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# Design of a Mini-Bioreactor System with Stimulating Devices for Cell Culture in Tissue Engineering (I)


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## 1. Introduction

This study has investigated the design of a mini-bioreactor system with bio-stimuli for culturing cells and tissue in tissue engineering. The present study in this paper was to review the several bioreactor systems related to cells culture in tissue engineering especially and to design a novel bioreactor system with bio-stimuli consisting of cell culture cartridge and stimulating devices such as ultrasound and electrical stimuli. A bioreactor system is the general term applied to a closed culture environment, that is usually mixed, that enables control of one or more environmental or operating variables that affect biological processes (Asenjo and Merchuk 1995). In biotechnology, the use of a bioreactors is to grow mammalian cells to high cell densities in order to produce a metabolic product and enzyme. In these applications of several bioreactor systems, the most common bioreactors applied to the batch stirred tank reactor as well as continuous stirred tank reactors, packed beds, and membrane bioreactors (Asenjo and Merchuk 1995).

Martin et al (2004) reported that bioreactors can be defined as devices in which biological or biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions (e.g., pH, temperature, pressure, nutrient supply, and waste removal). Especially, bioreactors were well established for the cultivation of mammalians cells (Ratcliffe et al., 2002). Important aspects for bioreactor design with respect to tissue engineering are used for different purposes such as cell proliferation on a small scale and on a large scale, generation of 3D tissue constructs from isolated and proliferated cells in vitro and direct organ support devices (Shachar et al., 2003).

In tissue engineering, bioreactors are used to give reproducible cell and tissue proliferation. Therefore, bioreactors are providing necessary mixing, mass transfer and a controlled environment for the organism to produce a desired biological product (Blanch & Clark 1996, Riet & Tramper 1991, Shuler & Kargi 2002).


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Many publications have been issued on the subject with a number of reactor configurations. However, few subjects only reported the growth of functional and reasonably cells and tissues (Martin et al., 2005). Especially, Mini-bioreactor systems have the advantages with co-culture and automation in the system design as well as cost reduction for medium constituents when cultivating cells and tissue.

The present study in this paper was to review the reported bioreactor systems based on the cell culture in tissue engineering, and then to suggest a novel bioreactor system with bio-stimuli for cell culture based on cells and scaffolds, including physically stimulating devices such as ultrasound and electrical stimuli.

## 2. Bioreactor Systems in Tissue Engineering

An overview of the culture systems and bioreactors used for the tissue engineering is illustrated schematically in Fig. 1 (Ralf Portner et al., 2005). Cell maintenance and proliferation is typically performed in the monolayer culture of adherent cells (T-flasks, petri dishes, multi well plates). These culture methods enable sterile handling procedures and are easy to use, disposable and low cost. However, controlling environmental parameters is generally impossible (Morgan et al., 1999).

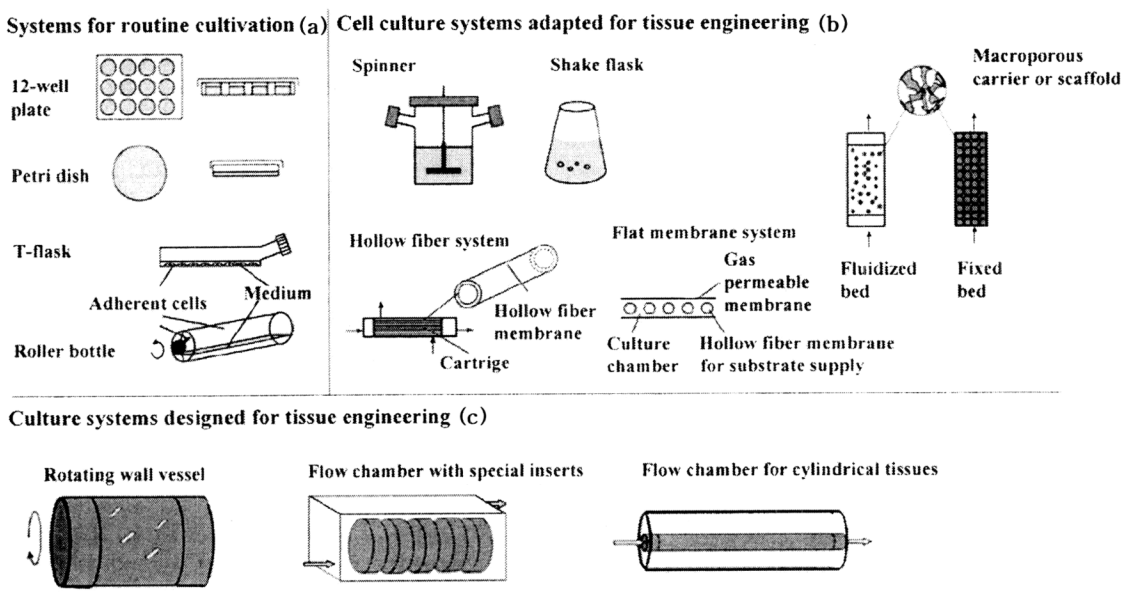



Fig. 1 An overview of cell culture systems used in tissue engineering (Ralf Portner et al., 2005). (a) Systems used for routine cultivation within an incubator (b) Culture systems developed mainly for cultivation of mammalian cells (c) Culture systems designed for tissue engineering mimicking the special demands of a 3D tissue.

In recent studies, small well-mixed bioreactors (e.g., shake flasks, stirred vessels, and spinner) have been suggested for cell proliferation, in which the cells are grown on microcarriers (Malda et al., 2004). Additionally, membrane based systems such as hollow fiber reactors or fluidized and fixed bed reactors were adapted for cultivation of tissue cells in three-dimensional structures. Culture systems of rotating wall vessel type with a low shear stress and a high mass transfer rate have been introduced (Freed et al., 1997). These types of bioreactor are very efficient for the long term cultivation of mammalian cells.

Bioreactors allow for different process strategies including the batch, fed-batch or continuous cultivation type. In particular, continuous perfusion bioreactor enables cultivation to be carried out under constant and controlled environmental conditions (Sittinger et al., 1997 ; Nehring et al., 1999). Perfusion culture of mammalian cells features high cell density, high volumetric productivity, and fast medium exchange, which are all advantages over traditional batch and fed-batch cultivation modes (Mercille et al., 2000; Voisard et al., 2003). Especially, the rate of medium replacement (perfusion rate) is one of the important operating parameters in a perfusion system (de la Broise et al., 1991).

## 3. Design Factors in Bioreactor System

In this study, requirements for bioreactor systems design in tissue engineering were investigated. Freed et al. (1997) reported that bioreactors should perform, at least one of, the following. (i) create an environment that enables cells to proliferate and differentiate as in vivo establish spatially uniform cell distributions on 3D scaffolds, (ii) maintain desired concentrations of nutrients, (iii) provide efficient mass transfer to tissue, and (iv) expose tissues to physical stimuli. The bioreactor systems should also be designed to operate under sterile conditions and preventing entry of infecting microorganisms (Sinclair and Ashley, 1995).

As a factor in bioreactor system design, scaffolds also need to be seeded with cells because cells are allowed to attach on the scaffold, and then seeded scaffold is inserted into the bioreactor. Dynamic seeding method was reported to higher attachment efficiencies and more uniform cell distribution on the scaffold for higher quality tissue rather than static seeding (Freed et al., 1997; Martin et al., 2004). Appropriate design of the scaffold and bioreactor should be undertaken in order to maximize the mass transfer processes. Critical issues of all bioreactor concepts involve mass transfer problems such as oxygen, nutrient supply, and removal of toxic metabolities (Griffith et al., 2002). Oxygen supply is particularly critical, as cell layers of 100-200 ㎛ can be supplied by diffusion (Fassnacht et al., 1999). Mass transfer, the diffusion of nutrients to cells through biomaterials, is a major limitation in tissue engineering (Yang et al., 2001).

Various designs have been aimed at increasing mass transfer rates in the bioreactor system. In addition to the these factors as stated above, stimuli of cells in the bioreactor system design is critically important in this study. Various studies showed mechanical compression, hydrodynamic pressure, and fluid flow. These reported that biomechanical stimulation can have a positive impact on tissue formation (Butler et al., 2000). Fluid flow affects the cell shape and cell function (Freed et al., 1997). Specific bioreactors are being developed to induce stimuli as cultures progress (Dennis and Kosnik, 2000).

Cells are subjected to and stimulated by mechanical, electrical, and chemical signals and gradients that influence their behavior, shape, properties, and the proliferation rate. However, cells cannot proliferate and form organized tissues if these stimuli are inappropriate. they were therefore dedifferentiated and become disorganized, which can eventually lead to cell death (Salgado et al., 2004).

Various physical and physicochemical factors influence the development, growth, and metabolic function of cells (Mow et al., 1992). These stimuli are to promote and increase cell reproducibility and productivity. Therefore, these stimuli in tissue engineering have to be required as main factor of bioreactor system design.

## 4. Design of Mini-Bioreator System with Stimulating Devices

4.1 Novel design of mini-bioreactor system with cartridge

Schematic layout of the bioreactor systems design with a cell culture cartridge for culturing cells and tissue in tissue engineering was described as shown in Fig. 2.

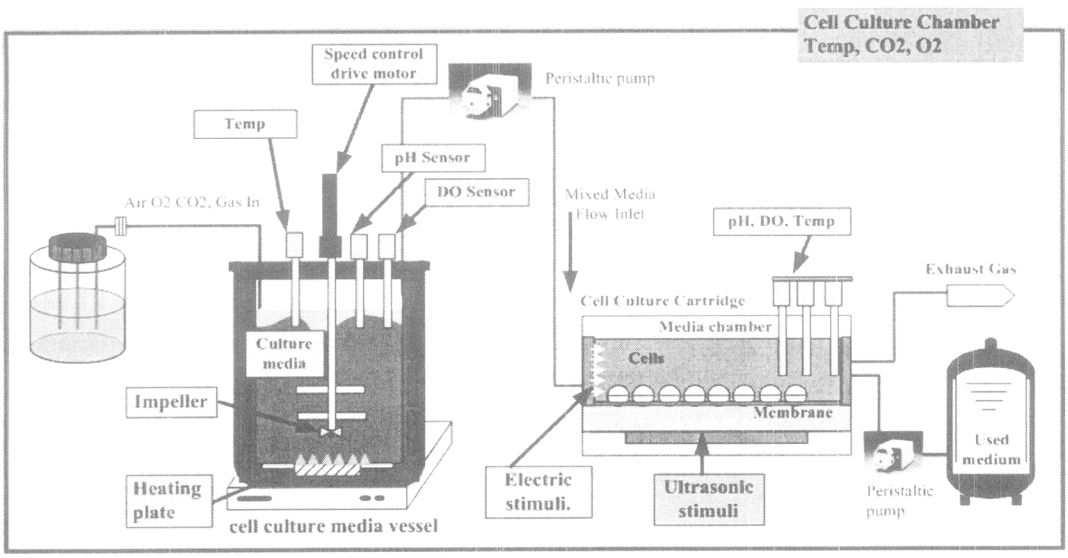
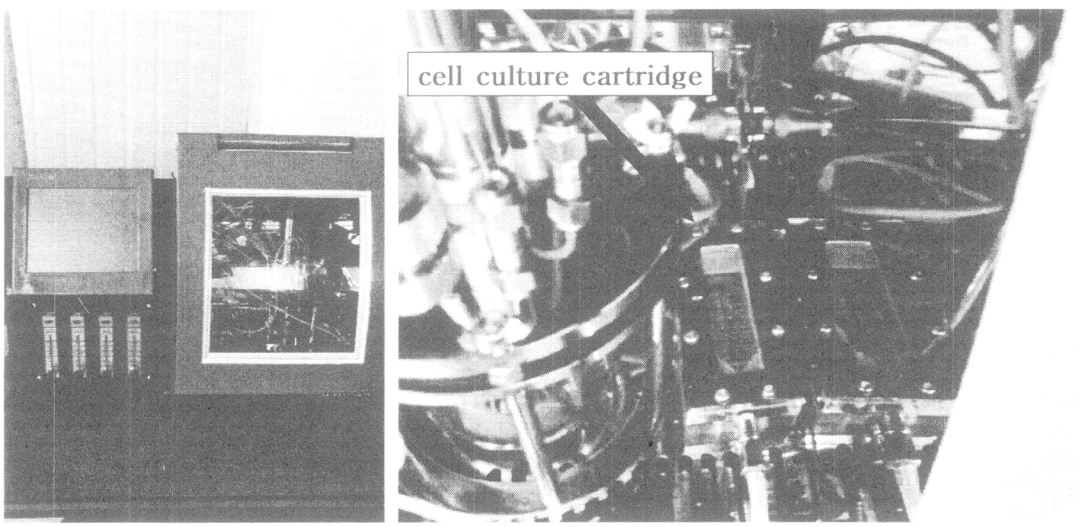



Fig. 2 Schematic layout of a mini-bioreactor system to be developed consisting of cell culture cartridge, cell culture media vessel, control peristaltic pump, and stimulating devices.

The essential conditions that should be taken into consideration in this system design are adhesive cells, as well as factors for mammalian cells such as media, nutrient, pH, temperature, dissolved oxygen, $CO_2$, $O_2$, especially, cell culture cartridge with stimuli technologies in the chamber. This system composed of cell culture cartridge, cell culture media vessel, control peristaltic pumps, vessels for fresh and spent medium, stimulating devices such as ultrasound and electrical stimuli. Ultrasound and electrical stimuli have principal role to cell differentiation by the effect permeability of cell membrane as well as uptake of nutrients and secretion of products by the increasing permeability of cell membrane in this study.

4.2 The prototype of mini-bioreactor system

We are presently developing a mini-bioreactor system prototype for cell culture in cooperation with BIOTRON, Inc. The first prototype of a bioreactor system fabricated in the BIOTRON, Inc was shown in Fig. 3. Cell culture media vessel and cell culture cartridges have a separate independent control device, which can perform a precise environmental control in chamber. A cell culture cartridge is mainly designed for adherent cells, which is constructed from polycarbonate. As there are 4 gas supplying ports for $O_2$, $CO_2$, $N_2$, and air inside the chamber, it can directly infuse gas quantities into the cell culture cartridge.




(a) (b)

Fig. 3 A mini-bioreactor system prototype to be developed in this study. (a) View of a mini-bioreactor system, TotiCell$^{TM}$ (BIOTRON, Inc); (b) The photo of a cell culture cartridge installed in the chamber of bioreactor system

## 5. Conclusions

This study has investigated the design of a mini-bioreactor system with stimulating

devices for culturing cells and tissue in tissue engineering. Bioreactor systems play an important role in tissue engineering because they enable reproducible and controlled changes in specific environmental factors. The present study in this paper was to review the several bioreactor systems related to cells culture in tissue engineering, and to design the novel mini-bioreactor system consisting of cell culture cartridge and stimulating devices such as ultrasound and electrical stimuli. A novel bioreactor system prototype for cell culture in tissue engineering in this study will be developed as a mini-bioreactor system with various culture cartridges and cell stimulating devices.

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