

Chondrocyte Growth and Function on HPBS/HA Composite Scaffolds: Static Versus Dynamic Culture

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Abstract. The objective of this study was to comparatively evaluate the responses of porcine chondrocytes to the surface-hydrolyzed poly(butylene succinate)/hydroxyapatite (HPBS/HA) composite scaffolds in static (in tissue culture plates) and dynamic (in spinner flasks) culture. The HPBS/HA scaffolds with an average pore size in the range of 500-1000 μm and varied weight ratios of PBS to HA, i.e., 100:0, 90:10, 80:20 and 70:30, were employed. The chondrocytes cultured on the scaffolds were assessed for their proliferation, cartilage-specific gene expression and glycoaminoglycan (GAG) synthesis after a 28-day culture period. The cell proliferation observed on all scaffolds in static and dynamic environment appeared insignificant different, except for that on the HPBS/HA-90/10 scaffold where the number of chondrocytes markedly increased when the dynamic culture was conducted. The RT-PCR results showed that the chondrocytes cultured in the spinner flasks expressed more type II collagen mRNA. Nevertheless, the gene expression in the cultured cells on all scaffolds was not much different under the dynamic environment. In addition, the dynamic culture of chondrocytes remarkably increased the secretion of GAG, especially when being cultured on the HPBS/HA-80/20 scaffold. These findings demonstrated that the dynamic culture of the porcine chondrocytes on the HPBS/HA composite scaffolds enhanced the expression of type II collagen gene and GAG synthesis more effectively, compared with the static culture.

Keywords: Poly (Butylene Succinate), Hydroxyapatite, Scaffolds, Chondrocytes, Static and Dynamic Culture

1. Introduction

Recently, tissue engineering has played an important role in therapeutic repair of damaged articular cartilage. A wide variety of scaffolds have been studied to produce cartilage *in vitro* due to their biodegradability, biocompatibility, non-cytotoxicity and processability [1]. Among the scaffolds, aliphatic biodegradable polyesters, e.g., poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and their copolymers, are extensively used [2]. Poly(butylene succinate) (PBS) is another promising biodegradable polyester being explored as a substrate for osteoblasts culture [3]. Hydroxyapatite (HA) is known for its superior biocompatibility. Several polymer/HA composite scaffolds have also been developed to increase the mechanical properties of the substrates and to improve cell interaction [4-5]. Surface hydrolysis by NaOH altered the hydrophilicity of a polymeric scaffold, enabling a scaffold to absorb culture medium more readily [6]. In general, in static culture, there are oxygen and nutrient gradients from the surface to the center of a scaffold, resulting in cell death at the center of the scaffold. Therefore, several bioreactor systems have been designed to mitigate this shortcoming [7-8].

In this study, a comparative study was performed on static and dynamic culture of porcine chondrocytes on surface hydrolyzed poly(butylene succinate)/hydroxyapatite (HPBS/HA) composite scaffolds with pore size in the range of 500-1000 μm and varied weight ratios of PBS to HA, i.e., 100:0, 90:10, 80:20 and 70:30.

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The chondrocyte proliferation, cartilage-specific gene expression and GAG secretion were assessed using Alamar blue assay, RT-PCR analysis and GAG assay, respectively.

2. Materials and Methods

2.1. Preparation of Surface-Hydrolyzed PBS/HA Composite Scaffolds

PBS (Mw \approx 260 kDa) was initially mixed with HA at varied weight ratios, i.e., 100:0, 90:10, 80:20 and 70:30, and then fabricated by a high pressure supercritical CO₂ technique. The resulting PBS/HA composite scaffolds with average pore sizes in the range of 500-1000 μ m were then hydrolyzed with 0.6 M NaOH solution at 60°C for 30 min, yielding HPBS/HA scaffolds. The product code was assigned based on the percentage of HA initially incorporated, e.g., HPBS/HA-80/20 was a hydrolyzed composite scaffold with 20 wt% HA initially incorporated.

2.2. Cell Culture

The sterilized sample discs were placed into 24-well culture plates. Each scaffold was seeded with porcine chondrocytes at concentration of 1.5×10^6 cells/specimen and then incubated overnight to allow the cells to attach. Afterwards, the scaffolds were divided into two groups for static (Group I) and dynamic (Group II) culture. In Group I, the cell-seeded scaffolds were still further cultured in 24-well culture plates. The cell-seeded scaffolds in Group II were placed in the chambers of spinner flasks; each of which contained 60 ml of medium and was magnetically stirred at 80 rpm for 3 hours/day. All the scaffolds in both culture conditions were incubated under 5% CO₂ atmosphere at 37°C for 28 days. The culture medium was regularly replaced every 3 days and 7 days for the static and dynamic culture, respectively.

2.3. Cell Proliferation Assay

Cell proliferation on each scaffold was assessed by Alamar blue assay, which is based on the detection of metabolic activity of cells. Typically, after a 28-day incubation period, the chondrocytes cultured on each scaffold were further incubated in a medium containing resazurin dye for 4 hours. Aliquots of 200 μ l of each medium were subsequently read at the fluorescence wavelength of 530/590 nm.

2.4. RNA Extraction and Semiquantitative RT-PCR

In brief, after a 28-day culture period, total RNA was extracted from the chondrocytes cultured on each scaffold using TRIZOL reagent (Invitrogen) following the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from 2 μ g of RNA using Prime RT Master synthesis Kit (GeNet Bio) in a 20 μ l reaction. PCR analysis was performed to determine the cartilage-specific gene expression. The mRNA levels of 18s rRNA were used as internal controls. The PCR products were identified by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining.

2.5. Glycosaminoglycan (GAG) Assay

The total GAG content secreted from the chondrocytes cultured on each scaffold was determined using 1,9-dimethylmethylene blue (DMB) dye solution. Briefly, after a 28-day culture period, each scaffold was lyophilized and digested with papain solution (5mg/ml) at 60°C for 18 hours. Aliquots of 100 μ l of each papain digested scaffold solution were mixed with 100 μ l of DMB solution. The absorbance was measured at 525 nm. The amount of GAG was determined against the chondroitin-6-sulphate standard curve.

3. Results and Discussion

Figure 1 shows the proliferation of chondrocytes cultured on each surface hydrolyzed composite scaffold. The results revealed that the number of chondrocytes found on each composite scaffold was not different much after being cultured for 28 days in static and dynamic conditions, except for that on the HPBS/HA-90/10 scaffold where the number of chondrocytes markedly increased when being cultured under the dynamic environment. Nonetheless, this degree of cell proliferation was insignificantly different from that observed on the other hydrolyzed composite scaffolds under the same dynamic condition.

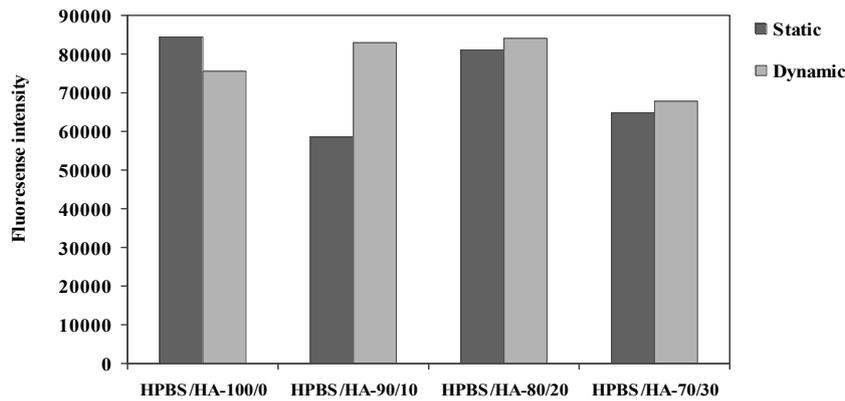


Fig. 1: Proliferation of chondrocytes cultured on different scaffolds in static and dynamic conditions at a 28-day incubation period.

The cartilage-specific gene expression in the chondrocytes cultured on the composite scaffolds was determined by RT-PCR analysis. As demonstrated in Figure 2, compared to those cultured in a tissue culture plate (a monolayer culture), the chondrocytes cultured on the composite scaffolds considerably enhanced their mRNA expression of type II collagen. The three dimensional constructs could absolutely stimulate the chondrocyte function under both static and dynamic culture. However, the bands of type II collagen gene expressed in the chondrocytes cultured on the scaffolds in the dynamic condition appeared more intense than those in the cells cultured under the static condition. This indicated that the dynamic culture could promote cartilage-specific gene expression; there were, however, no differences in the collagen band intensity found in all tested scaffolds.

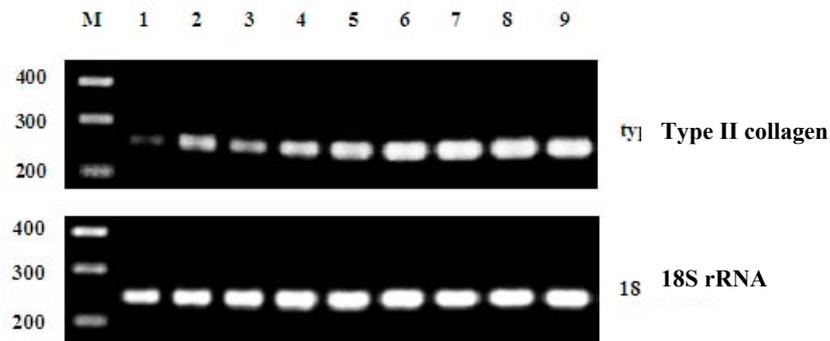


Fig. 2: Alterations in gene expression of type II collagen in chondrocytes in monolayer and on scaffolds cultured for 28 days; (lane 1) monolayer culture, (lane 2, lane 6) HPBS/HA-100/0, (lane3, lane 7) HPBS/HA-90/10, (lane 4, lane 8) HPBS/HA-80/20, (lane 5, lane 9) HPBS/HA-70/30, M = marker, lane 2-5 = static condition, and lane 6-9 = dynamic condition.

The amounts of GAG secreted from the chondrocytes cultured on the scaffolds are reported in Fig.3. Considerably greater GAG contents were apparently found in the chondrocytes cultured under the dynamic condition. In particular, that was secreted from the cells cultured on the HPBS/HA-80/20 scaffold; the total GAG content increased from 244 μg (produced under the static condition) to 911 μg (produced under the dynamic condition)

The results demonstrated above vividly indicated that the dynamic culture of the porcine chondrocytes on the surface-hydrolyzed composite scaffolds could significantly promote cartilage-specific gene expression (type II collagen) and extracellular matrix (GAG) production. This was attributed to more effective diffusion of oxygen and nutrient fluid through the whole porous substrates in the dynamic culture. The shear forces generated from the culture media stirring also enhanced the level of type II collagen gene expression and GAG secretion.

4. Conclusions

The proliferation of porcine chondrocytes cultured on the surface-hydrolyzed poly(butylene succinate)/hydroxyapatite (HPBS/HA) composite scaffolds with pore size in the range of 500-1000 μm under static and dynamic environment for 28 days appeared insignificantly different. However, compared with the static culture, the dynamic culture of the chondrocytes on the composite scaffolds in the dynamic condition could promote type II collagen gene expression and enhance the secretion of GAG more effectively, especially when being cultured on the HPBS/HA-80/20 scaffold.

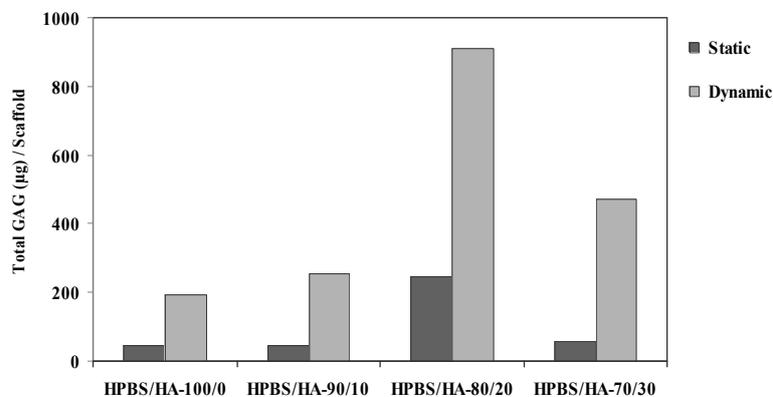


Fig. 3: GAG content secreted from chondrocytes cultured on different scaffolds in static and dynamic conditions after a 28-day incubation period.

5. Acknowledgement

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6. References

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