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A novel cartilage tissue construction based on artificial cells and matrix-shaping

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ABSTRACT

Cartilage tissue engineering through culturing chondrocytes in 3D porous scaffolds has potential for the repair of articular cartilage defects. To promote cell distribution in the scaffold, a novel construction method was proposed based on artificial cells and matrix-shaping. Results showed that the construct had better proliferation and ECM secretion with bFGF. Cells had the best proliferation, largest GAG and type II collagen content on day 10 in the 2D condition without bFGF, while these were enhanced by bFGF to day 15. Under 3D conditions, the highest proliferation and GAG content were on day 15 in bFGF-free medium while these two values were improved by bFGF, with the highest appearing on day 20. In conclusion, this construction method is efficient and provides a new and promising approach to cartilage regeneration.

1. Introduction

Articular cartilage is an avascular tissue that connects the joints and bones smoothly. Its only cells are chondrocytes, which synthesize the extracellular matrix (ECM) of cartilage. Many diseases occur if there is something wrong with the ECM. For example, osteoarthritis (OA) is caused by degradation of the ECM [1]. And lesions of cartilage are hard to heal due to poor regeneration of chondrocytes. One method that is often used to treat with such situations is autologous chondrocyte transplantation (ACT). However, ACT has some drawbacks in healing cartilage diseases. One is that this method is not suitable for defects larger than 4 cm^2 [2], and chondrocytes are prone to losing phenotype and forming non-functional fibrocartilage when cultured *in vitro* [1,3,4]. To solve these problems, the rapidly developing field of tissue engineering (TE) provides an alternative to organ transplantation by restoring, maintaining or enhancing existing tissues and organs with living cells [5]. Biomaterials, cells, and signal molecules such as growth factors are usually combined for use in TE [6,7].

Regarding biomaterials, most current scaffolds have the problem that the cells are not well-distributed in the scaffold. Here, we develop a new construction method using artificial cells and choose low melting point agarose (LMPA) as a filling matrix to embed artificial cells and shape the constructs. LMPA is a fully non-cytotoxic natural hydrogel with high water content and porous elastic structure [8]. And it can form hydrogel at 37 °C which does not harm to cells. Artificial cells, which are also called microencapsulated cells, can provide a liquid environment for cells [9] and maintain cell phenotype [10]. The resultant artificial cells can distribute in the matrix evenly. Artificial C5.18 cells and the corresponding constructs were prepared and cultured on a shaker (2D) and Rotary Cell Culture System (RCCS, 3D) for evaluating the effects of culture conditions with/without bFGF. A series of tests (CCK-8 for cell proliferation, DMMB detection for GAG, and type II collagen secretion) were all conducted.

2. Experimental

2.1. Fabrication of artificial cells and the construct

The fabrication of artificial cells was similar to the work we reported elsewhere by using a high-voltage electrostatic droplet generator [11]. The resultant artificial cells were mixed with 1.25% sterile LMPA (Suolaibao Bio-Technology Co., Ltd., China) and the homogeneous solution was injected into a PTFE template (Duolingfu Chemistry Co., Ltd., China) to form constructs 6 mm in diameter and height. Then, template was removed and the construct was obtained. The constructs were cultured under two different conditions, 2D (in a 24-well plate under a shaking speed

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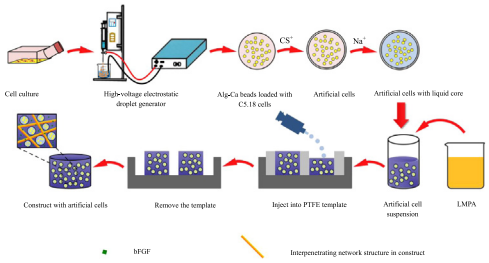

Scheme 1. Schematic representation of construct preparation.

of 100 rpm) and 3D (RCCS, NASA), with or without 100 ng mL^{-1} basic fibroblast growth factor (bFGF, PeproTech). A simple schematic of the preparation process is shown in Scheme 1.

2.2. Cell proliferation assay

A CCK-8 assay was used to determine cell proliferation [12]. The value of OD_{450} is directly in proportional to the number of living cells. Cells were obtained from the constructs and mixed with 110 μL medium containing 10 μL CCK-8. Then the solution was shifted to a 96-well plate and incubated for another 4 h at 37 °C according to the manufacturer's instructions. Absorbance was quantified at 450 nm through a SPECTRA MAX microplate reader (Thermo).

2.3. Glycosaminoglycan (GAG) release

The concentration of sulfated GAG in construct cells was assessed by dimethylmethylene blue (DMMB, Sigma) dye-binding assay. A standard curve was obtained using shark chondroitin sulfate (1–100 μg/mL, Sigma) as a standard. Cells were obtained from the constructs through disrupting the hydrogel and membrane, and GAG were digested by papain digestion. About 100 μL of each sample and 2.5 mL DMMB dye were added to a spectrophotometer cuvette, and absorbance was read on a spectrophotometer at 525 nm. GAG concentrations were calculated from the standard curve.

2.4. Type II collagen secretion

Type II collagen detection was carried out following the manufacturer's instructions (Cusabio, China). In brief, cells were obtained from the constructs and 100 μL of cell samples was added to each well followed by adding 100 μL of Biotin-antibody and HRP-avidin and, finally, TMB. And samples were evaluated on days 5, 10, 15 and 20.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation. Statistical significance was calculated by one-way ANOVA. $^{*}P < 0.05$ and $^{**}P < 0.01$ were considered significant. Each measurement reported was based on duplicate analysis of at least three independent experiments.

3. Results and discussion

3.1. Morphology of artificial cells and the constructs

As shown in Fig. 1a, the artificial cells were spherical with liquid core and smooth surface. The distribution of cells in the microcapsules was uniform and clearly visible. The particle size distribution was 150–280 μm, and the average particle size was 220 μm, which was suitable for cell cultivation. What is more, C5.18 cells presented a spherical morphology, which has been demonstrated to be relevant to the production of cartilage-related ECM proteins [13,14]. The constructs were transparent cylinders 6 mm in diameter and height and their size was uniform with a smooth surface and consistent mechanical strength (Fig. 1b). And artificial cells could distribute in the matrix evenly which could be seen in Fig. 1c.

3.2. Cell proliferation in the constructs

As can be seen in Fig. 2, cell proliferation in 2D and 3D constructs with bFGF was greater than without bFGF. Studies have revealed that bFGF is beneficial for enhancing cell proliferation [15] and retaining chondrocytes' phenotype [16–18]. Under 2D conditions, cells without bFGF attained their highest proliferation rate on day 10 ($OD_{450}=0.263$), while they reached their peak ($OD_{450}=0.375$) on day 15 with bFGF. Cell proliferation was improved by bFGF and significantly higher ($P<0.05$) than without bFGF (Fig. 2a). The same trend was observed under 3D conditions (Fig. 2b). The cells showed similar proliferation rates in the two groups without obvious decline. Cells achieved their highest proliferation on day 15 ($OD_{450}=0.29$) but this was extended to day 20 ($OD_{450}=0.326$) with bFGF.

3.3. Concentration of GAG

ECM is an important part of cartilage. A large quantity of GAGs and type II collagen demonstrate cells have a chondrogenic phenotype [19]. For 2D condition, significant differences were shown between the two groups (Fig. 3a). GAG concentrations in the construct cells increased steadily for 10 days (1.24 mg/mL) and

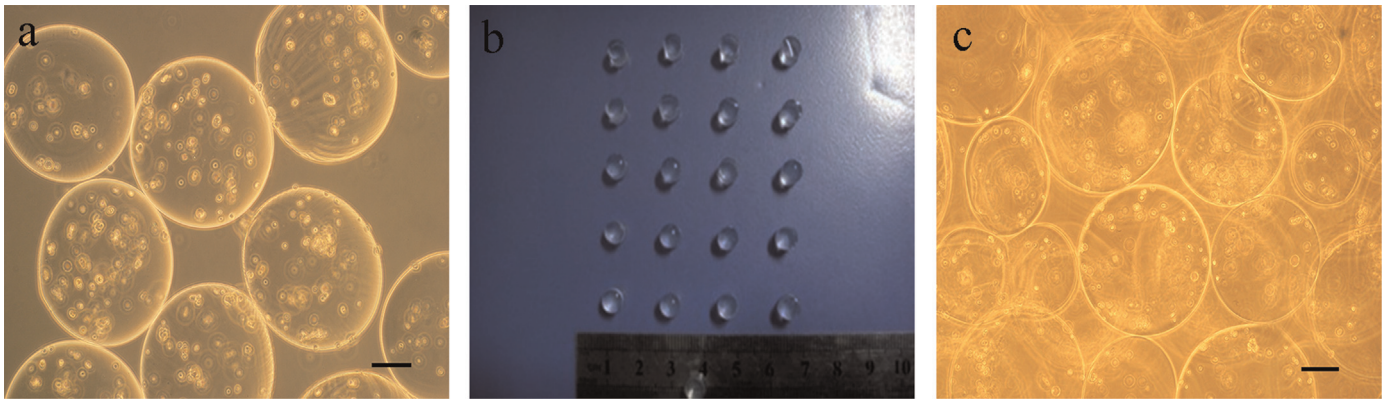

Fig. 1. Morphology of artificial C5.18 cells (a, bar=100 μm); the constructs (b) and artificial cells in construct (c, bar=100 μm).