CONFOCAL MICROSCOPY TO STUDY SPATIO-TEMPORAL CHANGES IN COLLAGEN ORGANIZATION IN CARDIOVASCULAR TISSUE ENGINEERED CONSTRUCTS

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Introduction
Cardiovascular tissues have a prominent load-bearing function. In particular the content and organization of collagen fibers in the extracellular matrix (ECM) contribute to the load-bearing properties and dominate overall tissue strength. In case of changes in mechanical demand, collagen content and organization in the tissue is actively remodeled to meet the new requirements. We use engineered tissue models to mimic and understand collagen remodeling. This knowledge will be used to control and optimize the load-bearing properties of engineered cardiovascular tissues. Here, we focus on visualizing spatio-temporal changes in collagen organization in living engineered tissues, using confocal microscopy and fluorescent labeling of the collagen fibers. The techniques are applied to tissue models incorporating different cell types and/or scaffolds.

Materials & methods
Tissue models consist of cell-seeded fibrin gels or electrospun nano-fiber scaffolds. These tissue models are attached, either by Velcro or gluing the scaffold, to the bottom of a well plate, which allows for visualization with confocal microscopy and time-lapse scanning. Attachment on two or four sides allows for uniaxial or biaxial constraint, whereas placement in a strain device is used to apply uniaxial or biaxial loading to the tissues. By changing mechanical demands, it is possible to induce changes in the collagen organization. A combination of life fluorescent probes (Cell Tracker Organe for cell cytoplasm and CNA35-OG488 for collagen) is used to visualize cell and collagen organization.

Human myofibroblasts (vascular derived cells for in vitro TE) and human endothelial colony forming cells (ECFCs) were studied in fibrin constructs, and human myofibroblasts on an electrospun nano-fiber scaffold.

Results & discussion
With this system changes in collagen organization can be induced and detected within days. In a confined biaxial setting myofibroblasts produce collagen in a random orientation. When attachments change over time from biaxial to uniaxial, collagen fibers align parallel to the direction of static strain. When cyclic strain is applied to the biaxially confined construct, collagen orients parallel to the direction of applied strain. ECFCs do not respond to strain, but form vessel-like structures. On an isotropic scaffold, myofibroblasts form collagen along the fibers of the scaffold, irrespective of the strain applied. These studies indicate that mechanically induced tissue remodeling can be followed in space and time using our confocal imaging methods, allowing to dissect between the various underlying mechanisms of collagen remodeling in engineered tissues, such as strain avoidance or contact guidance.