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Regeneration of subcutaneous tissue-engineered mandibular condyle in nude mice

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ABSTRACT

Purpose: To explore the feasibility of regenerating mandibular condyles based on cartilage cell sheet with cell bone-phase scaffold compared with cell-biphasic scaffolds.
Materials and methods: Tissue-engineered mandibular condyles were regenerated by the following: 1) cartilage cell sheet + bone-phase scaffold (PCL/HA) seeded with bone marrow stem cells (BMSCs) from minipigs (cell sheet group), and 2) cartilage phase scaffold (PGA/PLA) seeded with auricular chondrocytes + bone-phase scaffold seeded with BMSCs from minipigs (biphasic scaffold group). They were implanted subcutaneously in nude mice after being cultured in vitro for different periods of time. After 12 weeks, the mice were sacrificed, and the specimens were harvested and evaluated based on gross appearance and histopathologic observations with hematoxylin and eosin, safranin O-fast green and immumohistochemical staining for collagen I and II. The histopathologic assessment score of condylar cartilage and bone density were compared between the 2 groups using SPSS 17.0 software.
Results: The 2 groups' specimens all formed mature cartilage-like tissues with numerous chondrocytes, typical cartilage lacuna and abundant cartilage-specific extracellular matrix. The regenerated cartilage was instant, continuous, homogeneous and avascular. In the biphasic scaffold group, there were still a few residual PGA fibers in the cartilage layer. The cartilage and bone interface was established in the 2 groups, and the microchannels of the bone-phase scaffolds were filled with bone tissue. The score of cartilage regeneration in the cell sheet group was a little higher than that in the biphasic scaffold group, but the difference was not significant ($p > 0.05$). There was no significant difference in bone tissue formation between the 2 groups ($p > 0.05$).
Conclusion: Both the cartilage cell sheet group and the biphasic scaffold group of nude mice underwent regeneration of condyle-shaped osteochondral composite. Without residual PGA fibers, the cell sheet group might have less chance of immunological rejection compared to biphasic scaffold group.

1. Introduction

Temporomandibular joint (TMJ) osteoarthritis (OA) is a degenerative disease affecting both cartilage and subchondral bone (Glyn-Jones et al., 2015). The clinical symptoms include long-term chronic pain and difficulty in mouth opening and chewing etc., which could impact the quality of life for the patients. A previous study has shown that 50% of the population above 55 years old maybe affected by TMJOA (Machon et al., 2011). Since the articular cartilage has limited ability for self-repair, at the end stage of OA,

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when medication is not working, joint replacement is needed to relieve pain and restore function of the TMJ. However, so far, neither autogenous bone grafts nor alloplastic prostheses produce a biological TMJ (Khadka and Hu, 2012; Ren et al., 2011).

Tissue engineering is a promising technology that can regenerate osteochondral composites including the biological structure of cartilage, subchondral bone, and its interface in vitro as reported already (Nukavarapu and Dorcemus, 2013; Oka et al., 1997; Ding et al., 2013). However, most studies show limited ability to restore local defects, which is hard to repair, and poor integration with surrounding tissue forbearing biological forces (Nukavarapu and Dorcemus, 2013; Oka et al., 1997). In 2013, Ding et al. (2013) used a tissue-specific biphasic scaffold fabricated by computer-aided design and manufacture (CAD/CAM) technology to regenerate a biological goat femoral head. Chondrocytes and bone marrow stem cells (BMSCs) were seeded into the biphasic scaffolds for cartilage and bone regeneration respectively. It was the first time that the cell-scaffold composites of goat femoral head were successfully regenerated subcutaneously in nude mice. The regenerated femoral heads presented a well-integrated osteochondral interface. This study provides a promising method for condylar regeneration. However, there are no further reports on the application of this technology in large animals with complete immunity. Previous studies (Cao et al., 1998; Ylinen et al., 2002) have shown that cartilage phase scaffold of polyglycolic acid/polylactic acid (PGA/PLA), a non-biological synthetic material, produces acid degradation products leading to aseptic inflammation and failure. Cartilage cell sheet is a scaffold-free membrane based on chondrocytes stacked and extracellular matrix (ECM) secretion (Yang et al., 2005). It offers a promising alternative to repairing defects without the challenges caused by non-biological synthetic materials (Ge et al., 2016). It has been used to repair local cartilage defects in knee joints in an animal study in Japan (Sato et al., 2014). There are no reports of regenerating a whole condyle by combining cartilage cell sheet with bone-phase scaffolds.

In this study, we aimed to study the feasibility of cartilage cell sheet covering bone-phase scaffold to subcutaneously regenerate the mandibular condyle in nude mice, and compare it with the previously reported cell-biphasic scaffold composite to evaluate the structure of regenerated cartilage, bone, and osteochondral interface. If the cartilage cell sheet with bone-phase scaffold and BMSCs can regenerate mandibular condyle in nude mice, it can then be tested in immunocompetent large animals later for successful cartilage regeneration.

2. Materials and methods

2.1. Experimental design

All animal experiments were approved by the Independent Ethics Committee of the 9th People's Hospital (No. 201681). All animals received humane care in compliance with the “Guide for Care of Laboratory Animals” as detailed by the National Ministry of Science. In total, twelve 7-week-old athymic nude mice (Shanghai Slac Laboratory Animal Co., Ltd.) were used in this research. They were randomly divided into 2 groups with 6 mice in each group. In group 1, the nude mice were implanted with cell-biphasic scaffold composite (biphasic scaffold group), and in group 2 the nude mice were implanted with cartilage cell sheet covering cell-bone-phase scaffold (cell sheet group).

2.2. Isolation and culture of cells

Both chondrocytes and BMSCs were obtained from a 10-month-old minipig. Cartilage samples were harvested from auricular cartilage. Chondrocytes were isolated, cultured, and expanded according to reported methods (Rodriguez et al., 1999). Chondrocytes in passage 2 were harvested for regeneration of articular cartilage. BMSCs were isolated from the marrow blood of the iliac crest, cultured, and expanded as previously described (Shang et al., 2001). BMSCs in passage 2 were harvested for the construction of subchondral bone.

2.3. Preparation of cartilage cell sheet

Chondrocytes in passage 2 were seeded at a high density of 10×10^7 cell/mL onto the 6-well plate. The cell sheet membrane formed quickly after 24 h. After that, it was cultured under chondrogenic medium as described in previous literature (Ding et al., 2013) for 4 weeks. After 4 weeks of in vitro culture, the cartilage cell sheet had basic mechanical strength and could be easily manipulated by forceps.

2.4. Preparation and fabrication of cartilage phase scaffold

PGA/PLA scaffolds (Fig. 1E) were prepared according to our previously established method (Liu et al., 2008, 2010). Briefly, 100 mg of unwoven PGA fibers (provided by the National Tissue Engineering Center of China) with 10% PLA content was compressed into the surface shape of minipig mandibular condyles through male mould and female mould press formation. The fabricated PGA/PLA scaffold fit the PCL/HA scaffold perfectly (Fig. 1C and F). The PGA/PLA scaffold has good biocompatibility with over 85% cell seeding efficiency (Ding et al., 2013). Microstructure and cell adhesion of the scaffold was evaluated by scanning electron microscopy (SEM; S-2150 Hitachi Ltd, Japan).

2.5. Preparation and fabrication of bone-phase scaffold

Hydroxylapatite (HA) powder and polycaprolactone (PCL) particles were placed into a drying oven overnight and they were sufficiently mixed at 120 °C by blender. Computed tomography and digital reconstruction were used to scan and model the TMJ of the minipig (Fig. 1A and B). The bone-phase scaffolds (PCL/HA) were printed by a 3-dimensional (3D) printer at operating temperature (90 °C) (Fig. 1D) (Jiang et al., 2013). The PCL/HA scaffolds had the dimension of $13 \times 11 \times 10$ mm^3 (length × width × height), which was equal to the anatomic size of mandibular condyle of the minipig. To promote cell distribution and nutrient transportation towards the inside of the scaffold, the PCL/HA scaffold was designed to contain regular 3D interconnecting microchannels (200–400 mm in pore size) with a porosity of 54.6 ± 1.2% (Jiang et al., 2012).

2.6. Preparation of cells-scaffold complex

Chondrocytes in passage 2 (Fig. 2B) were seeded into the PGA/PLA scaffolds (Fig. 2A) at a density of 5.0×10^7 cells/mL. The cell suspension was dropped into the scaffolds and incubated for 4–5 h to allow sufficient cell adhesion (Liu et al., 2010; Yan et al., 2009). PGA/PLA scaffolds seeded with chondrocytes were cultured in vitro for 4 weeks in chondrogenic medium supplemented with 10 ng/mL of transforming growth factor-β1 (TGF-β1, Inter Gen, Burlington, MA), 40 ng/mL of dexamethasone (Sigma–Aldrich, St. Louis, MO, USA), 100 ng/mL insulin-like growth factor 1 (IGF-1; Sigma–Aldrich), and 1% insulin-transferrin-selenium (ITS; Sigma–Aldrich).

Similarly, BMSCs in passage 2 (Fig. 2F) were seeded into the microchannels of PCL/HA scaffolds (Fig. 2E) at a density of 2.5 × 107 cells/mL. The cell suspension was dropped into the microchannels and incubated for 4–5 h to allow sufficient cell adhesion. PCL/HA scaffolds seeded with BMSCs in scaffold

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