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Original Article

A Novel Culture Chamber Design and Cell Biomaterial Sheet Engineering for Reconstruction of Cartilage Tissue

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Abstract

Introduction*: In recent years tissue engineering developed to replace or repair damaged tissues using cell and biomaterial. One of the most important fields of tissue engineering is simulation of in vivo micro environment of body tissues. This study aimed to develop culture chamber and cell biomaterial sheet engineering for the reconstruction of cartilage tissue.*

Methods*: Stainless steel culture chamber was designed with mechanical factor affecting cartilage. Shear exerted on the wall of the chamber was predicted with computational fluid dynamic modeling with Fluent. The meshes were created with Gambit software.After isolation of chondrocytes from cartilage, cells mixed with natural biomaterial and hydrogel tissue construct cultured in chamber. Sections of resulted cell-biomaterial construct were examined with histological methods.*

Results*: The designed chamber mimicked synovial joint with perfusion flow. Maximum wall shear was predicted with fluent was 2.407× 10-3 Pa. Chondrocyte-scaffold was created as a thin sheet. Histological examination of chondrocyte biomaterial revealed morphology of native cartilage tissue with round cluster chondrocyte profile. Isogenic group of constract proliferated and expanded.*

Discussion*: According to simulation of in vivo environment of natural cartilage joint and simplification of tissue constructs production from chondrocyte and hydrogel scaffold, the novel strategy described here has great advantages to the improvement of cartilage tissue engineering.*

Keywords: Tissue engineering; Chamber; Chondrocyte; CFD.

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Introduction

In recent years tissue engineering developed to replace or repair damaged tissues using cell and biomaterial. The general principle of cartilage tissue engineering involves three fields: Cell, scaffold and mechanical stimuli to build a three dimensional (3-D) living constructs (1).

One of the most important fields in the tissue engineering is in vivo simulation of body tissues micro environment (2). Therefore, several bioreactor composed culture chamber have been developed. The successful application of culture chamber in tissue engineering requires careful consideration of their 3-D geometry, biocompatibility and biomechanics (3). In recent years, perfusion bioreactors with a culture chamber have been examined to build three-dimensional cartilage grafts (4,5). Shear exert on wall of chamber in perfusion bioreactors has been predicted with computational fluid dynamic modeling with fluent software (6).

Pioneering cell-sheet scaffolding from tissue engineering strategy was proposed to regenerate cartilage tissue; therefore, studies were designed to develop a culture chamber and cell biomaterial sheet engineering for reconstruction of cartilage tissue (7).

Here, we demonstrate some techniques to generate chondrocyte sheets with respect to their composition and mechanical properties. In addition, we demonstrate a methodology to chondrocyte sheet with culture chamber for potential cartilage graft.

Materials and Methods

All chemicals such as Trypsin, high glucose DMEM, 0.2% Collagenase type II, Penicillin, Streptomycin and Amphotricinwere purchased from Sigma-Aldrich (MO, USA) and used directly without further purification.

The shear stress imposed on the cells in the culture chamber is predicted by Computational Fluid Dynamics (CFD). Modeling and calculation of wall shear was performed by FLUENT software (Fluent Corp, USA). Gambit software created the meshes. The CFD model considered fluid flow entering the inlet reservoir, flowing through the culture chamber to the outlet reservoir. Characterization of the mechanical stresses was performed in an idealized chondrocyte cell subject to a range of laminar fluid flow regimes (8).

The specifications of our culture chamber have been reported in our previous article (9). Briefly, the stainless steel culture chamber designed simulating internal geometry of synovial joint with an inlet and outlet. Perfusion system simulating joint was used to examine shear on chondrocyte within the chamber. The system includes the inlet medium reservoir, culture chamber and exhaust medium. Entire chondrocyte-biomaterial within culture chamber is directly perfused with culture medium. Silicon tubing was used to connect inlet/outlet ports of the perfusion chamber.

Cell isolation: Fresh intact cartilage was obtained from white glossy nasal septum. Chondrocytes were isolated and expanded following the procedure described in our previous study (10). Since, the perichondrium and cartilage are molecularly distinct tissues; dense supportive connective tissue around the cartilage was removed to expose the white glossy

nasal septal cartilage. The Perichondrium is composed of cells and a firm matrix contains the cartilage cell precursors (chondroblasts).

Chondrocytes as well as cartilage slices were trypsinized first in room temperature for 2hrs. Then cartilage slices were transferred in high glucose DMEM composed of0.2% collagenase type II into an incubator (37°C, 5% CO2) for 16-20 hrs. Also antibiotic/antimycotic agents such penicillin (100 U/ml), streptomycin (0.1 mg/ml) and amphotricin B (250 µg/ml) were added. Under invert microscope, cartilage slices examined for digestion, were then carefully filtered through70 and 100 µm nylon filters (BD Falcon, USA) to eliminate undigested pieces. The filtered solution was washed several times with PBS. The suspension was transferred to a 50Ml falcon tube and centrifuged at 2500 rpm for 10 min to obtain a pellet (11).

Chondrocyte sheet: The cell pellet was redispersed in 5 ml of complete culture medium (DMEM/Ham's F-12). The medium supplemented with 5% FBS, ascorbic acid (50 g/ml), L-glutamine (29.2 mg/ml).

Briefly, first HEPES and NaCl powders were dissolved in deionized water, warmed up at 50-60°C. The alginate powder was added with constant stirring to prepare homogeneous solution. The solutions were sterilized via 0.45-µm filter and 2.0 % alginate solutions were prepared.

The cell pellet of cartilage were counted and suspended in culture medium. The cell suspension was aspirated into a syringe(21-gauge needle and 20 ml syringes) and the solution was released drop-wise into 100 mL of polymerization solution (102 mM CaCl2), maintained under gentle stirring by a magnet. The alginate beads were allowed to polymerize in102 mM CaCl2, washed in NaCl. Finally 50-60 alginate beads placed in each flask contain the complete culture medium.

The filtered polymerization solution was discarded, and beads were transferred into 150 mMNaCl solution and washed several times. The obtained tissue construct was cultured in culture flasks, then transferred to the culture chamber and dynamically cultured for 3–5 days under direct perfusion (12).

Alginate sheet preparation: Alginate solution was prepared as mentioned above. Two thin layer of sterile filter paper was prepared as a pocket and soaked in 102 mM CaCl2. The prepared alginate solution was released drop-wise into packet filter and transferred into CaCl2 solution. During polymerization of gels, the casting two-layer filter papers were separated spontaneously. The obtained tissue construct was cultured in culture flasks, then transferred to the culture chamber and dynamically cultured for 3-5 days under direct perfusion (13).

Histological evaluation: Culture alginate beads and sheets were then filtered and fixed in Bouin's Fixative. Tissue constructs were dehydrated, cleared, and then embedded in paraffin wax. Five to seven-micrometer sections were cut from tissue constructs. The sections were stained with hematoxylin and eosin for cell morphology. Statistical significance was determined by SPSS software. A value of P= 0.05 was selected as the threshold of statistical significance.

Results

The results of CFD modeling indicated peak velocity and maximum wall shear stress were 1.706×10-3 m/s and 2.407× 10-3 Pa (1 Pa= 10 dyn/cm(2) respectively. Pressure Contour (Pascal) and velocity magnitude (m/s) are depicted in figure 1. Fluid filled the chamber from the bottom opening; therefore, the highest flow speeds were found near the inlet of the fluid and the bottom of the tissue chamber.

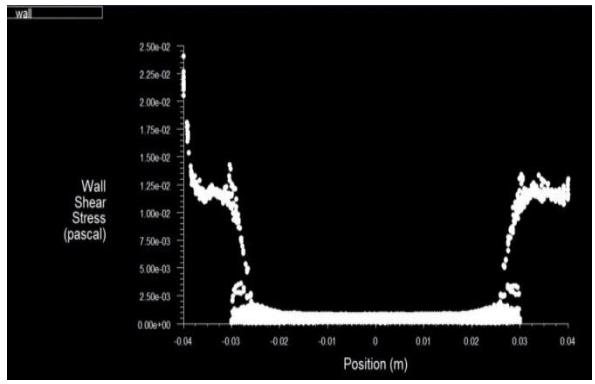

Figure 1. Wall shear exert on culture chamber predicted by CFD modeling showing flow speeds

Tissue construct successfully were cultured and have strength architecture to potential implantation. After alginate hydrogel polymerization, under invert microscopy, viable and round homogeneous chondrocytes were observed. The chondrocytes maintained a round shape similar to the original cartilage. The important morphological change within the hydrogel was cell agglomerates (Figure 4). This feature reflected the presence of strong cell division in alginate peals and sheets.

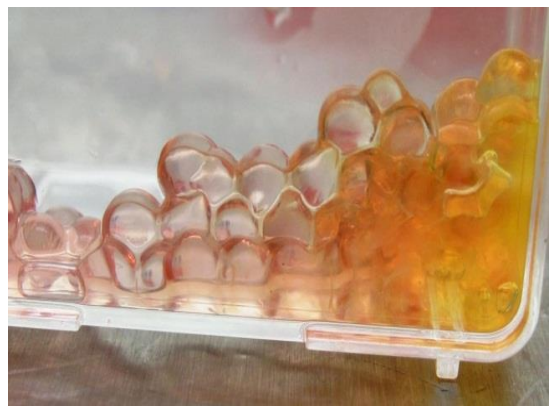

Figure 2. Photographs of beads composed of alginate and chondrocyte in a culture flask

Cartilage-like tissue derived from septal nasal chondrocytes was found in both alginate beads and sheets. Cartilage-like tissue subjected to shear showed similar feature of natural cartilage morphology such as lacuna a cartilage-like matrix. The extracellular matrix surrounding chondrocytes within a chondron is clearly was observed. Isogenic groups of chondrocytes in a single lacuna reflected chondrocyte

agglomerates as seen under invert microscopy. The lacuna housed mature and round-shaped chondrocytes (Figure 5, 6).

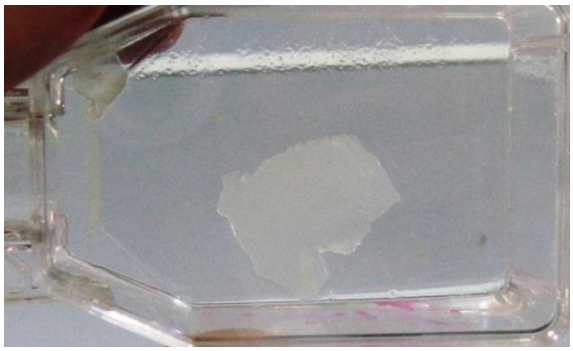

Figure 3. Photographs of sheet composed of alginate and chondrocyte in a culture flask

Figure 4. Photographs of tissue construct under invert microscopy. Several chondrocyte agglomerates are visible

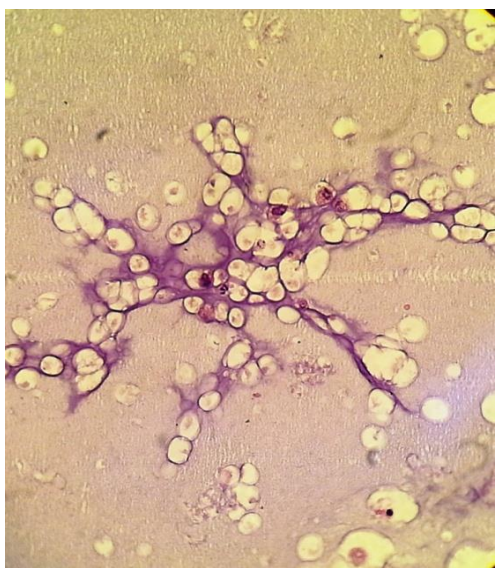

Figure 5. Microscopic view of tissue construct. Architecture of tissue construct is similar to natural cartilage morphology with lacuna and distributed chondrocytes magnification 40x

Discussion

In the present study we designed a stainless steel culture chamber that housed a hydrogel-chondrocyte sheet and beads. One of the best advantages of the perfusion system was in vivo simulation of synovial joint environments subjected to shear stress.

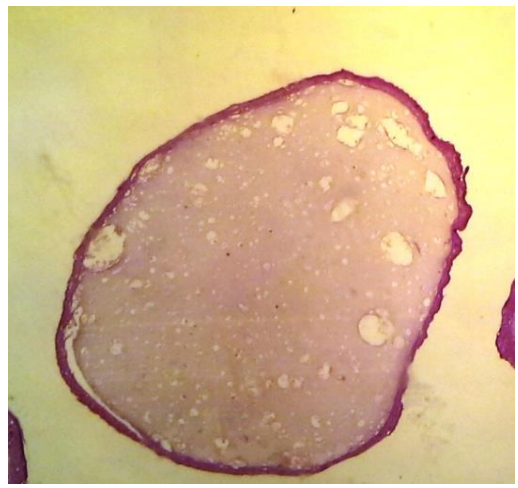

Figure 6. Examining tissue construct under a light microscope. Hematoxylin and eosin stained sections revealed cartilage-like tissue.

Previous studies revealed that culture environments perfusion, enhance cartilage extracellular matrix (ECM) production (14,15). Therefore, several culture chambers for cartilage tissue engineering were designed to enhance medium exchange through perfusion.

One of the great advantages of fluid flow culture system is providing more efficient nutrient supply and metabolite removal. The system gives favorable environment to promote the chondrogenesis (16,17).

The results of CFD modeling indicated maximum wall shear stress on surface of culture chamber were 2.407× 10-3 Pa. The highest flow speeds were found near the inlet of the tissue chamber. When compared with results of other studies, the shear stress was extremely low in the chamber.

During the first days of chondrocyte culture in three dimensional environment of hydrogel scaffold cell agglomerates appeared in alginate bead and alginate sheets. This feature reflected the presence of strong cell division. When the stained section of alginate beads and alginate sheets was examined, chondrocyte agglomerates appeared as isogenic groups (18).

Cartilage-like tissue subjected to shear showed similar feature of natural cartilage morphology such as lacuna a cartilage-like matrix. The lacuna housed mature and round-shaped chondrocytes (figure 5,6).

In conclusion, the novel method described here can improve tissue engineering strategies by simulations of natural cartilage joint and tissue construct fabrication from chondrocyte and biomaterial.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Fodor WL. Tissue engineering and cell based therapies, from the bench to the clinic: the potential to replace, repair and regenerate. Reprod Biol Endocrinol. 2003;1(1):102.
2. Freed LE, Vunjak-Novakovic G. Tissue engineering bioreactors. Principles of tissue engineering. 2000;2:143-56.
3. Grayson WL, Fröhlich M, Yeager K, Bhumiratana S, Chan ME, Cannizzaro C, et al. Engineering anatomically shaped human bone grafts. Proceedings of the National Academy of Sciences. 2010;107(8):3299-304.
4. Salzmann GM, Stoddart MJ. Bioreactor Tissue Engineering for Cartilage Repair. Developing Insights in Cartilage Repair: Springer; 2014. p. 79-97.
5. Freitas D, Almeida H, Bártolo P. Perfusion Bioreactor Fluid Flow Optimization. Procedia Technology. 2014;16:1238-47.
6. Hutmacher DW, Singh H. Computational fluid dynamics for improved bioreactor design and 3D culture. Trends in biotechnology. 2008;26(4):166-72.
7. Tran RT, Thevenot P, Zhang Y, Gyawali D, Tang L, Yang J. Scaffold sheet design strategy for soft tissue engineering. Materials. 2010;3(2):1375-89.
8. Raimondi MT, Moretti M, Cioffi M, Giordano C, Boschetti F, Laganà K, et al. The effect of hydrodynamic shear on 3D engineered chondrocyte systems subject to direct perfusion. Biorheology. 2006;43(3):215-22.
9. Gharravi AM, Orazizadeh M, Hashemitabar M. Direct Expansion of Chondrocytes in a Dynamic Three-Dimensional Culture System: Overcoming Dedifferentiation Effects in Monolayer Culture. Artificial organs. 2014;38(12):1053-8.
10. Gharravi AM, Orazizadeh M, Hashemitabar M, Ansari-Asl K, Banoni S, Alifard A, et al. Design and validation of perfusion bioreactor with low shear stress for tissue engineering. J Med Biol Eng. 2013;2:185-92.
11. Masuda K, Sah RL, Hejna MJ, Thonar EJ. A novel two step method for the formation of tissue engineered cartilage by mature bovine chondrocytes: The alginate recovered chondrocyte (ARC) method. Journal of orthopaedic research. 2003;21(1):139-48.
12. Lee DA, Reisler T, Bader DL. Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared to conventional monolayer techniques. Acta Orthopaedica. 2003;74(1):6-15.
13. Lee CS, Gleghorn JP, Choi NW, Cabodi M, Stroock AD, Bonassar LJ. Integration of layered chondrocyte-seeded alginate hydrogel scaffolds. Biomaterials. 2007;28(19):2987-93.
14. Grogan SP, Sovani S, Pauli C, Chen J, Hartmann A, Colwell Jr CW, et al. Effects of perfusion and dynamic loading on human neocartilage formation in alginate hydrogels. Tissue Engineering Part A. 2012;18(17-18):1784-92.
15. Schulz RM, Bader A. Cartilage tissue engineering and bioreactor systems for the cultivation and stimulation of chondrocytes. European Biophysics Journal. 2007;36(4-5):539-68.
16. Tortelli F, Cancedda R. Three-dimensional cultures of osteogenic and chondrogenic cells: a tissue engineering approach to mimic bone and cartilage in vitro. Eur Cell Mater. 2009;17:1-14.
17. Raimondi M, Boschetti F, Falcone L, Fiore G, Remuzzi A, Marinoni E, et al. Mechanobiology of engineered cartilage cultured under a quantified fluid-dynamic environment. Biomechanics and modeling in mechanobiology. 2002;1(1):69-82.
18. Bittencourt RAdC, Pereira HR, Felisbino SL, Ferreira RR, Guilherme GRB, Moroz A, et al. Chondrocyte cultures in tridimensional scaffold: alginate hydrogel. Acta Ortopédica Brasileira. 2009;17(4):242-6.