In vitro Cartilage Regeneration using Primary Chondrocytes Cultured within Porous Poly(lactide-co-glycolide) Scaffolds

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Abstract

Cartilage injuries are frequent nowadays. The previous surgical treatment of cartilage defect was limited. Another approach in the treatment of cartilage injuries is the use of reconstitute cartilage consisting of chondrocytes cultured in suitable biodegradable scaffolds. Current studies have demonstrated the compatibility of chondrocytes with different biomaterials and the chondrogenesis in various types of porous scaffolds. The cell ingrowth into the porous scaffolds is modulated by initial cell loading efficiency. Therefore, well-interconnected pore structure and even pore distribution of the scaffolds are essential for efficient cell seeding. According to our previous work, well-interconnected macroporous scaffolds can be prepared by gas-foaming/salt-leaching method using ammonium bicarbonate salt as porogen additives. In this work, primary chondrocytes were cultured in PLGA 65/35 scaffolds fabricated by using our method. Cells seeded in the scaffolds showed well distribution by agitated seeding method. Histochemical staining of proteoglycans present in the scaffolds was used to visualize the chondrocyte ingrowth in the scaffolds. At 3 weeks, the population of chondrocytes was increased for the most part of the scaffolds, and extra cellular matrix (ECM) secretion was increased as culture periods progressed.

Introduction

The rapid changes in people's life-style in modern age are considered as the causes for many various diseases. Among them, the diseases related to the weakened bone and cartilage tissues have become social problems. The previous surgical treatments of cartilage defect such as osteotomy, perichondral grafting, joint replacement were limited. Another approach in the treatment of cartilage injuries is the use of reconstitutes cartilage consisting of chondrocytes cultured in suitable biodegradable scaffolds. Current studies have demonstrated the compatibility of chondrocytes with different biomaterials and the chondrogenesis in various

types of porous scaffolds. The cell ingrowth into the porous scaffolds is modulated by initial cell loading efficiency. Therefore, well-interconnected pore structure and even pore distribution of the scaffolds are essential for efficient cell seeding. According to our previous work, well-interconnected macroporous scaffolds can be prepared by gas-foaming/salt-leaching method using ammonium bicarbonate salt as porogen additives. These scaffolds showed a high cell loading efficiency using rat hepatocytes as a cell culture model.

In this work, primary chondrocytes were isolated from rat costal bone and cultured in PLGA 65/35 scaffolds fabricated by using gas-forming/salt-leaching method. To efficient cell seeding into the scaffolds, pre-wetted PLGA scaffolds were immersed with cell suspension and incubated with shaking. Cells seeded in the scaffolds showed well distribution by agitated seeding method. Histochemical staining of proteoglycans present in the scaffolds was used to visualize the chondrocyte ingrowth in the scaffolds. At 3 weeks, the population of chondrocytes was increased for the most part of the scaffolds, and extra cellular matrix (ECM) secretion was increased as culture periods progressed.

Experimental Methods

Polymer scaffolds fabrication

Highly viscous polymer solution was prepared by dissolving PLGA in chloroform at a concentration of 200 mg/ml. subsequently, cold ethanol was added to the PLGA solution as a non-solvent to precipitate and concentrate the polymer solution[1, 2]. Ammonium bicarbonate salt particulates (weight ratios of NH_4HCO_3 to PLGA used were 10:1) were added to the precipitated PLGA gel and mixed homogeneously. The paste mixture of polymer/salt/solvent was cast into a disc shaped Teflon mold (10 mm diameter and 2 mm thickness). After chloroform was partially evaporated under atmospheric pressure, the semi-solidified samples were immersed into an excess amount of acidic aqueous solution at room temperature. Afterwards, the samples were then freeze-dried. Morphologies of the resultant scaffolds were examined by scanning electron microscopy (SEM) and their porosity and pore volume were determined by mercury intrusion porosimetry.

Isolation of rat chondrocytes

Rat costal cartilage samples were harvested from 6 week-old male Sprague-Dawley rats. The cartilage samples were diced into small pieces and rinsed with PBS. Next the samples were digested for 24 hr in DMEM with collagenase (2mg/ml). Released chondrocytes were filtered

with 100 mm nylon filter and centrifuged at 50 g for 10 min. Cell pellets were resuspended in DMEM containing 10 % FBS, 50 μg/L ascorbic acid and 0.4 mM proline. Cells were first cultured for multiplication under conventional monolayer culture condition for 3-4 passages in 3 weeks.

Cell seeding and culture on biodegradable polymer

Prepared PLGA scaffolds were pre-wetted by 0.22 μm filtered ethanol emersion and they were rinsed with PBS twice. Then scaffolds were transferred to the 100 ml glass flask containing 50 ml of cell suspension in culture medium. The flask was shake at 100 rpm in 37°C incubator. After 1 week, cell/scaffolds complexes were harvested and incubated in fresh culture medium with shaking at 50 rpm. Culture medium was changed every 3 days. Cell/scaffolds complexes were sampled at timed intervals between 1 to 4 weeks.

Analytical assays

Histochemical morphologies of chodrocytes cultured in PLGA scaffolds were observed by using H & E staining. ECM secreted from chondrocytes could be visualized by H & E staining. For phenotypic characterization, RT-PCR was performed to detect chondrocyte-specific collagen type II.

Results and discussion

Highly open porous biodegradable polymer scafffolds could be prepared by using by using ammonium bicarbonate as an efficient gas foaming agent as well as a particulate porogen salt (Figure 1). A binary mixture of PLGA-solvent gel containing dispersed ammonium bicarbonate salt particles, which became a paste state, was cast in a mold and subsequently immersed in a hot water solution to permit the evolution of ammonia and carbon dioxide within the solidifying polymer matrix. This resulted in the expansion of pores within the polymer matrix to a great extent, leading to well interconnected macroporous scaffolds having mean pore diameters of around 300-400 m, ideal for high-density cell seeding. Cell seeding efficiency determined by trypan blue exclusion, was up to 90 % after 24 hr. However, chondrocytes showed well distribution on the surface of the scaffolds but few cells were observed in the core of the scaffolds (Figure 2). This result was caused by interfering of large number of cell adhesion on a surface of the scaffolds with cell loading in the core of the scaffolds. But culture periods was increased, cross sectional view of cell/scaffold complex showed an increasing cell number and ECM secretion by using histochemical staining. At 3 weeks, proteoglycans produced by

chondrocytes, which present in the scaffold indicates that the cell ingrowth proceeded to the core of the scaffolds as shown in Figure 3. Phenotypic determination by using RT-PCR for collgane type II mRNA showed maintained similar level for 1 month. But cell viability in the scaffolds decreased as culture time progressed. It means that oxygen and nutrient supplement was not sufficient in agitated culture system. Furthermore, nutrient supplement could be hindered by cell number increment in the scaffolds. Therefore, it can be considered that effective cell/scaffold complex culture system is required for tissue regeneration *in vitro*.

References

1. Y. S. Nam, J. J. Yoon, T. G. Park, "A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a progen additive" (2000), J Biomed Mater Res (Appl Biomater) , 53(1), 1-7
2. J. J Yoon, T. G. Park, "Degradation behavior of biodegradable macroporus scaffolds prepared by gas foaming with effervesent salts" , J Biomed Mater Res, In press

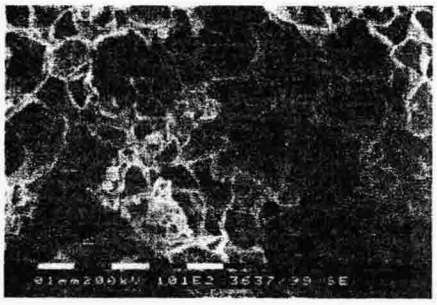


Figure 1. SEM image of porous structure of PLGA scaffolds prepared by gas foaming method.

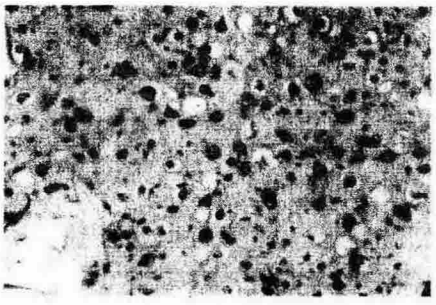
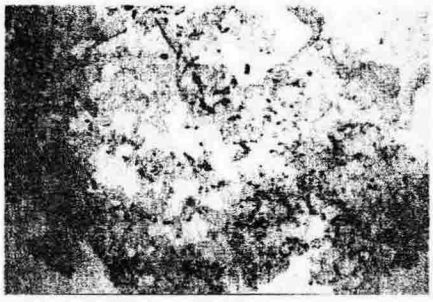


Figure 3. Histochemical section of chondrocytes/scaffold complex cultured for 3 week (H & E stained, 200 X).



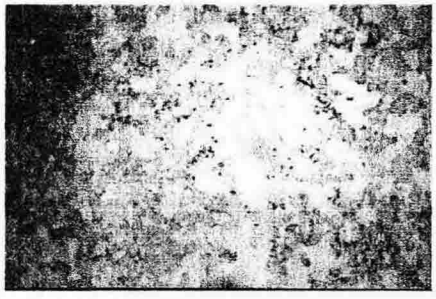


Figure 2. Rat chondrocytes distribution in PLGA scaffolds after seeding. Surface of the scaffold (above), core of the scaffolds (bottom).