Development of three-dimensional hybrid scaffold using chondrocytes encapsulated alginate hydrogel

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Statement of Purpose: Hydrogels are advantageous materials because of their chemical similarity to the extracellular matrix, the flexibility, rapid diffusion of hydrophilic nutrients and metabolites. Using the rapid prototyping (RP) methods, we are able to fabricate freeform three-dimensional (3-D) scaffolds which withstand specific loads. In this paper, we report the example of RP-based hybrid scaffold fabrication using alginate hydrogel encapsulated chondrocytes and growth factor for cartilage regeneration.

Methods: Although hydrogels are advantageous in its chemical and biological properties, it is very difficult to fabricate complex-shape of scaffolds and withstand specific loading environment. To develop 3-D hybrid scaffolds, we applied the microstereolithography system (Fig. 1) [1]. The microstereolithography apparatus consisted of a UV laser (λ=351.1nm, Spectra-Physics Beamlok 2065-4S), stage system (M-ILS50CC, Newport, USA), optic component with prism and mirror, a vertical elevator and controller (PMAC, Korea), and a liquid photopolymer reservoir. In order to create a 3-D structure, 2-D slicing shapes (slice data) of the desired 3-D data are required.

The biocompatible, biodegradable, photopolymerizable liquid prepolymer was prepared by the polymerization of trimethylene carbonate (TMC) with trimethylolpropane (TMP) and subsequently end-capped with an acrylate group [2].

A 5% solution of alginic acid in DMEM was dissolved using stirrer completely [3], and then counted chondrocytes put into them. A photo-polymerized 3-D scaffold was sterilized with 70% ethanol, washed with PBS, and air-dried. The Alginate solution encapsulated chondrocytes was injected into 3-D scaffolds and put in 5% $CaCl_2$ for crosslinking.

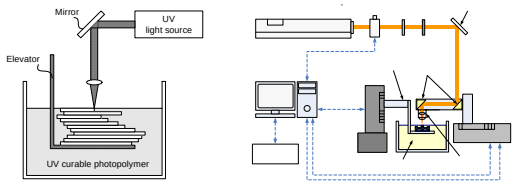

Figure 1.Schematic drawing of the principle and apparatus of developed microstereolithography system

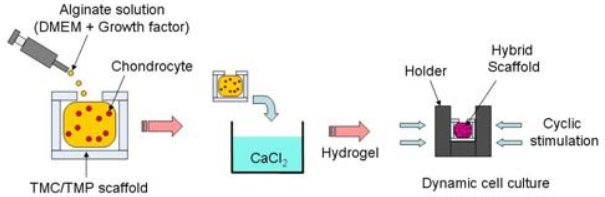

Figure 2.Schematic diagram showing the fabrication process of 3-D hybrid scaffold encapsulated cell

After gelation, all hybrid scaffolds were incubated with DMEM with 10% FBS, 100 units-penicillin/ml and 100 μg-streptomycin/ml at 37℃ in a humidified atmosphere of 5% CO_2 incubator.

To evaluate morphology of the prepared hybrid scaffolds, observation by scanning electron microscopy (SEM, JSM-6390LV, JEOL, Japan) and light microscopy (CKX41, Olympus, USA) was carried out. The authors of this paper, attempted to evaluate the feasibility of an in vitro cultured 3-D hybrid scaffolds inside chondrocyte encapsulated hydrogel.

Results/Discussion: We fabricated 3-D hybrid scaffolds using microstereolithography system (Fig. 3). Scaffolds of 4mm in height and 4.5mm by 4.5mm in length and width were fabricated with meshed framework which could be withstood the mechanical loading effectively. Their line depth (~150um), line width (~120um) could be controlled with varying laser power, scan path and scan speed. The images of chondrocytes-encapsulated hybrid scaffold and cultured results were shown in Fig. 4.

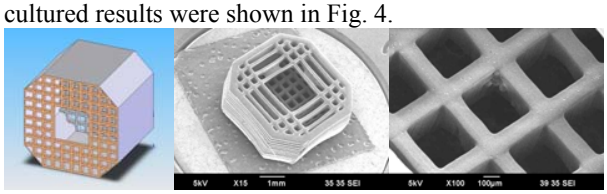

Figure 3.CAD design of hybrid scaffold and SEM images of fabricated hybrid scaffold

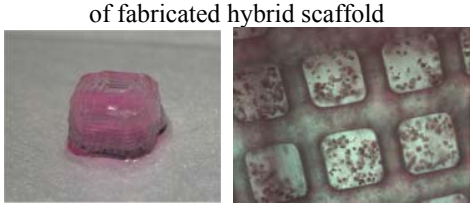

Figure 4.Images of 3-D hybrid scaffold: (a) chondrocytes encapsulated in hybrid scaffold (b) cultured chondrocytes after 3days

Conclusions: In this paper, we fabricated regular-shaped, mechanically stable and biomimetic hybrid scaffolds with chondrocytes encapsulated hydrogel. After cell culture, hybrid scaffold was effective in keeping phenotype of chondrocytes.

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References:

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