Engineered cartilage with internal porous high-density polyethylene support from bone marrow stromal cells: A preliminary study in nude mice

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Abstract

Tissue-engineered cartilage may have potential for the construction of clinical implants for the treatment of congenital deformities or post-traumatic defects. However, the lack of seed cells is a challenge, as is the maintenance of ideal shape and size. We have used bone marrow stromal cells (BMSCs) and a pre-shaped polyglycolic acid (PGA)-porous high-density polyethylene composite scaffold to solve these problems. High-density polyethylene was carved into cylindrical rods and encircled with PGA fibres to form scaffolds. Porcine BMSC were seeded into the scaffold and cultured in chondrogenic medium (high-glucose Dulbecco’s modified Eagle’s medium with 10% (v/v) fetal bovine serum, dexamethasone 40 ng/ml, transforming growth factor-β1 10 ng/ml, and insulin-like growth factor 50 ng/ml) for 3 weeks in vitro before the cell-scaffold constructs were implanted subcutaneously into nude mice. After 8 weeks of implantation, all specimens in the experimental group had formed mature cartilage around the polyethylene, and the prefabricated shapes and sizes were well maintained. The neoformed cartilage also grew into the pores of the scaffold with a fine interface between them; this gave the whole regenerated composite tissue characteristics similar to those of native cartilage. These results show that it is feasible to construct cartilage using BMSC and PGA-high-density polypropylene scaffolds. This may remove some of the obstacles that have prevented the clinical use of cartilage engineering such as limited volume, deformation, and a limited number of seed cells.

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Introduction

Grafting of cartilage is one of the most common treatments for congenital deformities and post-traumatic defects.1,2 Unfortunately, there is a lack of cartilage donors and operation can also cause scarring at the donor site.3 Tissue-engineered cartilage is currently being studied as a possible alternative for autologous cartilage grafting.4 However, the limited sources and lack of ability of chondrocytes to expand obviously hamper its development.5 Bone marrow stromal cells (BMSCs) can easily be isolated from autologous bone marrow and the numbers of cells considerably increased in vitro with multilineage (including chondrogenic) potential; they therefore become a promising source of cells.6

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Although BMSC chondrogenesis has been reported widely, two problems to remain to be solved before the technique can be used clinically. First, the regenerated cartilage usually fails to grow more than 2 mm thick because of limited nutrition in the centre, which obviously restricts the size and homogeneity of the engineered cartilage, and secondly it is difficult for engineered cartilage to retain its original shape during formation because of the weakness of the scaffold used and the increased expression of some contractile protein during chondrogenic induction.

To address these problems, we have used pre-shaped, porous high-density polyethylene with good biocompatibility and mechanical strength and accurate shape control as the internal support of a polyglycolic acid (PGA) scaffold, and the feasibility of regenerating cartilage of prefabricated shapes and sizes was investigated using these scaffolds with porcine BMSC.

Materials and methods

Isolation, culture, and expansion of cells

All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine. BMSC and auricular chondrocytes were obtained from newborn pigs and expanded as previously described. The first passage was used in our experiment.

Preparation of cell-scaffold constructs and grouping design

High-density polyethylene (Medpor, Porex, College Park, GA) was carved into cylindrical rods (3 mm in diameter and 5 mm long). Non-woven 25 mg PGA sheets 1 mm thick were then wrapped around the polyethylene rods to form the scaffold. In the experimental group (n = 8), porcine BMSC were seeded into the scaffold and cultured in regular medium (low-glucose Dulbecco’s modified Eagle’s medium (DMEM) plus 10% (v/v) fetal bovine serum (FBS)) for 5 days, The primary medium was then replaced by chondrogenic medium (high-glucose DMEM plus 10% (v/v) FBS, dexamethasone 40 ng/ml, transforming growth factor (TGF)-β1 10 ng/ml, and insulin-like growth factor (IGF-1) 50 ng/ml). Equivalent amounts of BMSC and chondrocytes were similarly seeded into the scaffolds and cultured in regular medium as negative and positive controls (n = 8 in each). All groups were kept in culture for another 3 weeks before being implanted subcutaneously into nude mice.

In vitro examination of the cell-scaffold constructs

The attachment and matrix production of the cells cultured on scaffolds were examined by scanning electron microscope (EM, Philips XL-30, Amsterdam, Netherlands).

Histology and immunohistochemistry

Nude mice were killed 8 weeks after implantation. The harvested samples were assessed histologically and immunohistochemically as previously described. Some of the specimens in the experimental and positive control groups were embedded in methylmethacrylate, cut with an SP1600 rotary microtome (Leica, Nussloch, Germany), and stained with Safranin-O.

Statistical analysis

The length and diameter of regenerated cartilage in the experimental and positive control groups were measured. The significance of differences in length and diameter between the two groups were analysed by Student’s t-test. Probabilities of less than 0.05 were accepted as significant.

Results

Culture of cell-scaffold constructs in vitro

Both BMSC and chondrocytes attached well to the PGA fibres with abundant deposition of extracellular matrix, indicating good biocompatibility between the cells and the scaffolds. Scanning EM showed that cells not only attached compactly to the PGA fibres but also grew into the pores of the high-density polyethylene (Fig. 1).

Gross view of in vivo engineered constructions

All the specimens in both the experimental and positive control groups maintained their original shape and size with no significant difference in length (mean (SD) 8.9 (0.34) compared with 9.3 (0.29) and diameter 5.9 (0.21) compared with...
6.2 (0.17)) of the engineered tissue. The specimens in both groups also formed cartilaginous tissue with a semitransparent white colour and smooth surface similar to that of native cartilage (Fig. 2). Cross-sectionally, neocartilage tightly conjugated with polyethylene and gradually grew into its pores (Fig. 3). As expected, the specimens in the negative control group formed no cartilage-like tissues.

**Histology of in vivo engineered construction**

All the specimens in the experimental and positive control groups showed typical histological features of cartilage with abundant lacunae (Fig. 4) and strong expression of type II collagen (Fig. 5). In the negative control group, however, only a little fibrous tissue was seen.

Slices of hard tissue that kept the polyethylene in the original site showed more cartilage-like tissue around the polyethylene core in the experimental and positive control groups. The neocartilage grew into the pores of the polyethylene and integrated compactly into the scaffold (Fig. 6), which
suggested good biocompatibility between the engineered cartilage and the high-density polyethylene.

Discussion

BMSC chondrogenesis has previously been reported. However, the neocartilage constructed by BMSC and biodegradable scaffolds have often been shown to be “hollow” if the thickness exceeded 2 mm and the shape could not be maintained. By using pre-shaped high-density polyethylene as the internal support for the PGA scaffold, we successfully constructed the cartilage with prefabricated shape and size using BMSC. The polyethylene was also biocompatible, which was shown by the regenerated cartilage growing in, which gave the tissue characteristics similar to those of native cartilage.

The thickness limit of engineered cartilage is closely correlated with the characteristic of cartilage itself. There is no blood vessel inside the cartilage and the transport of nourishment, metabolism and metabolised waste depends mainly on diffusion. Similar conditions were found when we engineered cartilage. After the outer cartilage has been generated, the transport of substances (including nourishment, induced factors, metabolised waste, and acidic material produced by degradation of PGA) in the centre of constructs is easily blocked, and necrosis and liquefaction can occur if the thickness exceeds the limit. In this study the thickness of the PGA was designed to be suitable for the permeation of nutrition and inductive factors and the polyethylene occupied the space in the centre. BMSC could therefore form cartilaginous structures without limiting thickness. Most importantly, neocartilage could conjugate the polyethylene tightly and grew into its pores, which gave the whole tissue the characteristics that made it similar to native cartilage and avoided the possible complication caused by direct implantation of polyethylene.

The main reason for the deformation in BMSC engineered cartilage is probably related to expression of α-smooth muscle actin, an underlying molecule that increases the forces of cell traction. Many studies have shown that TGFβ in chondrogenic medium could upregulate expression of α-SMA. However, the polyethylene had enough mechanical strength to withstand the contraction force caused by chondrogenic induction, and retained the original shape and size of the constructs.

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