Micrometer Scale Guidance of Mesenchymal Stem Cells to Form Structurally Oriented Extracellular Matrix
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Introduction
Current clinical techniques to repair injured cartilage do not offer a long-term solution. Tissue engineering is viewed as a favorable option for long-term repair of cartilage lesions, but tissue engineered cartilage constructs generally have inferior mechanical strength compared to native cartilage. Researchers attribute this problem to a lack of an oriented structure in the constructs at the microscale that is present in the native tissue. The goal of our research is to test the hypothesis that microscale features on the substrate will cause the differentiating mesenchymal stem cells (MSC) to arrange themselves preferentially and to create an oriented extracellular matrix on the microscale similar to native tissue structure. In this first set of experiments, human MSCs seeded in the channels were shown to be seeded selectively in the channels and to remain highly viable. In future experiments, these MSCs will be tested for the ability to differentiate chondrogenically and for improved mechanical strength of the resultant matrix.

Methods
To verify the hypothesis that microscale guidance channels can form structurally-oriented ECM, we first formed stable channels of varying microscale dimensions in collagen-based membranes. The channels were formed by a combination of microfabrication and collagen soft-lithography techniques. We further developed a new technique to selectively seed human MSCs in collagen channels by the use of pluronic F-108. We assessed cell attachment and viability in the channels.

Collagen Membrane Formation
Collagen-GAG membranes were produced using the standard filtration method over a period of 36 hours. To form micro channels within the membrane, microfabrication was used to produce silicon wafers containing the patterns shown in Figure 1. These wafers were then used to produce channels on the surface of the collagen membranes by a technique of collagen soft-lithography that involved selective solubilization, patterning, and pattern stabilization by 1-ethyl-3-(3-dimethyl-aminopropyl) carbodimide hydrochloride (EDC) crosslinking (Figure 2 left). The widths of the formed channels ranged from 25 µm to 1000 µm. After crosslinking, phase contrast images were taken of the channels and analyzed offline to determine the quality of the patterns.

Cell Culture
Adult human MSCs were cultured to confluence and were resuspended at concentrations of about 70 million cells/ml. The cell suspension was applied to the channels using a micropipette. After a 2 hour period to allow cell attachment, the membranes were immersed in TGF-β1 (10 ng/ml) containing chondrogenic medium. The medium was changed every 2-3 days.

Cell Viability and Adhesion Assessment
To evaluate cell viability within the channels, Live/Dead™ staining was used. The viability of the cells was tested 1 day after cell attachment. To confirm cellular attachment in the channels, cells pre-stained with Vybrant® were subjected to fluorescent imaging approximately 2-3 times a week. A total of 3 membrane pieces containing the three largest size channels and a total of 3 membrane pieces containing the smallest size channels were used for these experiments.

Selective Cell Attachment
To attach cells selectively to channels, the top surface of the patterned membranes were exposed to pluronic F-108, which repels cell adhesion. MSCs were seeded on to the membranes for 3 hours and unattached cells were washed out.

Results and Discussion
We developed two different designs for guidance studies (Figure 1 left and right). Figure 1 (left) shows a design that can be used to seed cells directly using flow, whereas the one on the right requires a selective seeding methodology.

Figure 2 (left) shows the new technique developed to form patterns of microchannels in collagen membranes. Regardless of the type of designs used, our results show that the technique is effective to obtain guidance channels made of collagen. The EDC-based technique is robust and can lead to channels with a precision as low as 25 micrometers. Figure 2 (right) shows a phase contrast image of 50 micron patterns. EDC-based technique offers several advantages when compared to other techniques such as glutaraldehyde crosslinking. These include low toxicity and very low background signal for fluorescence imaging.

Figure 3 (left) shows image analysis data where the widths of the channels are compared to the widths in the template. Overall, the method allows for micro channels of the smallest dimension we tested, 25 µm, to be produced accurately in the collagen membranes. Further, the membranes themselves are biocompatible: cells attach and retain very high viability (Figure 3 (middle) green; viable cells, red: dead cells).

Figure 4 (left) shows a schematic of the technique used to obtain selective seeding of MSCs. F-108 is non-toxic to cells and the new technique allows for highly improved selectivity (Figure 4 right vs. Figure 3 middle) of cell attachment in channels.

Our results demonstrate that guidance channels made entirely of collagen can be fabricated to study their effect on MSC chondrogenesis. The use of microfabrication to create the channels has the advantage of developing channels of various designs, shapes, and sizes. Micrometer-scale precision can be obtained, and MSCs can be selectively seeded in these channels and the guidance effects of the channels can be assessed. The cells attach onto these channels well with a very high viability. Future experiments involve culture of MSCs long term for chondrogenesis and mechanical strength assessment.