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Synergistic effect of TGFβ-3 on chondrogenic differentiation of rabbit chondrocytes in thermo-reversible hydrogel constructs blended with hyaluronic acid by *in vivo* test

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Abstract

In this study, a hydrogel composite, based on the thermo-reversible hydrogel of p(NiPAAm-co-AAc) and hyaluronic acid (HA) was used as an injectable cell and growth factor carrier for cartilage tissue engineering applications. Rabbit chondrocytes were embedded in blended hydrogel composites co-encapsulated with the transforming growth factorβ-3 (TGFβ-3). The blended hydrogel with the embedded chondrocytes and HA co-encapsulating unloaded growth factors and those with the thermo-reversible hydrogel were used as the controls to examine the effects of TGFβ-3 on neocartilage formation. The blended hydrogel loaded with TGFβ-3 embedded with chondrocytes were injected subcutaneously into the nude mice. The mice were monitored for 8 weeks after the injection. Both the differentiation and level of cartilage-specific ECM production were significantly higher in the presence of HA and growth factor than in the control without the growth factor. The level of cartilage associated ECM proteins was examined by immunohistochemical staining (collagen types II and X) as well as by Safranin-O and Alcian blue (GAG) staining. The results showed the potential application of blended hydrogel mixed with the growth factor to neocartilage formation.

Keywords: Transforming growth factorβ-3 (TGFβ-3); Thermo-reversible hydrogel; Hyaluronic acid (HA); Chondrocytes; Neocartilage

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1. Introduction

Recent studies on biomaterials in the fields of tissue engineering have focused on three-dimensional scaffolds for cell delivery and therapy (Guo et al., 2006; Lee et al., 2004; Perets et al., 2003; Radice et al., 2000; Sheridan et al., 2000). The main aim was to examine a suitable matrix that could be used as a scaffold framework for cell viability and proliferation, whilst maintaining the original cellular phenotype. In this viewpoint, a variety of natural and synthetic materials were examined as potential carriers of cells or therapeutic agents for cartilage repair (Rudert et al., 2005; Solchaga et al., 2005; Galois et al., 2004; Wozney and Seeherman, 2004). Of these, hydrogels are among the most promising alternatives because they can provide a temporary support structure during the repair process, possessing similar properties to the native cartilage. In addition, many hydrogel formulations can be cross-linked in situ and be used in minimally invasive surgical procedures (Aliyar et al., 2005; Thornton et al., 2004).

Several types of hydrogels for applications to cartilage formation have been attracted considerable attention because the direct injection of cells, formulated with the appropriate soft and biodegradable hydrogel carrier, into a defect site can avoid the need for open surgery procedure. Hyaluronate (HA) fibrin, collagen, alginate, and chitosan have been a major focus for potential applications to neocartilage formation for the healing the cartilage defects (Lindenhayn et al., 1999; Stove et al., 2002; Kenneth et al., 2000; Ting et al., 1998; Estrada et al., 2001; Miralles et al., 2001; Roughley et al., 2006). In particular, hyaluronic acid, which plays a key role in the retention of proteoglycans in the cartilaginous matrix, has been recently been developed as a cell carrier. These biomaterials have satisfactory biocompatibility, cytotoxicity, biodegradability, and the ability to offer a good fixation to subchondral bone and host cartilage. Although alginate, collagen, and chitosan have been used in articular cartilage, their applications are limited because of severe problems such as immune reactions when implanted, high cost, and adhesion properties on the operation site. One approach to solving these problems has been to enhance the properties of biomaterials such as hybrid matrix systems. This enhancement would have several advantages: they surround the chondrocytes and offer protection against immunity rejection and phenotype instability. They can also present some advantages for the survival, proliferation, differentiation and synthesis ability of chondrocytes, as well as be able to synthesize matrix components and reconstitute the hyaline architecture of the cartilage (Abe et al., 2005; Babensee and Paranjpe, 2005; Solchaga et al., 2005; Yoo et al., 2005).

This study prepared a new hybrid material that would act as a three-dimensional scaffold for the transplantation of chondrocytes. The overall aim was to encapsulate both chondrocytes and HA within the thermo-reversible hydrogel for the simultaneous delivery of cells and TGFβ-3. The specific aim of this study was to determine the synergistic effect of TGFβ-3 released from the hybrid hydrogel on chondrocyte proliferation and cartilage ECM production. Thermo-reversible gels includes tissue culture with the cells were chosen as three-dimensional scaffolds and cell delivery agents. The polymer gels at temperatures at or above body temperature. Hence, the polymer will solidify when exposed to simulated body conditions.

2. Materials and methods

2.1. Chondrocyte isolation and cell culture

Chondrocytes were isolated from rabbit stifle articular cartilage using a collagenase digestion method (Syftestad et al., 1985). Briefly, male rabbits weighing 250 g were sacrificed by an over-dose of Nembutal, and the non-fibrillated articular cartilage of the knee was removed by a sterile dissection. The cartilage was finely minced and suspended in calcium and magnesium free phosphate-buffered saline (CMPBS), and washed. The fragments were sequentially digested in 0.2% collagenase (Worthington Biochemical, Lakewood, NY) in CMPBS for 3 h at 37 °C. The cells obtained from the collagenase digests were pooled and passed through a cell strainer (70 μm nylon: Falcon, Franlkin Lake, NJ) to remove the undigested matrix. The cells released in the supernatant were collected by centrifugation (1700 rpm, 15 min) and washed twice with CMPBS. The number and viability of the cells were determined using a hemocytometer and the trypan blue (0.25%) exclusion dye test. The collected cells were suspended in Dulbecco's modified Eagles medium (DMEM: Gibco BRL, Grand Island, NY)