They consist of two or three very thin shutters which open and close in sequence with each heartbeat [42]. Their sealing allows for adequate operation pressures in the heart [24].The desirable characteristics of a heart valve grown *in vitro* would be a stable geometry with a potential for growth and regeneration within the patient [43].

Table 4 : Bioreactor applications for heart tissue culture

	G.C.Engelmayr Jr. et. al., 2003[44]	K.Schenke-Layland et. al., 2003[45]	S.P.Hoerstrup et. al., 2000[46]	A.Mol et. al., 2003[47]
Cell source	n/a	Endothelial cells and myofibroblasts from lamb carotid arteries	Endothelial cells and myofibroblasts from lamb carotid arteries	Human venous myofibroblasts
Scaffold	PGA and PGA/PLLA, both coated with P4HB	Decellularized porcine pulmonary valves	PGA coated with P4HB	PGA coated with P4HB
Type of the bioreactor	Fixed	Fixed	Fixed	Fixed
Flow	None	Pulsatile	Pulsed perfusion through the valve	None
Mechanical stresses	Cyclic flexural simulation	Dynamical stresses induced by the flow	Dynamical stresses induced by the flow	Increasing cyclic strain
Studied parameters	Effects of cyclic flexure on scaffolds	Repopulation potential of decellularized valves	Effects of pulsed flow vs constant flow	Effects of cyclic strain

G.C. Engelmayr Jr. *et al.* [44] studied stiffness and fatigue behaviour of a biodegradable polymeric scaffold with a bioreactor applying cyclic flexure. Every scaffold showed a decrease of mechanical properties over time in the culture medium, which can be explained by the polymer degradation. Other potential scaffolds are decellularized heart valves. K. Schenke-Layland *et al.* [45] evaluated their repopulation potential under a pulsatile flow. This mechanical improvement was underlined by S.P. Hoerstrup *et al.* [46] who studied the influence of pulsatile blood flow on the properties of heart valves. In fact, after two weeks, the mechanical and structural properties stopped improving, remained constant for a few days, than started decreasing. But when the pulsed valves were implanted *in vivo* in replacement of the pulmonary valve, they operated functionally for 20 weeks and their properties improved during this period.

CONCLUSION

Since the design of the first bioreactor, tissue engineering has improved immensely. The optimization of oxygen and nutrient supply, temperature, pH, transport of catabolites and metabolites and mechanical stresses stimulates the formation of the extracellular matrix and allows for cohesion between cells. It is now possible to grow tissues with specific geometries. By maintaining pseudo-physiological culture conditions specific to cultivated cells, bioreactors allow for the culture of well differentiated three-dimensional tissues with specific mechanical properties.From this review, it appears clearly that rotating bioreactors are used more often for the culture of fragile tissues, while non-rotating bioreactors are more adapted to the culture of tissues with a complex geometry that are normally submitted to higher mechanical constraints in the body.

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