Inverted Colloidal Crystals as Tissue Engineering Scaffolds

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Introduction

Three-dimensional (3D) geometry actively regulates the growth of cells, communication among cells, and interactions between cells and the matrix, as well as cell differentiation pathways. Until now various methods have been utilized to generate a 3D synthetic scaffold, but rarely uniform and ordered structure could be achieved, though it has great advantages. In the course of our study, a new type of scaffold with controlled 3D organization of the pores has been developed. [1,2] Highly ordered colloidal crystal "template" has been prepared from 100μm diameter polystyrene beads using modified methods of colloidal crystal preparation conventionally utilized for photonic materials synthesis. With respect to engineering, the architecture of inverted opals can significantly reduce the mass transport resistance around it. Also, three-dimensionally ordered uniform size of pore structure makes it possible to understand effects and conditions of 3D contacts on cell development in systematic fashion. Colloidal crystal scaffolds were prepared from sol-gel and polymerization methods. To test the 3D geometry effect and biocompatibility of the scaffold, human promyeloblast HL-60 and human thymus Hs202.TH cell lines were accessed.

Experimental

Conventional protocols of colloidal crystals preparation in sub-micron range (100-1500nm) took advantage of gravity sedimentation, capillary forces, gentle agitation, and hydrodynamic forces to facilitate generation of closely packed and ordered structure.[3] However, these methods proved inadequate when applied to micron size (>10μm) beads because of their relatively heavy mass and large volume. In other respects, there were two advantages. The first one was a relatively faster sedimentation in comparison to nano-meter beads which, due to their slow sedimentation rate, require application of pressure and fluid together to speed up precipitation. The other advantage was that beads' agitation by shear force worked more effectively because of their relatively large volume. Accordingly, we hypothesized that controlled slow sedimentation and gentle agitation during beads precipitation would assist in getting 3D ordered colloidal crystals in micron-range.

To control sedimentation rate, two or three aqueous beads were dropped 25 times through a long, Pasteur glass pipette in continuously 10 minute intervals. The glass pipette was used as a thin funnel and caused a bottle neck effect to precipitating beads. Gentle agitation assisted the beads' movement and positioned them at the lowest energy spot. Once the bottom area was covered with beads, their rugged surface served as a template in the second layer formation, in ordered fashion (Figure 1). As a result, 100μm beads were closely packed in highly ordered HCP structure. Prepared colloidal crystals were heat-treated at 120°C for 4 hours to guarantee well interconnected channels and increase the size of a channel.

As scaffolding materials, sodium silicate and poly (acryl-amid) were utilized. Sodium silicate solution, diluted with 50wt% pure water, was infiltrated into a colloidal crystal, assisted by centrifugation. Sodium silicate sol was transitioned to gel inside of a furnace at 300°C. 30wt%

poly (acryl- amid) solution with 5wt% cross-linker was prepared and infiltrated in the same manner. Within few minutes after adding initiator, polymerization was completed. Colloidal crystals infiltrated with scaffolding materials were put in THF for one day and polystyrene beads were completely dissolved by it. As a result, inverted colloidal crystals with 100μm pores were created. Particularly hydrogel scaffolds were coated with clay particles to improve cell adhesion before testing them. 0.5wt% Poly (acrylic-acid) (PAA), 0.5wt% poly (diallydimethylammonium) (PDDA), and 0.5wt% Clay particles were layered sequentially, using a layer-by-layer method.

Human promyeloblast HL-60 and human thymus Hs202.TH cell lines were cultured following ATCC protocols. Cells were seeded on scaffolds and cultured for one week inside of a rotary cell culture vessel. Then cells on a scaffold were observed by a fluorescence microscope, confocal microscope, and SEM.

Results

Highly ordered 100μm colloidal crystals were prepared by simple modification of conventional methods used in nano-range. From the bottom to top area, highly ordered HCP structure was acquired (fighre2. A, B). However, an uncompleted and a less ordered top layer were common defects. After infiltration of scaffolding materials and removing poly styrene beads, highly ordered 3D structure was maintained (Figure2. C.D). Size of the interconnected channel was around 20-25μm depending on the heat treatment temperature. Incomplete infiltration of sol-gel materials, due to its high viscosity, usually caused breakage of a scaffold into several pieces. Incompletely transitioned sodium silicate gel was slightly dissolved by media and resultantly increased pH to around 9. To reduce this effect, usually silicate scaffolds were washed several times with pure water before using them. Monomer solution was completely infiltrated into colloidal crystals resulting in better maintenance of the entire structure. To reduce shape deformation during the beads' dissolving step, relatively higher monomer concentration was applied.

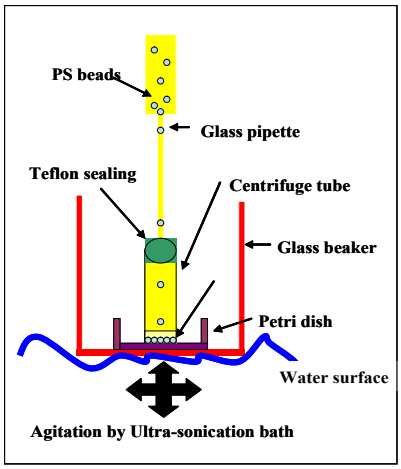

Figure 1. Schematic diagram of scale up system

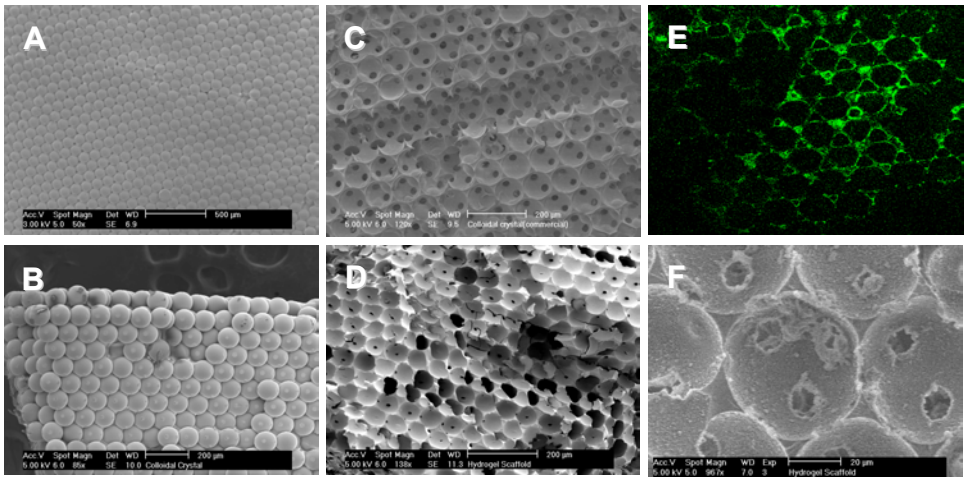

Figure 2. SEM image of colloidal crystal (100μm) (A) Bottom (B) Inside, SEM images of scaffolds (C) Sodium silicate scaffold (D) Freeze dried hydrogel scaffold, (E) Confocal image of Hs202.TH cells on silicate scaffold (F) SEM image of Hs202.TH and HL-60 cells inside of a hydrogel scaffold

Fibroblast cells (Hs202.TH) attached and grew well on the silica scaffold without any surface treatment (figure2. E). Clay particles were coated on the hydrogel scaffold to improve cell adhesion on its hydrophilic surface. Clay particles are biocompatible materials and their flat shapes can cover a surface area more effectively. A greater number of Hs202.TH cells were observed in clay particle coated hydrogel scaffold compared to without surface coated one. Suspension cells (HL-60) were introduced to test the 3D geometry of scaffold. Because the scaffold had highly ordered structure, it was expected that cells could travel a longer distance within the scaffold. The characterized dimension of detached fibroblast cells and suspension cells was between 10 and 15μm. The size of interconnected pores allowed floating cells to pass them one by one. Based on SEM images of hydrogel scaffold, a portion of cells traveled up to 8 layers (800μm) within the structure while some were clogged in the interconnected pores (figure2. F).

Discussion

Substantially higher degree of internal order of the scaffold generates uniform microenvironment and is especially important for stem cells and other similar cells sensitive to 3D surrounding. Also it extensively simplifies analysis of cell interactions and development inside of 3D scaffold. Furthermore, it makes the systematic study of them possible.

The preparation of highly organized 3D scaffolds was achieved without any specialized computer setup and equipments. Simply pipettes and an ultra-sonic bath were used to foster relatively slow sedimentation and gentle agitation of micron size beads. Also, an easy and flexible scaffold preparation method can conveniently adjust pore dimension and scaffolding materials depending on target cells. The size of pore can be controlled just changing the size of beads. Merely changing the amount of dropping beads and a time interval, beads' sedimentation rate can be faster or slower relatively. As long as materials have low viscosity and resistance to strong solvents such as THF, they can be applied as scaffolding materials. However, one important issue of the method is that large amount of high quality beads are required with a cheap price. Current work includes synthesis of micron-size polystyrene beads and the development microenvironment for the co-culture system using highly ordered 3D scaffold.

References

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