Porous Microbeads as Three-Dimensional Scaffolds for Tissue Engineering

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Abstract:

The goal of this project included fabricating uniform porous poly(lactic-*co*-glycolic) acid (PLGA) microbeads using a fluidic device, seeding fibroblasts into these beads, and culturing these cell-loaded beads in a mold of a desired shape. The fibroblasts attached and proliferated in porous beads with average pore diameters of 30 μm. These fibroblast-loaded beads connected to neighboring beads by a network of cells and extracellular matrices (ECM) to form the beginnings of a tissue construct inside the mold. These preliminary results have promising implications, such as the possibility of creating shape-specific tissues to better fit injury sites.

Introduction:

Tissue engineering seeks to create replacements for tissues and organs, in an effort to maintain, improve, or regain biological functions [1]. Three dimensional porous scaffolds are commonly used to enhance cell attachment, facilitate nutrient and oxygen transport, and create a suitable microenvironment for proper cell activities [2]. Recently, microbeads with interconnecting inner and outer pores have been studied as a viable carrier scaffold for better implants. Since scaffolds need to be biocompatible, biodegradable, and mechanically strong, porous microbeads are a viable choice to fulfill these basic requirements [3]. However, the difficulty lies in making microbeads with large enough pores for cells to penetrate and grow inside.

Porous microbeads promote cell migration and allow for nutrient and waste transport through the pores, protect cells growing inside, and are small enough to be used for many purposes, including cell and drug delivery, direct injection to injury sites, and as scaffolds to culture cells *in vitro* for improved implants [3].

Our group has recently reported the fabrication of uniform PLGA porous microbeads by introducing a phase-separated emulsion into a simple fluidic device [3]. This project was carried out to produce uniform PLGA porous beads with controllable pore sizes, and examine if mouse fibroblasts could successfully grow within these beads packed inside a specified mold.

Experimental Procedure:

To fabricate porous microbeads, we used a simple fluidic device consisting of a bent needle inserted into polyvinyl chloride (PVC) tubing with an attached glass capillary tube. A homogenized mixture of a 2% polyvinyl alcohol (PVA) and 15% gelatin solution in water and a 2% PLGA solution in dichloromethane (DCM) was filled into a syringe attached to the bent needle as the discontinuous phase. Another syringe filled with 2% PVA was used as the continuous phase, attaching to the PVC tube.

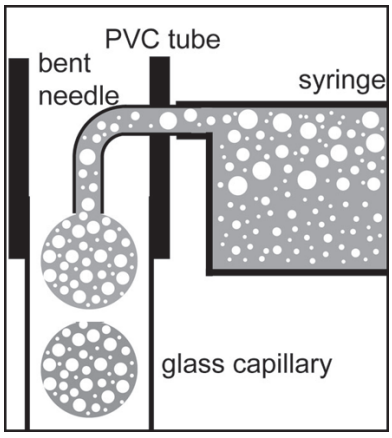

Figure 1: Fluidic device to fabricate porous microbeads [3].

Figure 1 shows a schematic of the bead fabrication process. The steady flow of 2% PVA aqueous solution from the syringe into the PVC tubing knocked off the beads leaving the tip of the needle. The pores in these microbeads were filled with gelatin particles from the emulsion. These beads were collected in a beaker filled with 0.2% ice-cold PVA aqueous solution, where the gelatin inside the beads hardened upon gelling. The beads were kept stirring overnight to evaporate DCM and solidify PLGA. By heating the beads, the gelatin dissolved, leaving only the PLGA beads with interconnected pores.

NIH/3T3 mouse fibroblasts were seeded into porous beads using a spinner flask for 24 hours. Various molds were fabricated using agar in the shape of small circular wells. A 15 mL centrifuge tube was also used as a mold. After placing

the cell-loaded beads in the molds to pack into a pellet form, the pellets were kept in culture for approximately 5-7 days before staining.

Scanning electron microscope (SEM) images confirmed the outer pore sizes of the beads and their size distribution. Both SEM and confocal microscope images were used to characterize cell attachment and proliferation within the beads, as well as the cell and ECM networks connecting neighboring beads together.

Results and Conclusions:

We successfully fabricated uniform PLGA porous microbeads with an average diameter of 300 μm and average pore sizes of 30 μm using a fluidic device (Figure 2).

From confocal micrographs of cytosol staining (Figure 3, where white dots indicate cells) and SEM images (Figure 4) of the cell-loaded porous beads, it is evident that the mouse fibroblasts attached to the porous beads and penetrated into the inner space to proliferate. The beads connected with each other by a network of cells and secreted ECM. These interconnected beads with cells agglomerated together, forming the beginnings of a construct inside the mold. These findings support the application of porous microbeads as scaffolds of specific shapes to promote, influence, and support cell growth, and as a support system to facilitate the healing and recovery process after an injury.

Future Work:

The success rate of porous bead fabrication must be improved by further optimizing and standardizing our methods. With enough porous beads, tissues could be grown on a larger scale, making transplants with these procedures more feasible. Eventually, studies will be done with animal models to better understand the *in vivo* effects of these porous beads. Our findings must also be substantiated with long-term cell cultures using human cells, as the use of a patient's own cells will eliminate possible complications associated with transplantation.

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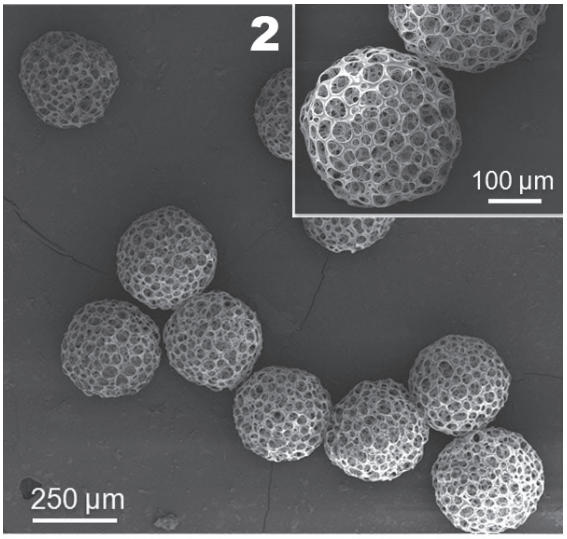

Figure 2: SEM images of uniform porous microbeads. Inset: magnified view.

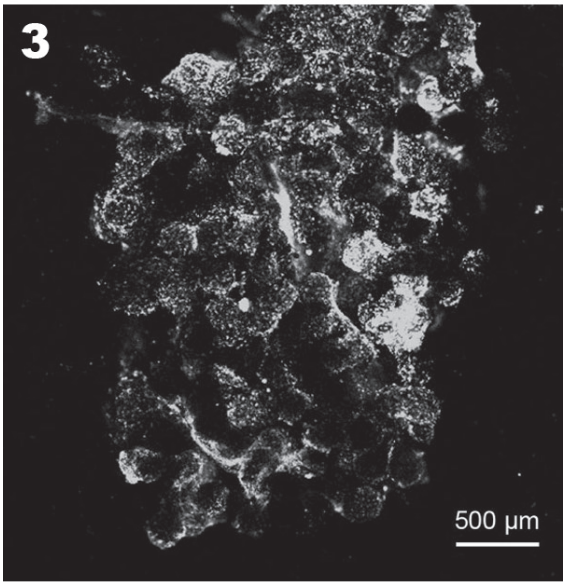

Figure 3: Confocal micrograph of a cell-bead construct at day six in culture.

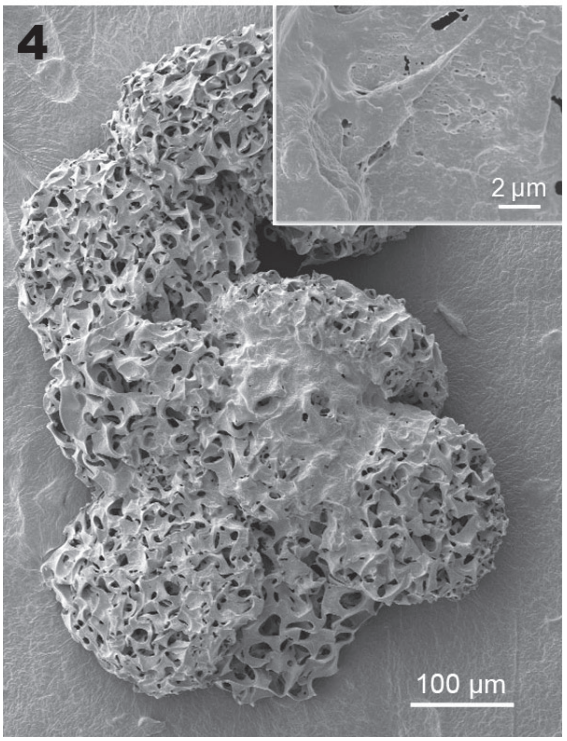

Figure 4: SEM images of a cell-bead construct. Inset: magnified view.