NOVEL BIOREACTORS FOR OSTEOCHONDRAL TISSUE ENGINEERING

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ABSTRACT

Tissue engineering is a new approach for articular cartilage repair, but the integration of engineered cartilage into the host subchondral bone is a major problem. One approach for solving this problem is to make osteochondral tissue engineering instead of cartilage tissue engineering only. The aim of the present paper was to describe two patented newly designed bioreactors for tissue-engineered osteochondral graft. The first bioreactor is double-chamber bioreactor, which is made of glass and is completely transparent. The whole system consists of one chamber for culture of chondrocytes and the other chamber for osteoblast culture. One important role for this bioreactor is to co-culture osteoblasts and chondrocytes at the same time in a biphasic scaffold. The bioreactor is modified from spinner flasks. The stirring of the magnetic bars provides medium mixing and mechanical stimulations for the cells. The second bioreactor is modified from perfusion chamber. The driven force of the medium flow is produced by siphon phenomenon. The bioreactor is composed with two parts. The first part is a modified siphon tube which can hold the biphasic scaffold for osteochondral tissue engineering. The second part is a medium reservoir bottle, which contains large amount of medium and can connect to multiple siphon tubes at the same time. The medium reservoir bottle is placed higher than the siphon tubes. The gravity will drive medium into the siphon tubes. The siphon phenomenon will make the cell-seeded scaffold covered with the medium. When the height of medium reach the height of outflow tube of the siphon tube, the medium will drain out, and the scaffold will be exposed to the air in incubator, which provides oxygen exposure. Then the gravity will make the medium refill again, the scaffold will be immersed in medium until next cycle of medium drainage out. The curve shape of the siphon tube will prevent backward bacteria contamination. The flow of the medium from reservoir through the siphon tube will produce an effect like traditional perfusion chamber bioreactor; however no power supply is necessary in this system.

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1. TISSUE ENGINEERING AS A NEW APPROACH FOR CARTILAGE REPAIR

Articular cartilage tissue itself lacks a blood supply to support repair and remodeling. Because of the limited capacity for spontaneous repair, minor articular cartilage injury can lead to progressive damage and degeneration of the synovial joint. In young patients with focal articular cartilage injury,

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autogenous chondrocytes implantation (ACI), mosaicplasty, or marrow stimulating techniques, such as microfracture or multiple drilling, are better alternatives than joint replacement, but the results are variable and the techniques have certain limitations [1]. Marrow stimulating techniques result in the formation of fibrocartilage, which has a lower mechanical strength than hyaline cartilage and only limited repair capacity [2]. With mosaicplasty, the integration of subchondral bone into the host tissue is excellent, but the limited amounts of autogenous tissue and donor site morbidity are major problems. In addition, this procedure involves destroying healthy non-weight bearing tissue to treat diseased tissue, and both the donor site and treated area would be expected to degenerate [3-4]. ACI with periosteal graft has shown encouraging results, but the predictability and reliability of hyaline or fibrocartilage formation are still questionable [5]. Some animal studies provided evidence that ACI might result in poor integration into the host cartilage and subchondral bone. This poorly integrated area might be a stress riser, ultimately resulting in degeneration of the repaired tissue [6-7].

Recently, tissue engineering has emerged as a new method that involves combining cells, scaffold, and bioactive agents to fabricate functional new tissue to restore, maintain or improve tissue function [8]. As mentioned above, current clinical therapies for cartilage repair have certain problems as regards integration. Two kinds of integration should be considered, integration into host cartilage and integration into subchondral bone. An ideal tissue-engineered approach to cartilage repair should offer good integration into both the host cartilage and subchondral bone. Using a biphasic scaffold for an engineered osteochondral construct has the theoretical advantage of rapid recovery after bone union as the cartilage cap is mechanically mature and supported by the subchondral construct [9]. Our goal is therefore to make a tissue-engineered osteochondral graft which integrates well with host subchondral bone and cartilage. Engineered osteochondral constructs could have a major impact on the treatment of articular osteochondral defect. Further, this technology could overcome the source limitations that restrict autogenous osteochondral transplantation. Currently there are no bioreactor can be used for tissue engineering of osteochondral graft. We have therefore designed two new types of bioreactors to support osteochondral tissue engineering.

2. THE ROLE OF BIOREACTORS IN CARTILAGE TISSUE ENGINEERING

Bioreactors are generally defined as devices in which biological and/or biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions (e.g. pH, temperature, pressure, nutrient supply and waste removal) [10]. The in vitro culture of 3D cell-scaffold constructs in bioreactor under conditions that support efficient nutrition of cells, possibly combined with the application of mechanical forces to direct cellular activity and phenotype, is an important step toward the development of functional grafts for the treatment of lost or damaged body parts (i.e. functional tissue engineering) [11]. Bioreactors can also provide many important functions, such as improvement of cell seeding on 3D scaffold , increase of mass transport and providing mechanical conditioning.

Incorporation of bioreactor in the fabrication process of cartilage tissue is very important. Static loading of chondrocytes was reported to have low seeding efficiencies and non-uniform cell distributions within scaffold [12-13]. Significantly higher efficiencies and uniformities were obtained when tri-copolymer scaffold were seeded in spinner flask bioreactor [14]. Another kind of bioreactor, perfusion chamber, which flow a cell suspension directly through the pores of 3D scaffold, can produce even higher cell seeding efficiencies and more uniformity. Perfusion seeding can be readily integrated into a perfusion bioreactor system capable of performing both seeding of the scaffold and subsequent culturing of the construct. This culture system in cartilage tissue engineering can increase cell content and matrix synthesis [15].

Bioreactor can also increase mass transport in cartilage tissue engineering. Culture of bovine chondrocytes on scaffold in spinner flask induced an increase in both the synthesis of glycosaminoglycan (GAG) and the fractions of GAG accumulated within the central construct regions [16-17]. Cartilage construct cultured in rotating wall vessel bioreactors had biochemical and biomechanical properties superior to those of static or spinner-flask cultures and approaches those of native cartilage [13]. Perfusion chamber can perfuse the culture medium directly through the pores of the cell-seeded 3D scaffold, thereby reducing the mass transfer limitations both at the construct periphery and within its internal pores [10] in cartilage tissue engineering. The GAG synthesis and accumulation by chondrocytes were increased [15, 18].

Mechanical stimulations are important modulators that might increase the biosynthetic activity of cells in bioartificial matrices and possibly improve in vitro tissue regeneration [b]. In cartilage tissue engineering, loading or shearing forces will stimulate GAG synthesis and increase the mechanical properties of the

engineered cartilage construct [19-21].

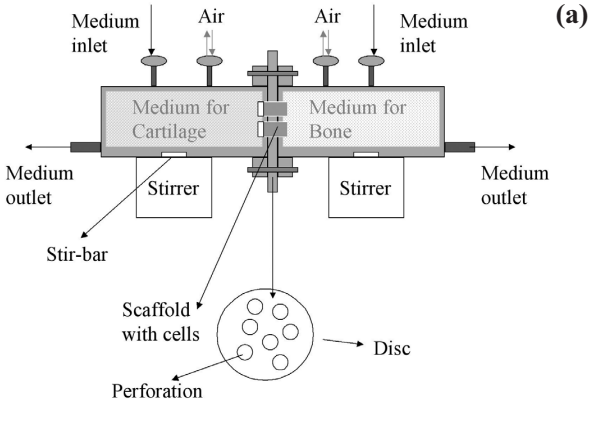
Bioreactors can provide controlled environment for reproducible and accurate application of specific regimes to tissue engineered construct, thus has a very important role in cartilage tissue engineering. As new tissue engineered construct is being developed, such as osteochondral tissue engineering, new type of bioreactors for this purpose will be created for different purpose. We have developed two new types of bioreactors for osteochondral tissue engineering, which are modified from spinner flask and perfusion chamber, the design and rationale of these bioreactors will be described.

3. DESIGN OF, AND RATIONALE FOR, THE DOUBLE CHAMBER BIOREACTOR

The double-chamber bioreactor consists of two tubular shape glass chambers, each of which has four branch tubes (medium inflow and outflow, oxygen ventilation, one surplus and reserved for other functions, where necessary). The whole bioreactor is autoclavible. An aseptic ventilation filter is connected to one branch tube one for each chamber under laminar flow. The chambers are separated by a silicone-rubber septum with multiple holes to hold the biphasic scaffold. The scaffolds are press fitted into a short plastic tube. The junction between the tube and the silicone membrane is sealed with heated silicone glue. Magnetic-bar stirring provides mixing of the medium and mechanical stimulation, such as shear stress for the engineered cartilage. The bioreactor has two independent medium-circulation systems [Fig. 1(a) and (b)] and, thus, can be used to supply various types of culture media to support co-culture of different kinds of cells, or, different induction media to induce mesenchymal progenitor cells (MPCs) to differentiate into chondrocytes and osteoblasts in the same biphasic scaffold (e.g., media containing beta-glycerophosphate and dexamethasone for induction of bone formation, or, transforming growth factor-beta 1 for induction of cartilage formation). Limited diffusion of the media may occur inside the scaffold, which may facilitate micro-interactions between different cells at the interface; however, little exchange takes place between the two chambers. In addition, as the cells secrete increasing amounts of matrix, diffusion will progressively decrease. The entire system, including bioreactor and magnetic stirring devices can be put into the incubator. The gaseous environment is 5% CO_2.

4. DESIGN OF, AND RATIONALE FOR, THE SIPHON BIOREACTOR SYSTEM

The second bioreactor is a modified perfusion chamber. The driven force of the medium flow is produced by siphon phenomenon. The bioreactor is composed with two parts. The first part is a modified siphon tube which can hold the biphasic scaffold for osteochondral tissue engineering (Fig.2 (a) and (b)). The second part is a medium reservoir bottle, which contains large amount of medium and can connect to



Perforation holes on the septum to hold scaffolds

(b)

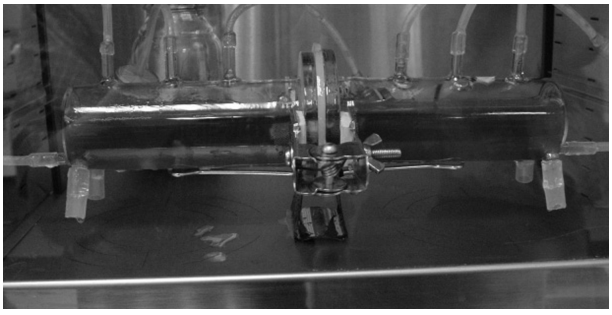


Fig.1 (a) Design of the double-chamber bioreactor: The double-chamber bioreactor consisted with two glass chambers. Each chamber contains three branch tubes for medium inflow, medium outflow and oxygen ventilation. The two chambers were separated with a silicon septum with multiple holes to hold the biphasic scaffold. Magnetic bar stirring provided the mechanical stimulation for the scaffold. The bioreactor contains two independent medium circulation systems. (b) The double-chamber bioreactor in the incubator.

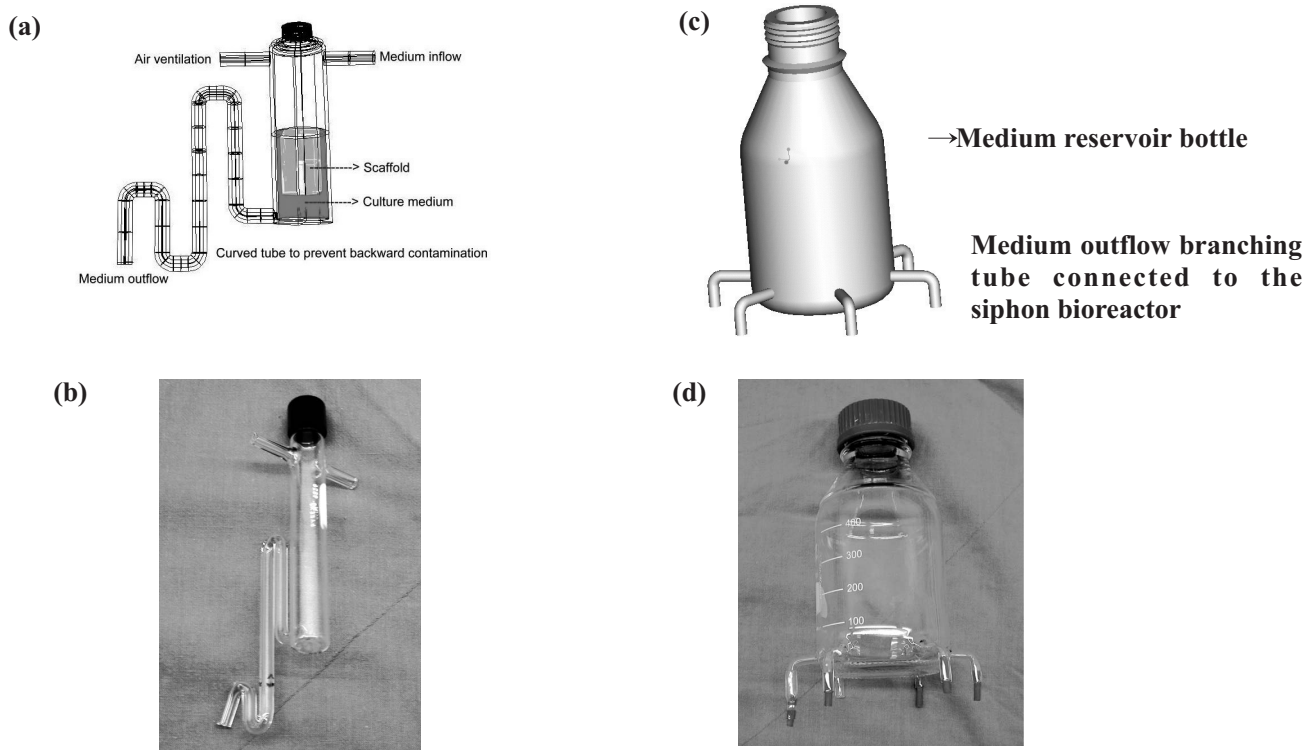


Fig.2 (a) Design of the siphon bioreactor: The siphon bioreactor consisted with main tube to hold scaffold and a S-shape branching tube for medium outflow. The upper part of the main tube contains two branch tubes for medium inflow and oxygen ventilation. The dropping of the medium will provide mechanical impaction on the cell-seeded scaffold. When the medium in the main tube reaches over the height of the outflow branching tube, the medium will be drained out due to the siphon effect. (b) The siphon bioreactor. (c) Design of the medium reservoir bottle: The bottle had multiple medium outflow tubes, which can be connected to the siphon bioreactors. Each bottle can supply multiple bioreactors at the same time. (d) The medium reservoir bottle.

multiple siphon tubes at the same time (Fig.2 (c) and (d)). The medium reservoir bottle is placed higher than the siphon tubes. The gravity will drive medium into the siphon tubes. The siphon phenomenon will make the cell-seeded scaffold covered with the medium. When the height of medium reach the height of outflow tube of the siphon tube, the medium will drain out, and the scaffold will be exposed to the air in incubator, provide oxygen exposure. Then the gravity will make the medium refill again, the scaffold will be immersed in medium again until next cycle of medium drainage out. The curve shape of the siphon tube will prevent bacteria contamination. The flow of the medium from reservoir through the siphon tube will produce an effect like traditional perfusion chamber bioreactor; however no power supply is necessary in this system. This bioreactor can be used both in cell seeding and culturing engineered construct. As for osteochondral tissue engineering, osteoblasts will be seeded first, with cells in medium perfuse through calcium phosphate scaffold. Then chondrocytes pellets will perfuse onto the surface of the osteoblast-seeded calcium phosphate scaffold result in an osteochondral construct through organ printing effect. The medium from the reservoir will drop on the surface of the osteochondral construct; the impaction of the medium drops will provide mechanical stimulation for cartilage cultivation.

5. DISCUSSION

According to our previously published paper, we had designed a novel bioreactor to culture this biphasic scaffold, with the scaffold made biphasic during fabrication, not sutured together or inserted separately

at a later stage. We had demonstrating successful culture of hyaline-like cartilage in a gelatin scaffold on a calcium phosphate surface. The engineered cartilage remained integrated with the surface of the calcium phosphate scaffold [22]. On the basis of the success of this *in vitro* study, we also plan to use the double chamber bioreactor to co-culture chondrocytes and osteoblasts, or, to induce simultaneous differentiation of MPCs into chondrocytes and osteoblasts in future *in vitro* studies.

Different approach for osteochondral tissue engineering had been reported. Kreklau et al., (1999) developed a biphasic cartilage transplant by fixing a polyglycolic acid/polylactic acid (PGA/PLA) scaffold to a calcium carbonate variant with fibrin and thrombin, using a perfusion chamber as a bioreactor to successfully culture hyaline cartilage on the surface of the calcium carbonate [23]. Schaefer et al., (2000) designed an osteochondral composite, suturing together chondrocytes-seeded (PGA) and periosteal cells-seeded scaffold (PLA/PGA/polyethylene glycol), and culturing it in an orbital shaker [24]. Coutts et al., (2001) sutured rib perichondrium on to cores of demineralized bone matrix to make a biphasic implant plug [25]. Gao et al., (2002) published an in vivo study of osteochondral tissue engineering using a two-phase injectable scaffold. This composite material consisted of an injectable calcium phosphate (ICP) and a hyaluronan (HA)-derivative sponge [26]. The osteochondral defect, filled with the ICP then the HA sponge, with or without loading of autologous bone marrow-derived MPCs, was inserted into the defect on top of the ICP as it hardened. In their animal study, the scaffolds were inserted separately and only MPCs were used. Despite the fact that they are either sutured together, or sequentially implanted, they may still be acceptable for restoration of subchondral bone tissue. The design of the siphon bioreactor is for sequentially organ printing of bone and cartilage tissue to make of engineered osteochondral construct. The osteoblasts were perfusing into the calcium phosphate scaffold first, followed by chondrocytes pallets printing. The dropping of medium will provide mechanical stimulation. The patent of this new bioreactor has been filed, and we will plan an in vitro study, followed by an in vivo study.

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