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ADJUSTING THE MICROMECHANICAL AND MORPHOLOGICAL DIFFERENCES OF COLLAGEN BASED MATERIALS TO INFLUENCE THE CELL-SUBSTRATE INTERACTION

MOTIVATION

Collagen has natural crosslinks, caused by enzymes, which prevent the dissolving of the collagen tissue within the body. However, when using collagen as a medical product, crosslinks need to be introduced to regulate the resorption in the body and to stabilize the structure of the collagen scaffolds or membranes. The most favored crosslinkers are carbodiimides, like 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and aldehydes, like glutaraldehyde (GDA). An alternative is the physical crosslinking by dehydrothermal (DHT) treatment. These crosslinking processes represent two different groups, the zero-length crosslinking (EDC, DHT) and crosslinking via a linker (GDA).
Within these two different groups, the properties of the collagen material might be different as well. The aim of this work is to reveal the changes of the collagen material, caused by the different crosslinkers. These effects were correlated with the cell behavior of three different cell types.

METHODS

Crosslinking

Carbodiimid and **aldehyde** crosslinking:

- Increasing concentration of EDC or GDA in buffer solution at pH 8.0
- Incubation of the acid soluble collagen (ASC) sheets in the crosslinking solution for 4 hours
- Washing three times and drying at ambient conditions

DHT crosslinking:

- Dry collagen sheets were wrapped in an aluminum sheet and placed in a vacuum oven for 24h at different temperatures

Cell types used for adhesion and proliferation tests

Fibroblasts	L929
Endothelial	Human umbilical vein endothelial cells (HUVEC)
Chondrocytes	Human chondrocytes (HCHON)

Static Atomic Force Spectroscopy and QI™ imaging

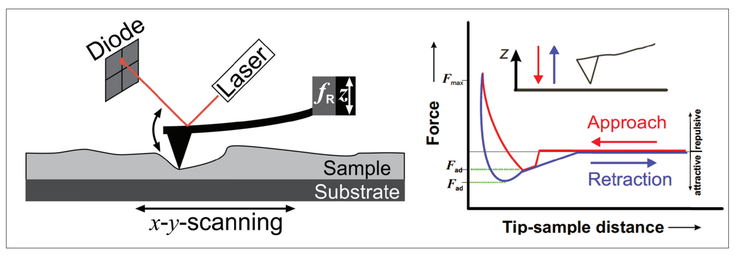

Fig. 1: Setup and function of the atomic force microscope (AFM) and the atomic force spectroscopy

- Atomic force microscope enables a nanoscale resolution of the mechanical properties of the collagen foil.
- QI®-Mode (JPK Instruments AG) enables a simultaneous measurement of the mechanical (stiffness) and topographical (roughness) properties in one single measurement run.

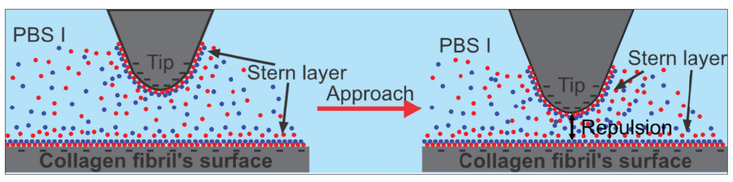

Fig. 2: Model to reconstruct the stiffness from the force measurement on a collagen foil under physiological conditionsforce spectroscopy

- Ability to measure the nano-mechanical properties under different conditions, e.g. under physiological conditions in phosphate buffered saline (PBS)

RESULTS

Influence of the stiffness on cell behavior

- **With increasing crosslinker concentration the stiffness increases**

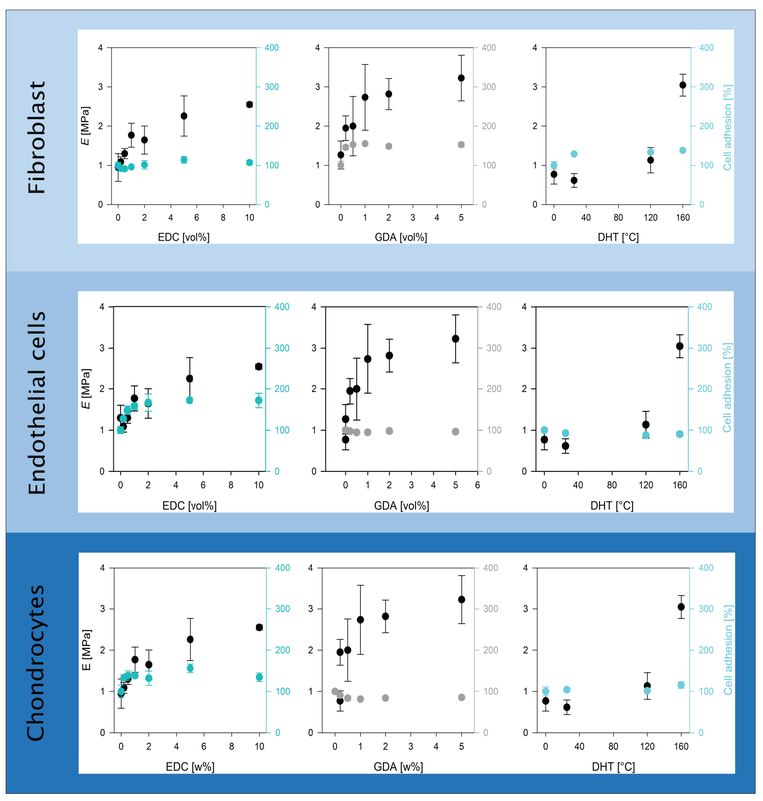

Fig. 3: Differences in cell adhesion of fibroblasts, endothelial cells and chondrocytes upon increasing stiffness.

- The adhesion of fibroblasts, chondrocytes, and epithelial cell is effected in case of EDC crosslinking
- No dependencies of the adhesion are visible for GDA and DHT

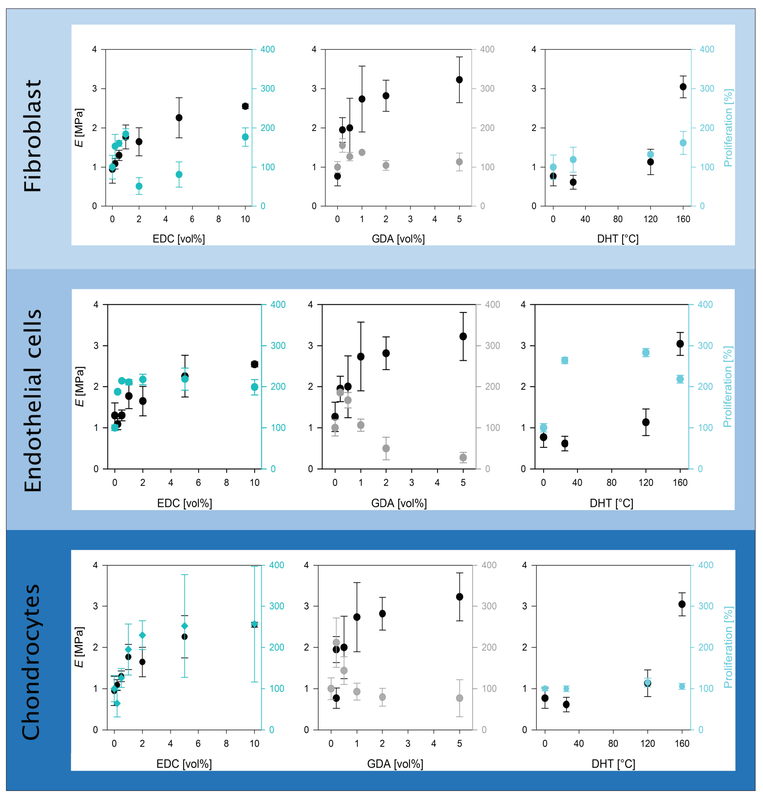

Fig. 4: Differences in cell proliferation of fibroblasts, endothelial cells and chondrocytes upon increasing stiffnesstes upon increasing stiffness.

- For EDC cross-linked samples, with increasing stiffness the cell proliferation of endothelial cells and chondrocytes increased.
- Fibroblast show a decrease in cell proliferation with increasing stiffness for all crosslinking methods
- Endothelial cells show an increasing proliferation for DHT crosslinked samples
- GDA crosslinked samples show a negative effect on the cell proliferation for all cell types

Influence of the surface structure on cell behavior

- Zero length cross linker (EDC and DHT) decreases surface roughness
- Cross linker with a linker (GDA) increases surface roughness.

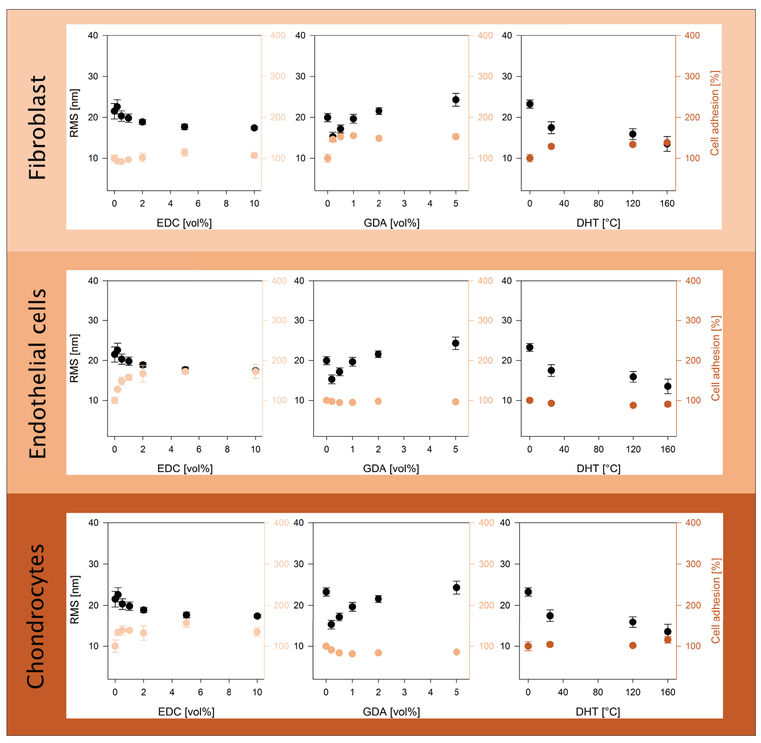

Fig. 5: Variation of the surface roughness (RMS) effects the cell adhesion of fibroblasts, endothelial cells and chondrocytes.

- Endothelial cells and chondrocytes show a positive adhesion behavior with decreasing roughness of EDC crosslinked samples
- Fibroblasts are not affected by changes in the surface roughness

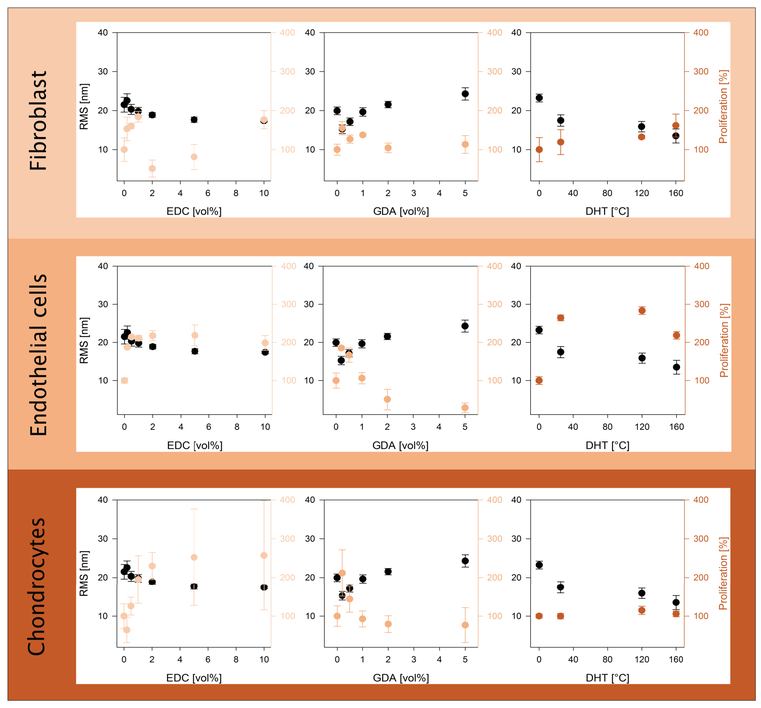

Fig. 6: Variation of the surface roughness (RMS) effects the cell adhesion of fibroblasts, endothelial cells and chondrocytes.

- Endothelial cells show a better proliferation with decreasing roughness (EDC and DHT)
- The proliferation of chondrocytes is positively affected by EDC crosslinking only

CONCLUSIONS

-> EDC and DHT crosslinking has positive effects on cell adhesion and proliferation of chondrocytes and endothelial cells
-> Roughness and stiffness are important parameters for designing scaffolds
-> Is it possible to measure the length of the GDA linker by AFM?
-> Fibroblast adhesion and proliferation is not influenced by crosslinking

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