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 ’U’ 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

Collagen from soluble collagen which have skin-like, tendon-like, or an elastic 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.

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° C in CO2 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 scaffold to affect cell migration. Cells migrated significantly faster on GF-supplemented aligned matrix.

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 melanomas 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.

Three days after implantation

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

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

Suture after cell seeding

Three weeks after implantation

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

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.