Bioreactors for 3D tissue engineering

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Abstract

The bioreactors for 3D tissues systems could be an advantageous alternative in terms of low contamination risk, easiness of handling and scaling-up. These equipments are specifically designed in order to provide a better control of the process as well as a safe and reproducible production of tissue construct. They can offer the technical means to perform controlled studies aimed at understanding the specific biological, chemical or physical effects.

An important problem that has to be solved is the bioreactors scaling-up from laboratory scale to industrial level. This transposition requires the bioreactors to be specialized and adapted for a standardized production process.

Keywords: bioreactor, cell, tissue, bioengineering, transfer process, scaling-up.

Introduction

The loss and damage of the tissues cause serious health problems. In the US, almost one-half of the costs for medical treatment are spent on implant devices annually. Worldwide, 350 billion USD are spent for substitute of organs [1]. The substitution of tissues (such as bone or cartilage) or joints with allograft materials includes the risk of infections by viruses (such as HIV, hepatitis C) or a graft rejection. New therapy concepts for practical medical applications are required. To this end, tissue engineered substitutes generated *in vitro* could open new strategies for the restoration of the damaged tissues. The generation of 3D (three-dimensional) tissue substitutes in vitro requires not only a biological model, but also the further development of new culture strategies including bioreactor concepts.

Tissue engineering has been defined as the application of principles and methods of engineering and life sciences for the development of biological substitutes for restoring, maintaining or improving the natural tissue function [2]. In one of the most typical approaches, 3D tissue structures are generated by the association of cells (autologous or allogeneic) with porous scaffolds, which provide the template for tissue development, being degraded or resorbed at defined rates.

In vitro culture of 3D cell-scaffold, carried out under conditions that efficiently support cell nutrition, and possibly combined with the application of mechanical forces, is an important step towards the development of functional grafts for the treatment of lost or damaged body parts (functional tissue engineering) [3-6].

Tissue engineering's goal is to find a new solution to the current problem of organ shortage and biomaterial failures. The ultimate goal of the 3D tissues engineering *in vitro* is not always to generate grafts, but to obtain non-implantable structures that can be used as external organ support devices when a compatible donor is not readily available. In the future, it could offer a more accurate treatment of tissue and organ diseases by reducing graft rejection and, therefore, increasing the life quality of all patients. For example, 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 years life time.

Bioreactors are generally defined as devices in which biological and/or biochemical processes are developed under closely monitored and tightly controlled environmental and operating conditions (pH, temperature, pressure, nutrient supply and waste removal). The high degree of reproducibility, control and automation introduced by bioreactors for specific experimental bioprocesses has been the key for their transposition to large-scale applications.

For tissue engineering, the bioreactors have already improved the processing and the final results of skin and cartilage healing, but in the present only two lab-grown products are commercially available [2,7].

Some *in vivo* studies are currently in progress to test on human subjects bioengineered corneas, bones, urethras and pancreatic cells. Most of the regenerated tissues are actually tested *in vivo* on animals (blood vessels, muscles, heart valves, tracheas, ears, livers, kidneys, pancreas, bladders, intestines, salivary glands).

Three major strategies are considered to control the regeneration of 3D tissues, as follows [2,8-10]:

1. The implantation of an acellular matrix to encourage the formation of a new tissue. *In vivo* studies have shown that it is difficult to encourage cell migration into the scaffold, resulting in poor tissue formation.

2. The encouragement of the self assembly of cells. Although much effort has been consumed, only several studies have been reported, and no functional tissue has been yet regenerated with this method. The reasons are the lack of cohesion between cells, dedifferentiation and an inadequate tissue shape. In fact, external guides and signals, such as mechanical stress and strain, are essential to make cells to grow into the functional 3D implantable organs, and these guides are difficult to be applied to the non-supported cells.

3. The use of a scaffold offers the possibility to tailor the initial properties of the construct and offers an easier application of mechanical conditions on the young and fragile construct at the beginning of the regeneration. This approach consists on seeding one or more kinds of cells on a scaffold (natural or polymeric) configured to the appropriate shape. The construct is then inserted into the culture media, in presence of growth factors, and transferred to specific intracorporeal conditions in a bioreactor, for allowing the cells to colonize its. Eventually, if these scaffolds are biodegradable, they will disappear, thus leading to a highly coherent, totally biological and functional tissue. The resulted regenerated tissues have been already successfully implanted *in vivo*.

This paper is an overview on the implication of bioreactors in key-processes for the *ex vivo* engineering of 3D tissues based on cells and scaffolds, including cell seeding of porous scaffolds, nutrition of cells in the resulting constructs, and mechanical stimulation of the developing tissues. The proper bioreactors allow not only to rigorously controlling the 3D tissues production, but also to reducing the manufacturing costs and to facilitating the tissues broad clinical use.

Specific bioreactors and conditions for 3D tissue engineering

In vitro, cultivated mammalian cells require physical (temperature, partial pressure of oxygen, partial pressure of carbon dioxide, pH, shear force) and biochemical conditions of their micro- and macro-environment. These requirements are of the major interest in all scientific activities on cells biology and technology. All investigations try to find homogeneous and constant conditions for micro- and macro-environment for the entire cells population.

Differences between tissues suggest that the bioreactor design considerations and operating conditions can be different when dealing with a specific tissue. However, every type of tissue has to benefit by controlled shear stresses, optimal nutrient availability and wastes elimination.

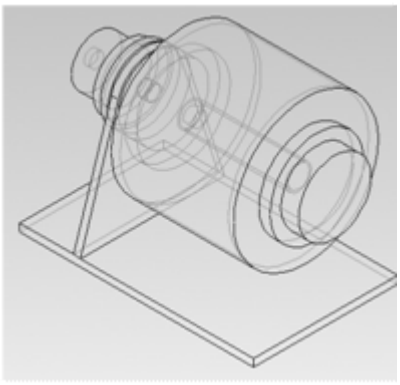
Mammalian tissues are the most difficult to be grow tissues *in vitro* under bioreactor conditions, because of their important nutrient needs, their sensitivity to the nitrogenated wastes and their high sensibility to shear stresses [11]. The real requirements in nutrients, waste elimination, and shear stress also vary widely for the different types of tissues. For example, the connective tissues, such as ligament and cartilage, can support *in vivo* stresses of up to 20 MPa. Obviously, these large stresses are not present in the majority of tissues in other parts of the human body. The density of cells in such connective tissues is typically much lower than the density of cells found in liver and kidney tissues, for example. Contrarily to other tissues, connective tissue cells grow in a hydrated, negatively charged matrix and the tissues formed are practically avascular (no blood vessels) and aneural (without nerves).

Specific bioreactors are essential for the researches in tissue engineering field. In the body, cells are always stimulated by mechanical, electrical and chemical signals, these influencing their behavior. If these signals are inadequate or absent, cells dedifferentiate becomes disorganized and could lead to the cells death. In fact, biological tissues adapt their structure and composition to surrounding specific and functional demands. By putting cells alone or only in contact with materials in culture medium is not enough to obtain a functional tissue. Therefore, bioreactors are particularly crucial for the regeneration of complex 3D tissues.

Current bioreactors can be divided into two main classes: rotating and non-rotating ones [2]. **The rotating bioreactors** have a culture chamber permanently in rotation, which promotes the uniform growth of the tissues (Figure 1) [12].

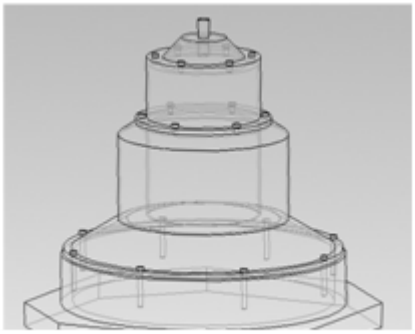
Figure 1. Rotating bioreactor with the culture chamber in permanent rotation.

Also, the rotation speed can be adjusted to produce a free-falling state. Thus, it protects the fragile tissues, because it decreases shear stresses and avoids the contact between cells and the walls of the bioreactor.



The non-rotating bioreactors have a motionless culture chamber which allows for the culture of complex tissues (Figure 2) [13]. 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.

Figure 2. Non-rotating bioreactor.



Both for nutrient needs and waste elimination, mass transfer to and from tissues is a critical issue in any reactor design.

In vivo cultures, the cells benefit from the proximity of blood capillaries for their mass-transfer requirements. In the most tissues, the cells are no more than 100 μm from these capillaries. Also, the small diameter of capillaries (between 6 and 8 μm) ensures a residence time long enough in tissues to allow the radial diffusion of chemical species [14].

In the bioreactor environment, increasing the tissues size, the cell proliferation increases the mass transfer requirements, thus limiting the final size of the tissues grown. For example, for hepatocyte culture, it has been shown that even a bioreactor is designed to offer the proper diffusion conditions for the oxygen mass transfer, the cells must be within 150–200 μm of an oxygen source to survive and proliferate.

Oxygen is one of the most important nutrients for cells, having the major role in all aerobic metabolic cycles. However, it is often the limiting nutrient in successful tissue growth *in vitro*. The reason for this arises from the difficulty of bringing sufficient amounts of oxygen to the surface of the cells mainly because of the poor solubility of oxygen in culture media. Indeed, hypo- and hyperoxic stresses have been implicated as causes of programmed cell death or apoptosis, which appears to be the main mode of cell death in many cells cultures. Low oxygen tension (40 mmHg) and low pH (6.7) have been associated with anaerobic cartilage cell metabolism, while higher oxygen tension (80mmHg) and higher pH (7) were associated with more aerobic cell metabolism [15].

Shear stress is another particularly sensitive issue in mammalian cell cultures. Many cell types respond to shear stress. For example, it was found that shear stress affected the endothelial cells proliferation and that they are oriented according with the flow direction. Moreover, cells existing in aggregates are exposed to higher shear stresses than the single cells, due to their large particle diameter. It is widely accepted that the shear stress has a determinant impact on tissue function and viability [16].

Different values are reported for the maximal sustainable shear stress of different types of cells. For cell suspensions, the rheological experiments were demonstrated that for a wide variety of mammalian cell lines the shears of 1 dyn/cm^2 were damaged the cells, the shears of 0.1 dyn/cm^2 were ideal, and the shears of 0.01 dyn/cm^2 were insufficient to promote the growth. For comparison, the heart valve leaflets must sustain temporary in vivo stresses of up to 22 dyn/cm^2 during systole; smooth muscle cells were studied under well-defined flow conditions between 5 and 25 dyn/cm^2, at the higher stress values, the growth being significantly reduced [16,17].

Bioreactors have to allow optimal mass transfer rates of molecules such as oxygen, carbon dioxide and urea, to and from any type of *in vitro*-grown tissue. In addition, they must restrain the shear stresses which can act on the fragile clustered cells. Many bioreactors used in mammalian tissue engineering are designed to produce 2D constructs, as the skin. For example, a patient with a large burned skin surface will require between 60 and 100 cultivated epidermal auto-grafts (50 cm^2 of skin).

For the above examples, the first-generation of “bioreactors” were the simple Petri dishes. The use of **simple static flasks** and **magnetically stirred flasks** into which the seeded scaffolds can be suspended is also reported as first-generation bioreactors (Figure 3) [18].

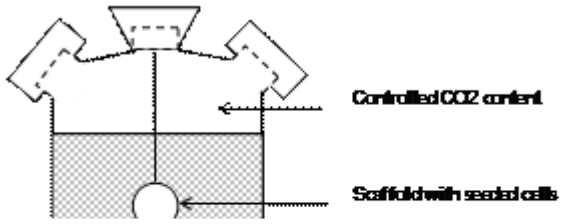


Figure 3. Bioreactor of stirred flask type.

A spherical tea ball is sometimes used to maintain suspended the tissue construct. Stirred-flask bioreactor induces mixing of oxygen and nutrients within the culture medium and reduces the boundary layer at the construct surface. For the stirred bioreactor system, the mixing speed of 50 - 80 rpm is typical. Cartilage constructs were grown in these reactors with reasonable results. When the constructs are fixed within the reactor, turbulence can be induced around the constructs. Turbulence has a beneficial effect on mass transfer. But there exists a delicate balance between the increasing of mass transfer and the modulating of shear stresses to an optimal (needed) level.

In the case of these bioreactors, significant higher efficiencies and uniformities have been obtained when poly-glycolic acid on in-woven meshes form was used for seeding in stirred-flask bioreactors. By mixing the dilute cell suspension around the stationary scaffolds suspended from the mouth of the flask allow the cells to be transported into the scaffolds by convection

Spinner-flask bioreactors have been used for the seeding of cells into 3D scaffolds and for subsequent culture of the constructs [19,20]. During seeding, cells are transported to and into the scaffold by convection. During culture, medium stirring enhances external mass-transfer but also generates turbulent eddies, which could be detrimental for the development of the tissue (Figure 4).

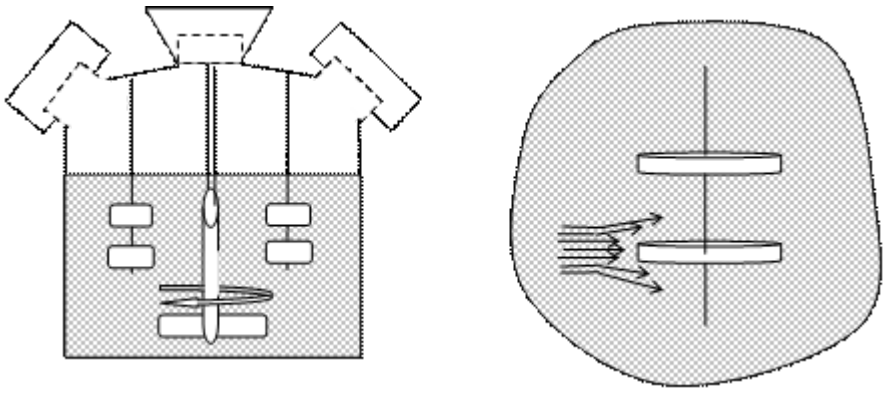


Figure 4. Flow streams inside the spinner-flask bioreactor.

Rotating-wall bioreactors (STLV, slow turning lateral vessel) provide a dynamic culture environment to the constructs, with low shear stresses and high mass-transfer rates (Figure 5) [19]. The vessel walls are rotated at a rate that enables the drag force (Fd), centrifugal force (Fc) and net gravitational force (Fg) on the construct to be balanced. Thus, the construct remains in a state of free-fall through the culture medium.

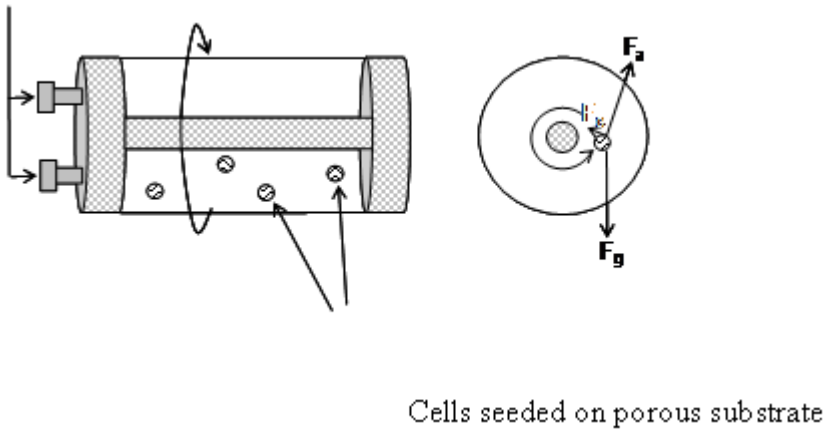


Figure 5. Rotating-wall bioreactor.

A bioreactor very similar to the STLV has also been developed at NASA's Johnson Space [19] Center. The aspect of the vessel is close to the STLV, but its design lowers the speed necessary to maintain the constructs stationary (typical speeds of 12 - 15 rpm) and enhances the gas exchange, by means of the presence of a 78.5 cm^2 gas exchange membrane at one end of the vessel, the area for exchange being more than twice as large than the one in the STLV (Figure 6). The operation principle of this reactor is identically to that of the STLV. Both reactors have been used to grow a large variety of tissue types such as cartilage and heart tissues.

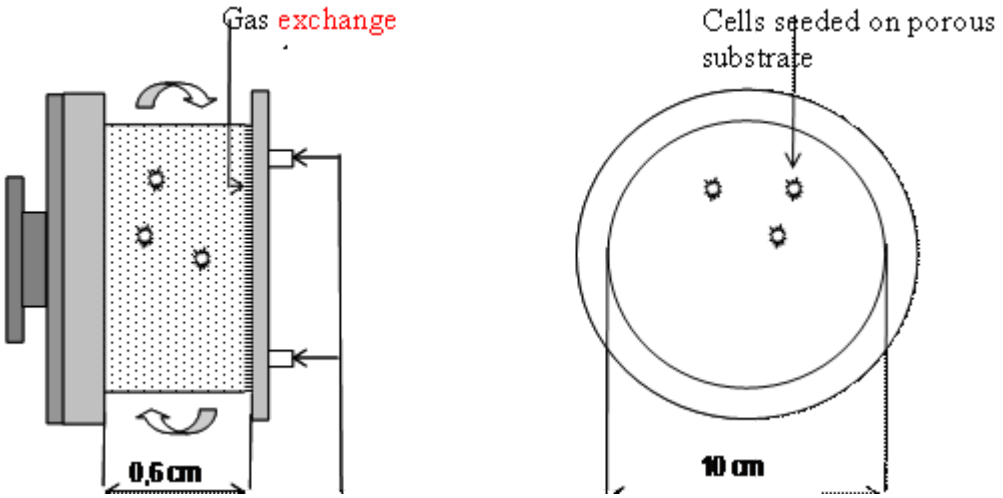


Figure 6. Rotating-wall bioreactor with gas exchange membrane.

Hollow fiber bioreactors, mostly used for the production of proteins made by mammalian cells, have also been proposed for the in vitro production of 3D mammalian tissues (Figure 7) [2,21,22].

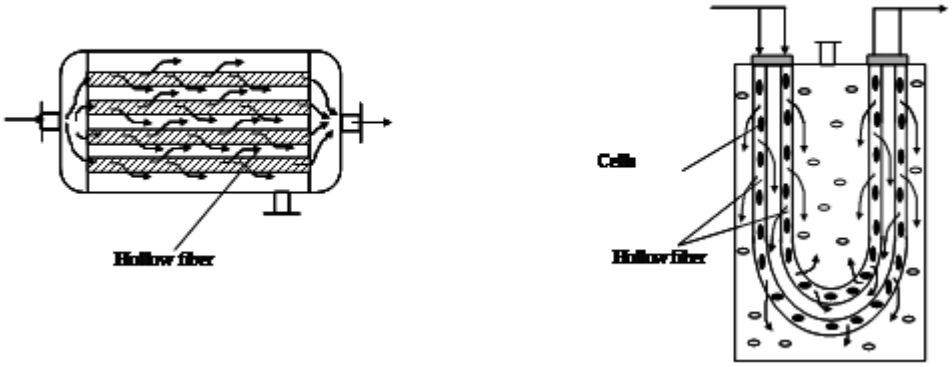


Figure 7. Hollow fiber bioreactors.

A hollow fiber reactor consists of a closed vessel filled with medium and mammalian cells. A bundle of semi-permeable hollow fibers is inserted inside the media (Figure 8). The hollow fiber provides the nutrients to the cells and eliminates their wastes, thus simulating the *in vivo* blood vessels.

cultured cells

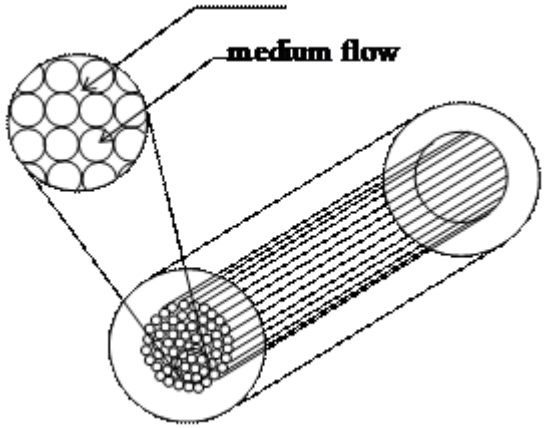


Figure 8. Hollow fiber.

The main advantage of this bioreactor is that it allows to providing nutrients to the center of the growing tissues (Figure 9).

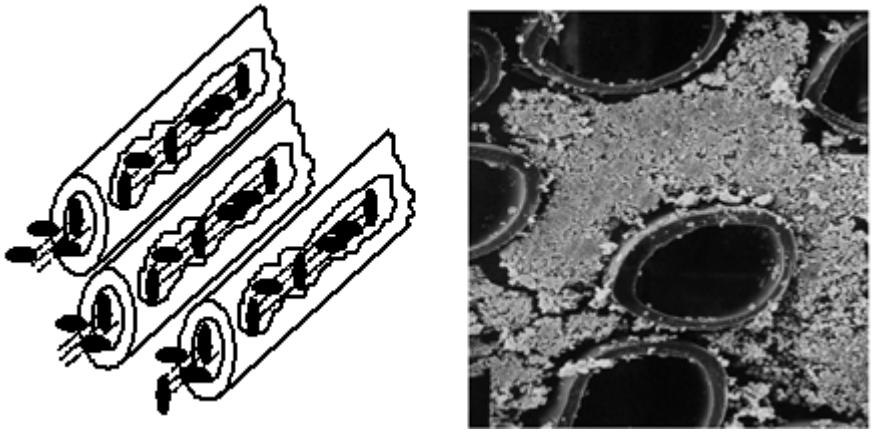


Figure 9. Hollow-fiber cartridge in cross-section and culture of lymphocytes in hollow fiber bioreactor.

An elevated level of intracapillary medium re-circulation can drive a secondary flow in the extracapillary space (where cells are cultivated). This flow results from a portion of the intracapillary medium entering the extracapillary space near the entrance of the reactor, due to the natural hydraulic permeability of the fibers and to

the pressure distribution within the reactor. The medium flows on the length of the bioreactor extracapillary space and re-enters the intracapillary flow near the end of the reactor.

Various other type of bioreactors have been investigated for mammalian cells cultures. Among these are **the airlift bioreactors**, described in Figure 10, in which air is fed at the bottom of a vessel {19,22]. The aeration respectively the air bubbles formation and circulation, can affect the tissues integrity, therefore a draft tube is required to prevent the direct contact between the tissues and the upcoming air. In these bioreactors, the micro-carrier beads of glass can be used for supporting the cells.

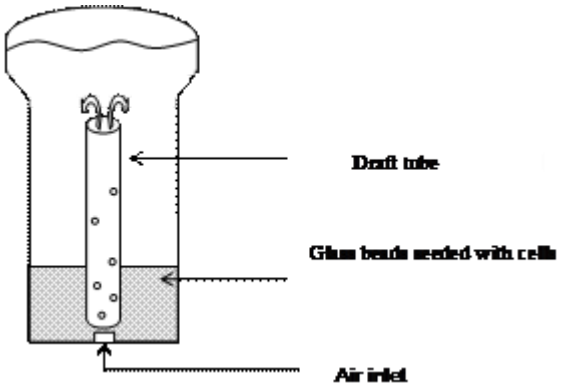


Figure 10. Airlift bioreactor.

Miniature stirred bioreactors (MSBR) can reduce the work intensity and materials cost of the vast number of cell cultivations necessary in bioprocess development (the working volume is generally of 10 to 20 ml). These bioreactors design is based on the conventional stirred bioreactors, they being developed as an alternative to the shaken systems for early-stage process development and cells characterization [23,24]. Typically these devices are closely modeled on lab-scale bioreactors and thus permit greater potential for monitoring and control than other miniature bioreactor platforms (Figures 11 and 12).

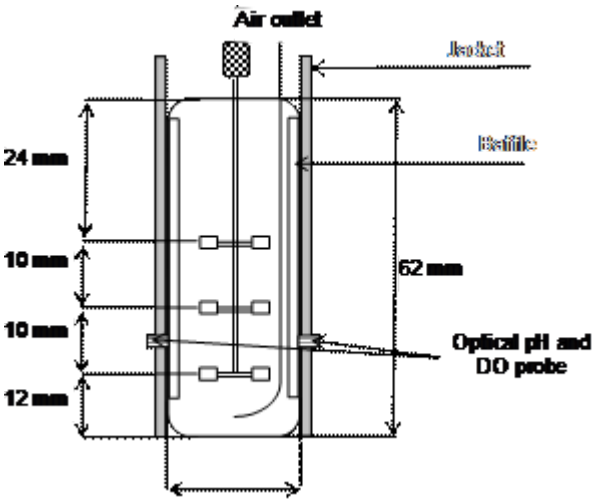
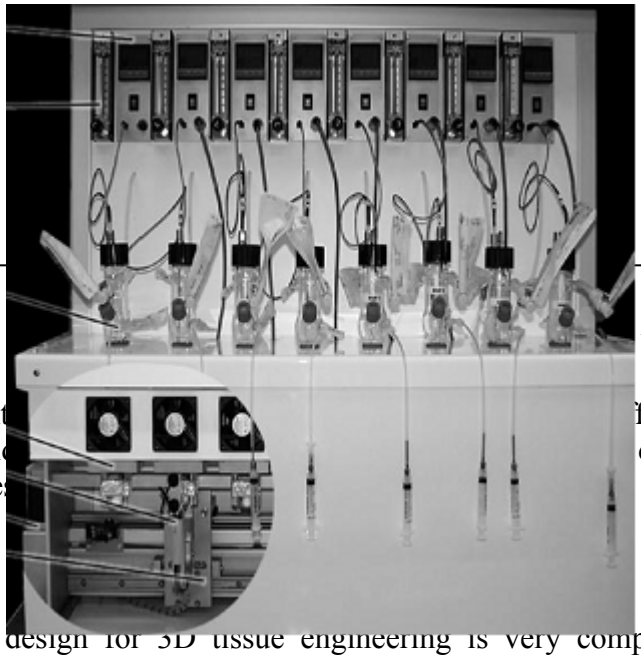


Figure 11. Miniature stirred bioreactor.



battery.

This equipment ficiency and oxygen transfer capability. The agitation rate coul damage of the shear-sensitive mycelia organisms through exce

Conclusions

The bioreactor design for 3D tissue engineering is very complex and still at an early stage of its evolution. The bioreactors for the generation of 3D tissue constructs are specially designed by taking into account different demands of the cells during the cultivation cycle, for providing a better process control and a safe and reproducible production of tissue construct. Furthermore, they can offer the technical means to perform controlled studies aimed to understanding the specific biological, chemical or physical effects. These bioreactors can also be used to study the effects of the shear forces and/or hydrostatic pressure on the tissues growth.

For the future clinical applications, the bioreactors for 3D tissues systems could be an advantageous alternative in terms of low contamination risk, easiness of handling and scaling-up. Therefore, the very intimate collaboration between engineers and biologists will lead to an accurate fundamental knowledge of the complex aspects that can have a major impact on tissue formation in these bioreactors. On the other hand, the bioreactors scaling-up from the laboratory scale to the industrial one requires the specialized bioreactors to be adapted and adequate for a standardized production process. These advances will offer the assurance that the tissue engineering will respect the expectations for revolutionizing the medical care.

References

1. VASALA A., PANULA J, BOLLÓK M., ILLMANN L., HÄLSIG C., NEUBAUER P., *Microb. Cell Fact.*, **5**, 8 (2006).
2. CAŞCAVAL D., GALACTION A.I. (eds.), *Biotechnologia intre stiinta si arta*, Venus, Iasi, 2007.
3. JU L-K., ARMIGER W.B., *Biotechnol. Prog.*, **7**, 323 (1991).
4. FREED L.E., LANGER R., MARTIN I., PELLIS N.R., VUNJAK-NOVAKOVIC G., *Proc. Natl. Acad. Sci. USA*, **94**,13885 (1997).
5. PALOMARES L.A., GONZALEZ M., RAMIREZ O.T., *Enz. Microb. Technol.*, **26**, 324 (2000).
6. COLIGAN J.E., KRUISBEEK A.M., MARGULIES D.H., SHEVACH E.M., STROBER W. (eds.), *Curent Protocols in Immunolog*, John Wiley & Sons, New York, 2003.
7. SEN A., KALLOS M.S., BEHIE L.A., *Develop. Brain Res.*, **134**, 103 (2002).
8. ALTMAN G.H., LU H.H., HORAN R.L., CALABRO T., RYDER D., KAPLAN D.L., STARK P., MARTIN I., RICHMOND J.C., VUNJAK-NOVAKOVIC G., *J. Biomech Eng.*, **124**, 742 (2002).
9. YU X., BOTCHWEY E.A., LEVINE E.M., POLLACK S.R., LAURENCIN C.T., *Proc. Natl. Acad. Sci. USA*, **101**, 11203 (2004).
10. BETTS J.I., BAGANZ F., *Microb. Cell Fact.*, **5**, 21 (2006).
11. PORTNER R., NAGEL-HEYER S., GOEPFERT C., ADAMIETZ P., MEENEN N.M., *J. Biosci. Bioengin.*, **100**, 235 (2005).
12. BILODEAU K., COUET F., BOCCAFOSCHI F., MANTOVANI D., *Artificial Organs*, **29**, 11 (2005).
13. MARTIN Y., WENDT D., HEBERER M., *Trends Biotechnol.*, **22**, 2 (2004).
14. MARTIN Y., VERMETTE P., *Biomaterials*, **26**, 7481 (2005).

15. PARK J., BERTHIAUME F., TONER M., YARMUSH M.L., TILLES A.W., *Biotechnol. Bioeng.*, **90**, 5 (2005).
16. LEE C., GRAD S., WIMMER M., ALINI M., *Topics Tissue Eng.*, **2**, 1 (2005).
17. CHEN R., CURRAN S.J., CURRAN J.M., HUNT J.A., *Biomaterials*, **27**, 4453 (2006).
18. TIERNEY C.M., JAASMA M.J., O'BRIEN F.J., *Proc. Conf. Bioengineering In Ireland*, Galway, 2006.
19. WEUSTER-BOTZ D., PUSKEILER R., KUSTERER A., KAUFMANN K., JOHN G.T., ARNOLD M., *Bioproc. Biosys. Eng.*, **28**, 121 (2005).
20. FRACHON E., BONDET V., MUNIER-LEHMANN H., BELLALOU J., *Appl. Environ. Microb.*, **72**, 5225 (2006).
21. SUN T., NORTON D., HAYCOCK J.W, RYAN A.J, MACNEIL S., *Tissue Eng*., **11**, 11 (2005).
22. CAŞCAVAL D., ONISCU C., GALACTION A.I., *Inginerie biochimica si biotehnologie. 2.Bioreactoare*, InterGlobal, Iasi, 2002.
23. LAMPING S.R., *Design of a miniature bioreactor and its use in fermentation cell culture*, Ph.D. Thesis, University of London, 2004.
24. LAMPING S.R., ZHANG H., ALLEN B., AYAZI SHAMLOU P., *Chem. Eng . Sci.*, **58**, 747 (2003).