3D Patterned Microenvironments Created through Assembly of Discrete Collagen-Chitosan Tissue Modules

David J. Caldwell, Rameshwar R. Rao, Jan P. Stegemann
Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

Purpose: Modular tissue engineering creates larger macroscopic structures from repeating modular subunits. We have shown that discrete protein-polysaccharide microenvironments composed of cell-seeded hydrogel microbeads can be constructed from collagen and chitosan and combined into multiphase tissue constructs. Additional constituents such as hydroxyapatite (HA) can be added to the hydrogel microenvironments to modulate their properties. The current study describes the use of centrifugation and vacuum molding to accomplish the assembly of cohesive multiphase tissue constructs from distinct modular subunits.

Methods: Microbeads composed of a mass ratio of 65/35 collagen/chitosan were generated using a water-in-oil emulsion technique. Powdered HA was added to selected microbead formulations at a concentration of 2.5 mg/mL. Fluorescent microspheres were added to microbeads for visualization and retrovirally labeled fluorescent fibroblasts were added at a concentration of $1.0x10^6$ cells/mL. Multiphase tissue constructs were created from populations of formed microbeads either by centrifugation or vacuum molding as shown in Fig. 2 and Fig. 3. PDMS molds were used to generate desired geometries for the vacuum molding technique. Microbeads were imaged using light microscopy. Constructs were imaged using standard photography and confocal microscopy. Cell-seeded constructs were imaged on day 1 and 7 using confocal microscopy.

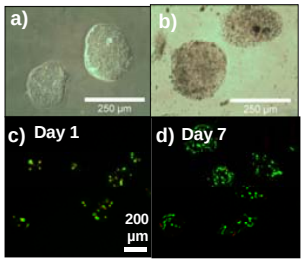

Fig. 1 - Images of 65-35 wt% collagen/chitosan microbeads without (a) and with (b) HA. Viability of mesenchymal stem cells embedded microbeads at day 1 (a) and day 7 (b) of culture.

Results: Microbeads fabricated both with and without HA are shown in Fig 1. Addition of HA resulted in denser and more opaque microbeads with homogenously distributed mineral. Microbeads supported viability and growth of the cells embedded inside of them (Fig. 1c-d). Micro-beads were combined into layered constructs using a centrifugation technique shown schematically in Fig. 2a. The number and composition of layers could be prescribed to create multiphase constructs with desired layers.

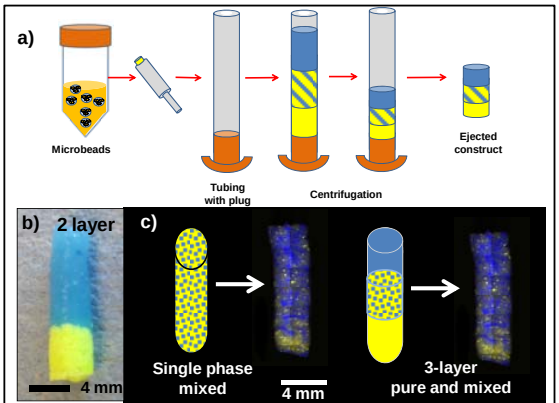

Fig. 2 - a) Schematic illustration of centrifugation method. b) Image of 2-layer construct created using blue and yellow microbeads with hydroxyapatite. c) Fluorescence imaging of constructs. Schematic shows intended pattern and fluorescent images show the construct created.

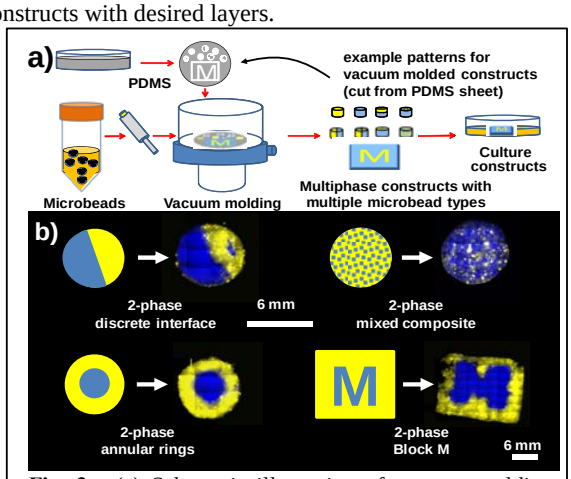

Fig. 3 - (a) Schematic illustration of vacuum molding method. b) Fluorescence imaging of patterned multiphase constructs using mixed and pure microbead preparations. Schematic shows intended pattern and fluorescent images show the construct created.

Microbeads were also combined into cohesive constructs using vacuum filtration in pre-formed molds, as illustrated in Fig. 3a. This technique allowed varied of geometries with horizontal and vertical divisions to be made (Fig. 3b). Both simple and more complex patterns could be created, with the resolution depending on the microbead size. Gradients could also be created by vacuum molding, and Fig. 4 shows multiphase gradient constructs created using microbeads containing fluorescently labeled fibroblasts.

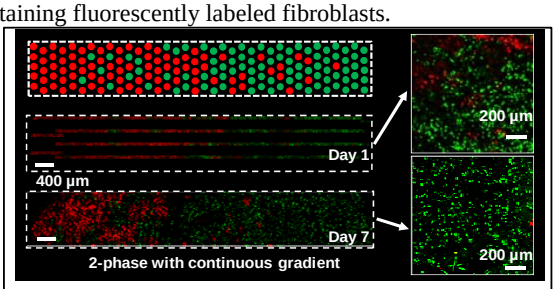

Fig. 4 - Graded microbead constructs containing red- and green-labeled fibroblast cells at days 1 and 7.

Conclusions: Cell-seeded protein-polysaccharide microbeads were assembled and patterned into larger-scale tissue constructs with prescribed architecture. Microbeads supported the viability of cells embedded within them, and cells remained viable after assembly of larger-scale constructs. These methods may have applications in patterning of cells and microenvironments into tissue-like structures. Such models could be used to study cell-cell communication and the effects of defined co-cultures on cell function. In addition, the assembly of microenvironments into prescribed architectures could have utility in creating more complex engineered tissues, in which the spatial composition can be tailored to direct cell function and achieve improved tissue performance.

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