ABSTRACT.
Using a novel computer controlled process, we have prepared sheets of collagen from soluble collagen which have skin-like, tendon-like, or an aligned braided structure on glass and plastic substrates. The process provides uniform structures over large area as confirmed by AFM, SEM, laser diffraction, and polarized microscopy. In some experiments, parallel microgrooves have been created during the deposition process while preserving fibril structure.
Various cell types when plated on these matrices preferentially align and migrate along the fibrils or along the crimped ridges depending on the geometrical parameters of the surface nanostructure, including fibroblasts, stem cells (adipose-derived and bone marrow-derived), periodontal ligament fibroblasts and osteosarcoma cells. Over the course of a few days myoblasts fuse to form aligned myofibrils. Cells cultured on collagen scaffolds were monitored by light microscopy and analyzed for patterns of protein and gene expression by immunofluorescent staining and qPCR. Primary corneal keratocytes align along fibrils and show gene expression more characteristic of a differentiated phenotype vs. phenotype observed in wounded cornea, suggesting that this collagen scaffold may improve the quality of the corneal wound repair. In addition, supplementing collagen scaffolds with a variety of growth factors (attached via glycosaminoglycans) induces faster cell migration and show a potential for a more controllable cell growth system in vitro.

Behavior of Cells on Tissue-like Collagen Matrices

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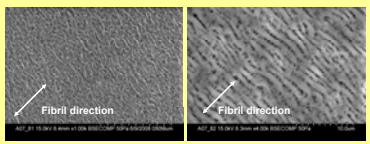
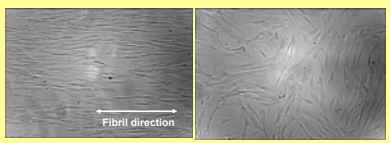
INTRODUCTION. Fibralign has pioneered the production of bioequivalent reconstituted tendon-like matrices made from solutions of purified monomeric collagen. These novel matrices are a) biocompatible, b) have high mechanical strength and "J" elasticity characteristics in the fibril direction, c) defect-free over a large area (50 x 250 mm), d) biomimetic (i.e. approximating specific native structure (i.e., ligament, cornea)—at the nano- through macro-scales), and e) biodegradable depending on the level of crosslinking. The complex crimp and aligned-braided structure of the matrices can be further tuned to incorporate growth factors with a desired spatial gradient.

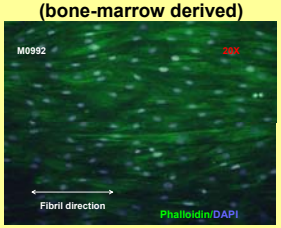
SEM of Tendon-like Scaffold



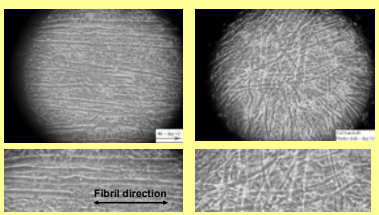
Human Fibroblasts



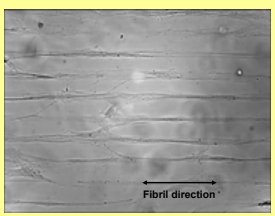
Human mesenchymal stem cells (bone-marrow derived)



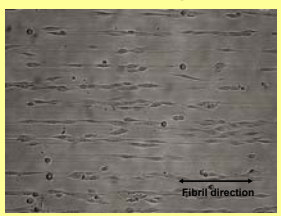
Myoblast Fusion: Myotube Formation



Adipose-derived mesenchymal stem cells



Osteosarcoma cells align and migrate on tendon-like collagen scaffold

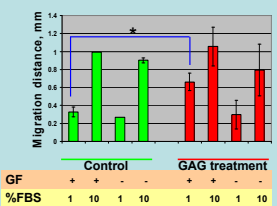


CELL MIGRATION ASSAY. We have developed a simple assay to test for cell migration on aligned collagen matrices. A 4-µl drop of cell suspension is applied to the substrate, and the cells were allowed to adhere for 30 min at 37° in CO_2 incubator. After that, the cells are incubated in DMEM supplemented with 1 or 10% FBS, with images taken at certain time points using light microscope. On aligned substrates, cells only migrate along the fibrils, and the difference between the cell front positions at day 2 and day 4 is calculated from the images taken. We have also included heparin as a component of the matrix, and tested this matrix with further added growth factors, for its ability to affect cell migration. Cells migrated significantly faster on GF-supplemented aligned matrix.

Cell Migration Assay

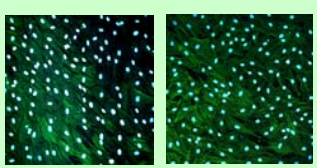
Enrichment of Tendon-like Scaffold with Growth Factors

GF-Enriched Scaffold Facilitates Cell Migration

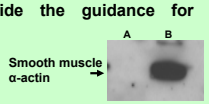


Cornea models reconstructed by tissue engineering include stroma composed of keratocytes within a collagen gel or a biomaterial, on which epithelium is placed. Endothelial cells can be added to form a reconstructed cornea. These cell-scaffold composites improve corneal wound healing. Gels have the advantage of being translucent but have no mechanical resistance, whereas most of existing biomaterial models are stronger but lack the transparency. Our matrices provide both transparency and mechanical strength, and in combination with cells may also provide the guidance for collagen fibrillogenesis.

Bovine keratocytes

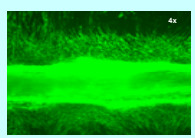


Phalloidin and Hoechst staining of corneal fibroblasts grown on aligned collagen and glass coverslip.



When the cornea sustains injury, quiescent corneal fibroblasts undergo a phenotypic change to repair fibroblasts, which are more proliferative and express more contractile proteins such as smooth muscle α-actin. The repair fibroblasts invade the wound region and secrete disorganized collagen which eventually forms scar tissue with decreased transparency. Smooth muscle α-actin is a marker for the repair phenotype of keratocytes and its expression suggests that cells exposed to control surface are more "fibrotic" than ones grown on Fibralign substrates.
A: Fibralign surface with 30 nm parallel fibers
B: Glass coverslip coated with collagen 1.

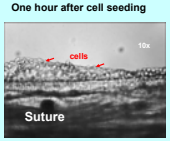
CELL DELIVERY DEVICE. Using an aligned collagen matrix sheet as a starting material, we manufactured a "pseudofiber" which can function as a suture. This suture provides a high surface area allowing for high number of cells to attach. This format allows for easy handling of the material and may serve as a cell delivery device. Cells adhere well to the suture and, provided a favorable immediate environment, migrated from the suture into this environment. In a "proof-of-concept" experiment we seeded melanoma cells on sutures and transplanted them subcutaneously in mice. Three weeks after implantation, a number of melanoma tumors were found on each implanted suture. These data prove the possibility of using our material for cell delivery. The data also show a good biocompatibility, cell migration into the suture, and limited angiogenesis as of three week post-implantation.



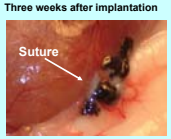
Cells attach, proliferate and migrate from the suture

Cell delivery device

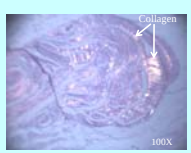
One hour after cell seeding



Three weeks after implantation

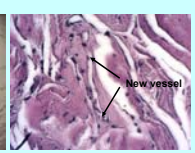


Three days after implantation



Suture cross-section under polarized microscope. The bright white sections of the suture show the intact (undigested) collagen.

Suture cross-section stained with H&E



Suture cross-section stained with H&E, larger magnification

Fibroblasts, myoblasts, and mesenchymal stem cells show excellent proliferation, migration, and alignment on these matrices. The tendon-like matrices can be formed into either fiber structures or multi-layer composite scaffolds. These scaffolds can be repopulated with patient stem cells and factors derived from the patient's platelets to substantially reduce healing time and improve their efficiency. The invention and development Fibralign's technology has made possible production of native tissue scaffolds outside of the body.