# Collagen remodeling at the micro level in engineered tissues

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## Introduction

Collagen is the main load-bearing component of the extracellular matrix of cardiovascular tissues and easily adapts its organization and load-bearing function in response to mechanical loading. It is therefore a key factor when creating functional tissue engineered cardiovascular constructs. The underlying mechanisms of collagen remodeling are not yet fully understood. **Therefore we developed a 3D *in vitro* model system to study collagen remodeling at the cell-matrix level in real-time.** Here we apply the model to investigate remodeling in tissues engineered without a carrier material, or scaffold.

## *In vitro* model system

The model system consists of myofibroblasts seeded in a fibrin gel, which allows for inducing remodeling under static and dynamic loading conditions (Figure 1).

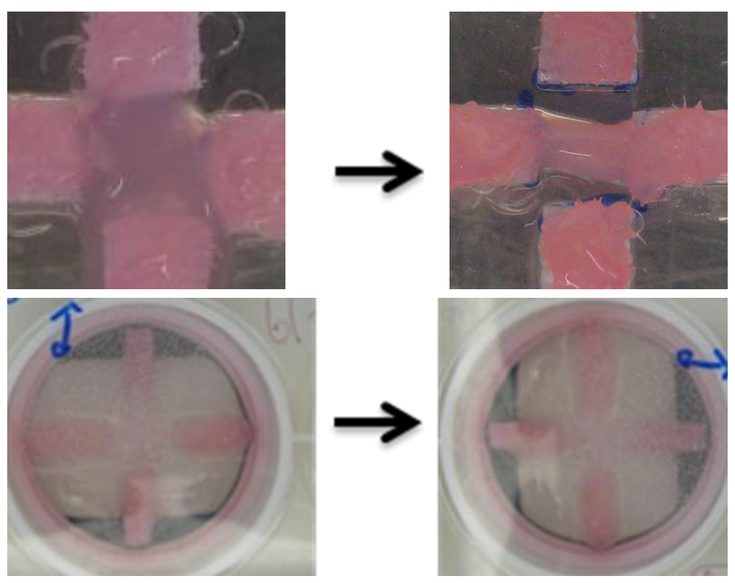

Figure 1. A fibrin gel is seeded with cells, constrained in two directions with Velcro. Velcro is glued to a flexible membrane in a six-well plate, which allows for straining with a Flexcell device. To induce static remodeling the tissue is released in one direction (upper pictures) after 12 days of culturing. For dynamic remodeling the strain direction is changed into horizontal direction (lower pictures), after 5 days of static strain, followed by 7 days of vertically applied intermittent strain (3 hours on/off, 1 Hz, 4% strain).

## Results

Statically strained samples show that collagen remodels from a random orientation to a more aligned orientation in the direction of the static strain in 5 days (Figure 2).
Dynamically strained samples show alignment perpendicular to the direction of strain – a process called strain avoidance. The superficial tissue layer remodels to be almost perpendicular to the new direction of strain within 2 days (Figure 3).

1. Rubbens, M.P., et al., *Quantification of the temporal evolution of collagen orientation in mechanically conditioned engineered cardiovascular tissues. Ann Biomed Eng, 2009.* ***37(7): p. 1263-72.***
2. Gauvin, R., et al., *Dynamic mechanical stimulations induce anisotropy and improve the tensile properties of engineered tissues produced without exogenous scaffolding. Acta Biomaterialia, 2011.* ***7: p. 3294-3301.***

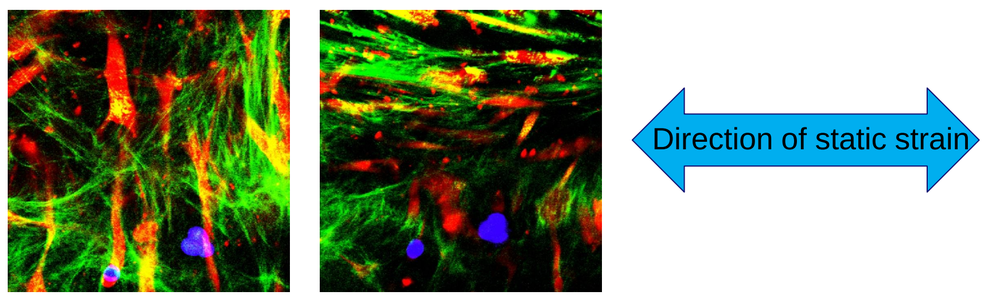


Figure 2. Confocal images of static remodeling, with cells (red), collagen (green) and beads (blue, used as internal reference coordinates). Left: an image before induction of remodeling with random collagen orientation, which remodeled to be more aligned in the direction of the static strain (right).

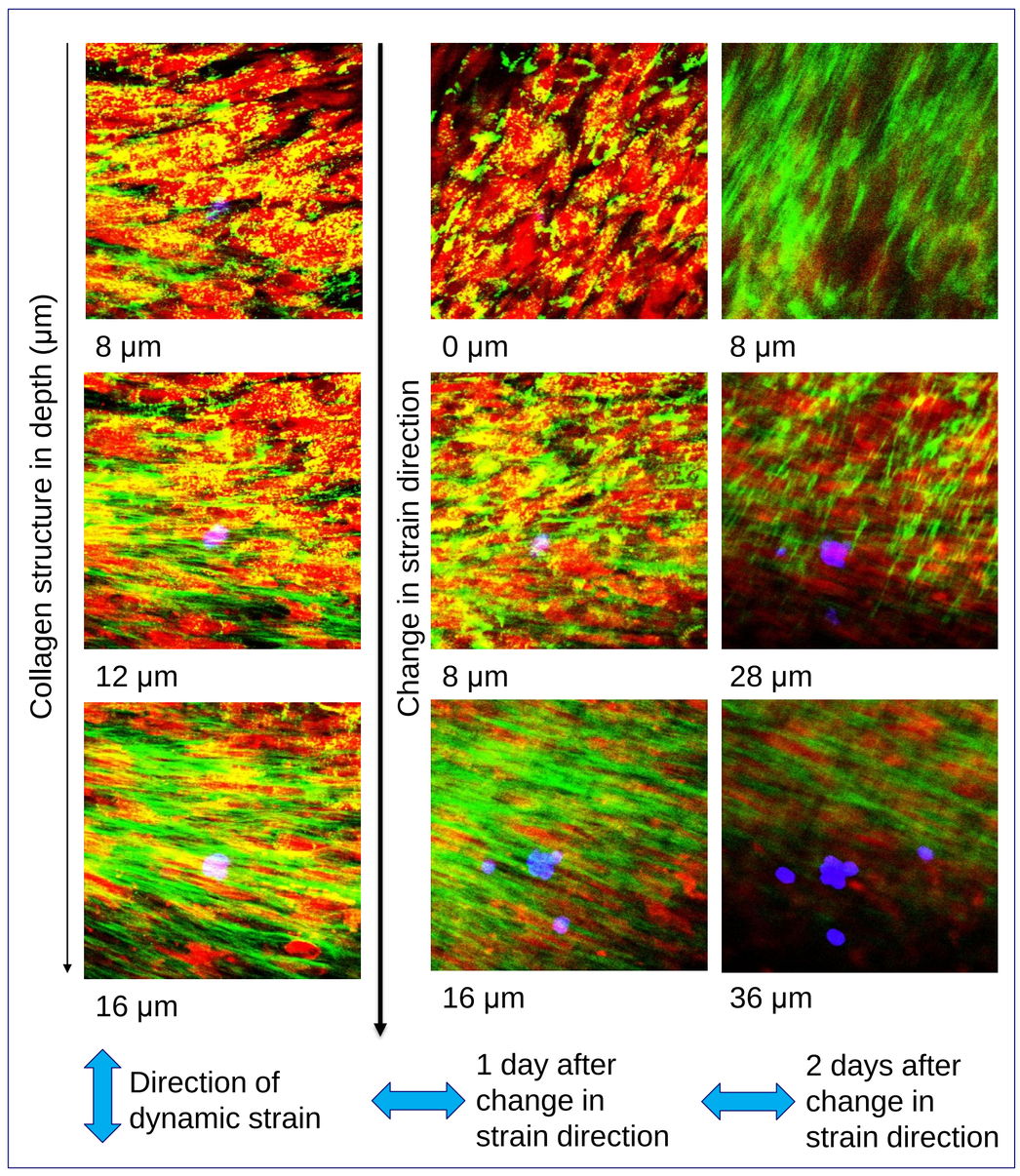


Figure 3. Confocal microscopy images of dynamic remodeling, with cells (red), collagen (green) and beads (blue). Collagen and cells are oriented perpendicular to the vertical strain. With changed strain direction the superficial tissue layer remodels to be almost perpendicular to the new direction of strain in 2 days.

## Conclusions

- Dynamic culture results in more tissue formation than static culture.
- Remodeling occurs faster with intermittent dynamic strain than with static strain.
- Collagen and cells align parallel to static strain, but perpendicular to dynamic strain. We hypothesize that this is due to the constraints in 2 directions. Other studies, which use constraints in 1 direction, show that alignment is parallel to the strain, with and without scaffold [1,2]. Further research is needed to elucidate more on this.