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Development of Three-Dimensional Hybrid Scaffold Using Chondrocyte-Encapsulated Alginate Hydrogel

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Hydrogels are useful materials because of their chemical similarity to the extracellular matrix and their ability to rapidly diffuse hydrophilic nutrients and metabolites. Using rapid prototyping (RP) methods, we fabricated freeform three-dimensional (3-D) scaffolds with chondrocytes encapsulated in an alginate hydrogel. The 3-D hybrid scaffold was developed as a combination of two components, a trimethylene carbonate (TMC)/ trimethylolpropane (TMP) framework and an alginate hydrogel containing encapsulated chondrocytes. To develop 3-D hybrid scaffolds, we employed a microstereolithography (MSTL) system. A biocompatible, biodegradable, and photopolymerizable liquid prepolymer was prepared by the polymerization of TMC/ TMP, and was subsequently end-capped with an acrylate group. The results of analyzing a cell culture indicate that because of the biomimetic nature of the encapsulated chondrocytes, scaffolds effectively retain the phenotypic function of the chondrocytes within their structure. The proposed 3-D hybrid scaffolds can be used for cartilage regeneration.

1. Introduction

For the generation of large and complex-shaped cartilage tissues, scaffolds must possess several structural features that are difficult to achieve using conventional scaffold design and fabrication technologies. The precise control of scaffold porosity and internal pore architectural parameters (*e.g.*, pore geometry, size, interconnectivity, and

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orientation) will be necessary to maximize nutrient diffusion and interstitial fluid flow to control cell growth and functions as well as to optimize the mechanical functionality of the scaffold along with the mechanical properties of the regenerated tissue. The ability to design and manufacture tissues utilizing a range of materials will be essential for the control of the chemical and physical properties that strongly affect cellular biology and ultimately tissue formation within a scaffold architecture.[1–6]

More recently, tissue engineers have started to design and study hybrid or biphased scaffolds. Hydrogels are useful materials because of their chemical similarity to the extracellular matrix and their ability to rapidly diffuse hydrophilic nutrients and metabolites. However, although hydrogels have advantageous chemical and biological properties, they are not well suited to the fabrication of complex scaffold shapes capable of withstanding loading. Hence, in this study, we employed a microstereolithography (MSTL) system to develop three dimensional (3-D) hybrid scaffolds for cartilage regeneration.

2. Materials and Methods

2.1 *Scaffold fabrication system*

To develop the 3-D scaffolds, we applied MSTL. Since there is no commercially available MSTL apparatus, the entire equipment was set up from scratch. The fabrication technique we used is based on conventional stereolithography (SL), in which a UV laser beam irradiates the surface of a UV-curable liquid photopolymer, causing it to solidify. Microstereolithography allows the microarchitecture of a device or scaffold design and the precision microshaping of surfaces for advanced medical procedures.[2–6] In the MSTL system, a laser beam of 5–10 μm in diameter is used to solidify a very small area of the photopolymer. To fabricate very small 3-D scaffolds, a precision control system and an optical system are needed. The MSTL system has to be designed systematically to fabricate the desired 3-D structures because the system and fabrication process are strongly affected by various key factors including the viscosity of the photopolymer, optical alignment, and elevator movement. We redesigned our previous MSTL system using an axiomatic approach for the fabrication of 3-D scaffolds. In our analysis, we identified the key factors affecting microstructure fabrication, and an improved MSTL system was constructed to produce 3-D scaffolds. Figure 1 shows a schematic drawing of the new scaffold fabrication apparatus. Figure 2 shows a photograph of the constructed scaffold fabrication system. The most significant difference between the previous system and the new one is that the design matrix of the previous system is coupled, whereas the new one is not. This is due to the use of a separate x-y stage and a z stage instead of an x-y-z stage.

2.2 *UV-curable biomaterial*

The scaffolds must be manufactured in a material that is biocompatible and biodegradable. In this regard, we synthesized trimethylene carbonate (TMC)/ trimethylolpropane (TMP) prepolymer because it has good weight loss, water uptake, swelling depth, and mechanical properties (maximum Young's modulus = 200 MPa).[7]

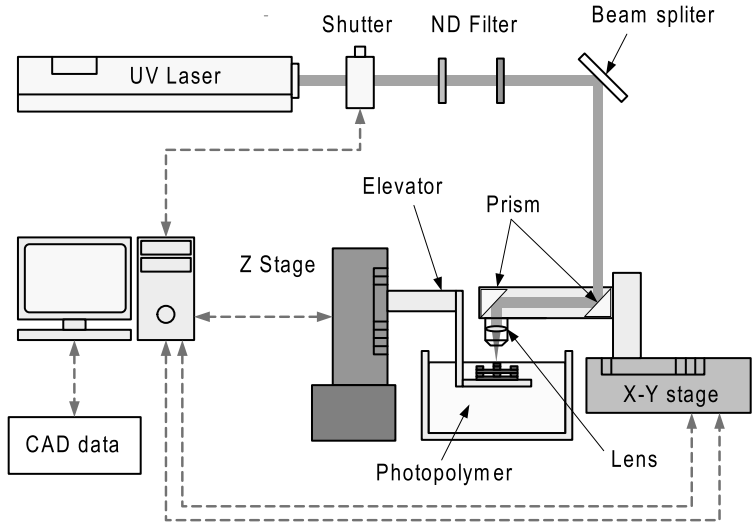


Fig. 1. Schematic drawing of new scaffold fabrication apparatus.

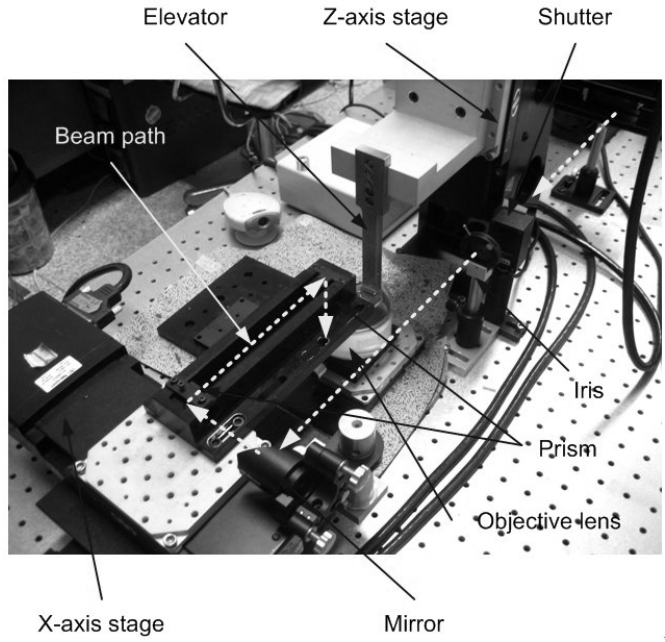


Fig. 2. Photograph of the constructed scaffold fabrication system.

Figure 3 shows the sequence of reaction steps for the preparation of a (co)oligomer, the acrylation of the (co)oligomer at both terminal ends, and photocuring. A series of TMC-based (co)oligomers was synthesized by ring-opening (co)polymerization using TMP

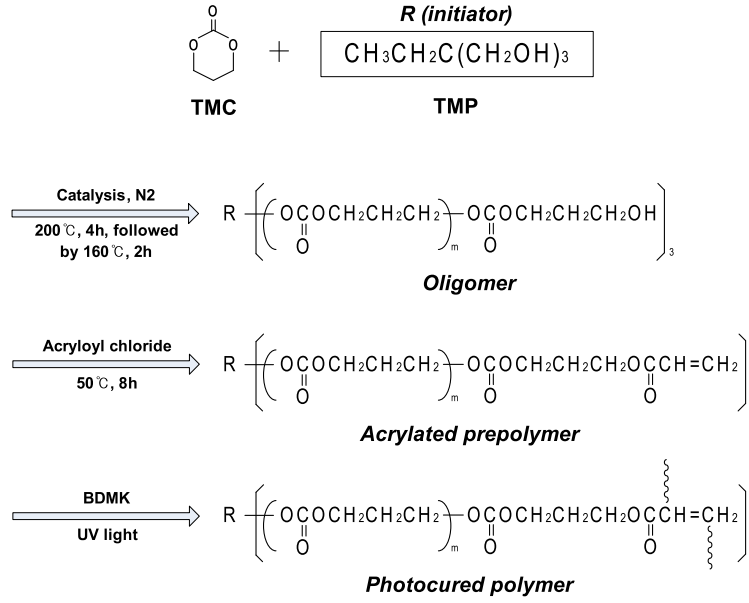


Fig. 3. Schematics of preparation processes of (co)oligomer and acrylate-end-capped prepolymer and photocuring.

as an initiator and stannic octanate as a catalyst. The TMP-initiated oligomer has three branches.[2]

3. Cell-Encapsulated Hybrid Scaffold

The hybrid scaffold was developed as a combination of two components, a TMC/TMP framework and an alginate hydrogel containing encapsulated chondrocytes. Figure 4 shows the design of the hybrid scaffold framework and a scanning electron microscopy (SEM) image of the manufactured TMC/TMP scaffold framework. The top and bottom layers are made of a lattice, which provides a frame so that alginate hydrogel does not leak out before gelation. To inject the liquid alginate solution into the hybrid scaffold framework, a square hole was opened at the centre of the top layer. Figure 5 shows a schematic diagram of the experimental procedure for fabricating hybrid scaffolds.

A 5% solution of alginic acid in Dulbecco's modified Eagle's medium (DMEM; Gibco) was dissolved completely using a stirrer and then a known number of chondrocytes was placed into the solution. A photopolymerized 3-D scaffold was sterilized with 70% ethanol, washed with phosphate buffered saline (PBS), and air-dried. The alginate solution containing encapsulated chondrocytes was injected into the 3-D scaffold, which was placed in 5% $CaCl_2$ to induce crosslinking. After gelation, all hybrid scaffolds were incubated with DMEM containing 10% fetal bovine serum (FBS; Gibco),

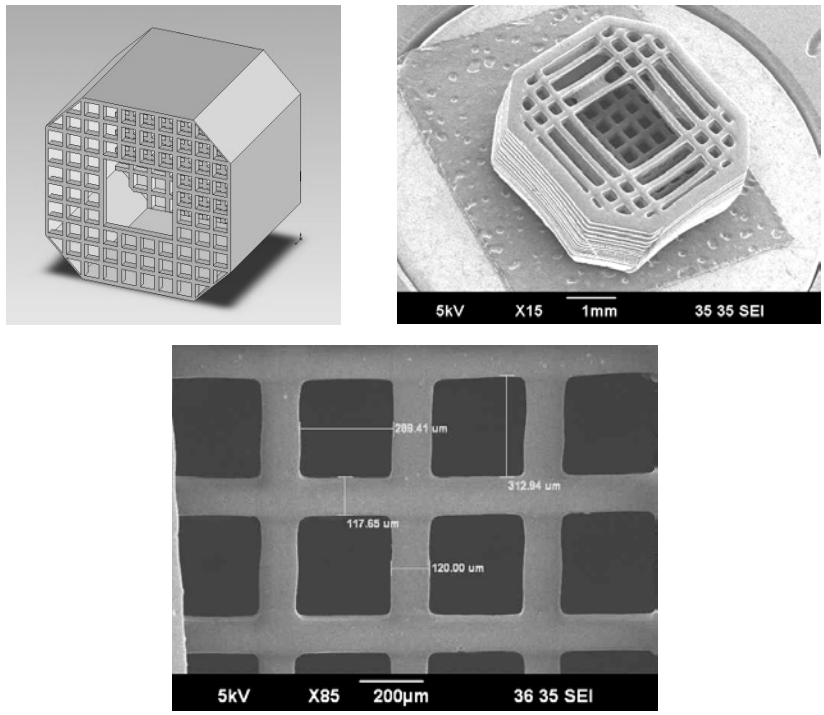


Fig. 4. 3-D CAD model and SEM image of TMC/TMP framework used for hybrid scaffold.

100 units penicillin/ml, and 100 µg streptomycin/ml at 37°C in a humidified atmosphere of 5% CO_2.

To evaluate the morphology of the prepared hybrid scaffolds, observation by SEM and light microscopy was carried out. We attempted to evaluate the feasibility of culturing *in vivo* 3-D hybrid scaffolds containing chondrocyte-encapsulated hydrogel.

4. Results

The meshed framework of 4.5×4.5×4.0 (mm) scaffolds effectively withstood mechanical loading. The line depth (~150 µm) and line width (~120 µm) could be controlled by varying the laser power, scan path, and scan speed.

We used light microscopy to examine the 3-D hybrid scaffold after 4 weeks of *in vivo* cultivation. It was observed that the cartilaginous extracellular matrix was expressed in the specimen. Figure 6 shows the result of *in vivo* cultivation.

Histological observation using Alcian blue and by haematoxylin and eosin (H&E) staining of the hybrid scaffold after four weeks of implantation was performed. After four weeks *in vivo*, all cell-encapsulated hybrid scaffolds showed a cartilage-like tissue morphology with typical lacunae formation (Fig. 7). It was found that the growth of chondrocytes in the alginate hydrogel was promoted.

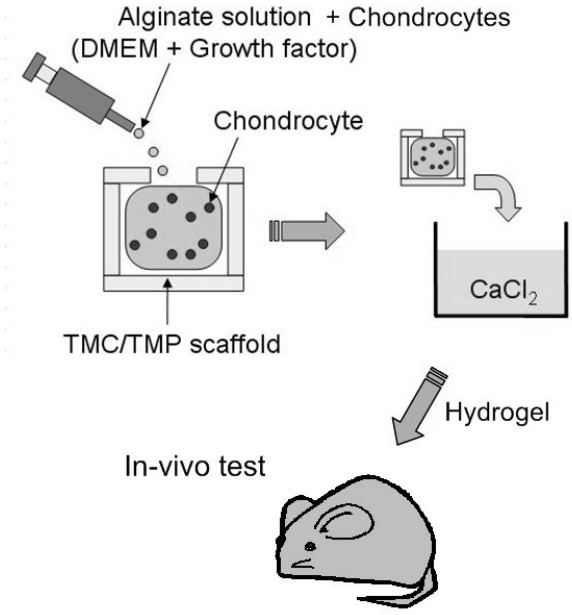
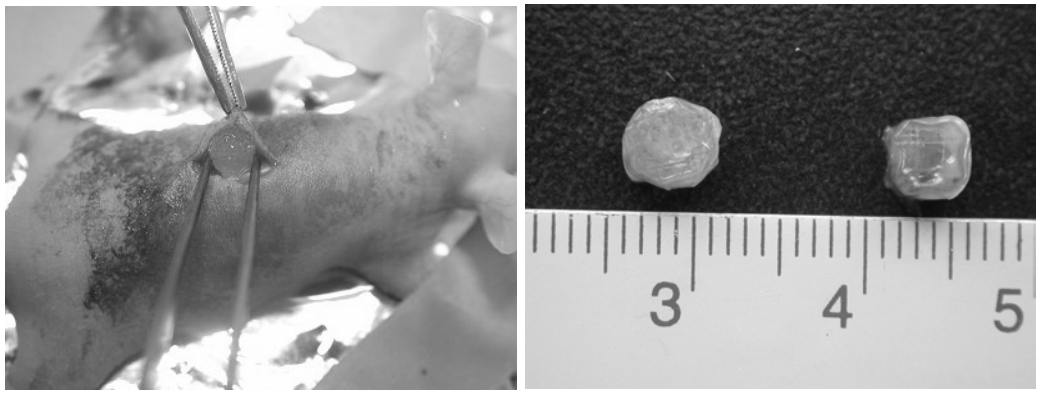


Fig. 5. Schematic diagram showing the fabrication process of 3-D hybrid scaffold containing encapsulated cells.

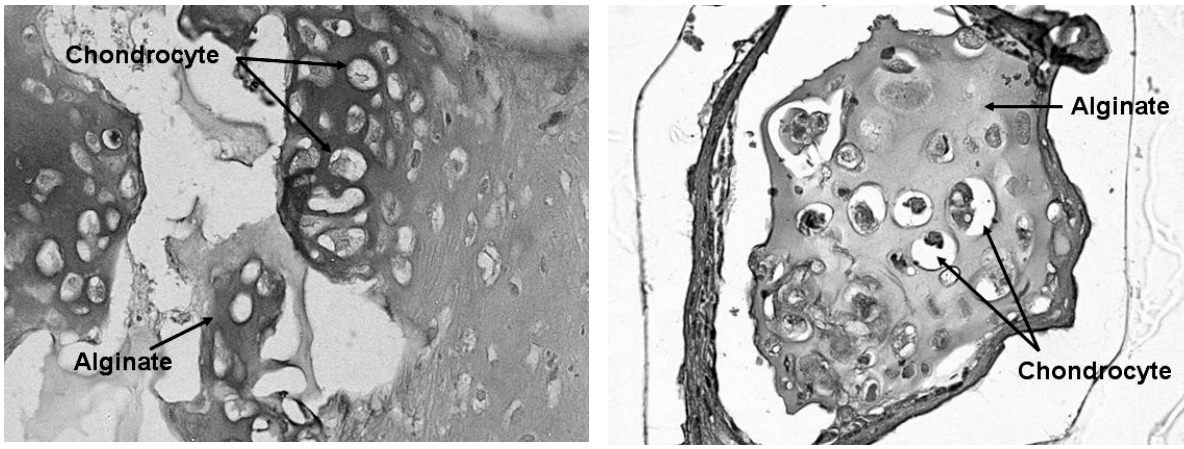


(a) (b)

Fig. 6. *In vivo* tissue growth test: (a) implantation and (b) hybrid scaffolds after four weeks.

5. Conclusions

In this paper, we fabricated regular-shaped, mechanically stable and biomimetic hybrid scaffolds using chondrocyte-encapsulated hydrogel. Cell-culture results indicate that because of their biomimetic nature, the chondrocyte-encapsulated scaffolds



(a) (b)

Fig. 7 Histological evaluation of chondrocytes after four weeks implantation: (a) Alcian blue staining image (400×) and (b) H&E staining image (400×).

effectively retain their phenotypic function within the scaffold structure. The proposed 3-D hybrid scaffolds can be used for cartilage regeneration.

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