

STIFFNESS OF VITAL-AVITAL COMPOSITE IS INCREASED BY MECHANICAL STIMULATION

K. Alberti^{1,2}, G. Raeber¹, E. Karamuk¹, G. Ettl¹, E. Wintermantel³ and J. Mayer¹

¹*Biocompatible Materials Science and Engineering, Swiss Federal Institute of Technology, Zurich, Switzerland,* ²*Humboldt University, Berlin, Germany,* ³*Zentralinstitut für Medizintechnik der TU München, Germany*

INTRODUCTION: Tissue engineering offers the possibility of replacing damaged tendons and ligaments by the means of functional vital/avital composites that consist of a biodegradable matrix and autologous cells¹. Mechanical stimuli represent one of several concepts to induce differentiation of vital/avital composites towards functional tissues². The aim of this study was to investigate the effects of cyclic mechanical stimulation on a vital/avital composite that consists of a woven fabric as a scaffold and confluent fibroblasts as a vital matrix. The response to mechanical stimulation was evaluated by force measurement, fluorescence staining of the cytoskeleton and SEM analysis.

METHODS: The vital/avital composite consisted of a textile cell carrier which was seeded with 3T3 fibroblasts at a density of 600cells/mm² and harvested for two weeks on a shaker at normal cell culture conditions. The poly(ethyleneterephthalat) (PET) monofilament mesh (mesh opening 190 µm, filament diameter 36 µm) was subjected to a temper procedure and O₂- plasma activation prior to seeding in order to reduce residual stresses in the textile and increase hydrophilicity.

The matured composites were installed in the six strain modules of the TissueTens apparatus³ with the filament axis at a 45° angle with respect to the direction of the uniaxial stimulation and subjected to a 2% sinusoidal stretch. Each stimulation cycle comprised a 1 hour exertion followed by a 6 hour recreation phase where the composites were stimulated at 0,5Hz and 0,01Hz, respectively. A Mesh without cells but otherwise identically treated was used as a reference.

Piezoelectric sensors monitored continuously the stiffness of the composites until they were removed from the strain modules after up to 12 stimulation cycles and prepared for Rhodamin-Phalloidin, Ethidiumbromid-/FDA and DNA staining (Hoechst 33258) as well as for SEM analysis.

The orientation of f-actin filaments with respect to the local strains within single meshes of the textile

cell carrier was quantitatively determined by analyzing CLSM image stacks with an autocorrelation algorithm.

RESULTS: The stiffness of the vital/avital composites increased during each exertion phase compared to the reference. The subsequent relaxation phase lead to a stiffness reduction. However, there was considerable memory effect leading to an overall gain in stiffness (*Fig.1*). F-actin filaments of samples taken in the middle of the exertion phase were aligned to a high degree parallel to the orientation of the main strains. The recreation phase induced a reorganization of the cytoskeleton, and caused a more random f-actin filament distribution as seen by image analysis (*Fig.2*).

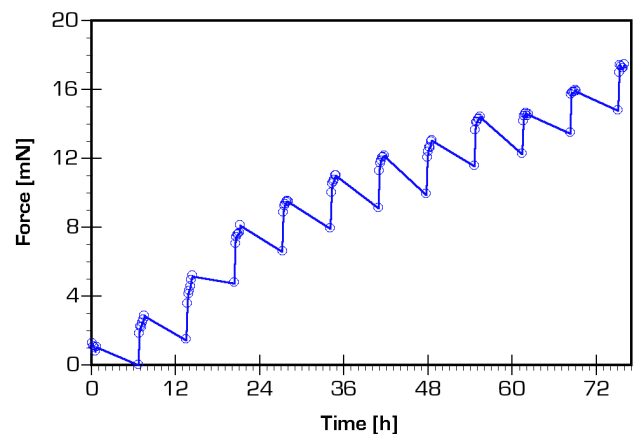


Fig. 1: Effect of uniaxial mechanical stimulation on the stiffness of vital/avital composites. In contrast to the stiffening of the composite throughout the exertion phase, stimulation at 0.01Hz during recreation caused an increase of the compliance.

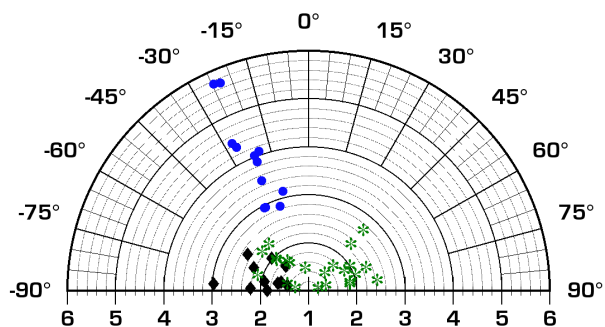


Fig. 2: Polar diagram of f-actin orientation. Radius: Degree of orientation, Angle: Direction of f-actin filaments (• cells during stimulation, ♦ cells during recreation, * unstimulated cells)

DISCUSSION & CONCLUSIONS: Our experimental setup allowed a continuous force monitoring with a resolution of $5 \cdot 10^{-5}$ N and showed a time-dependent increase of stiffness of the vital/avital composite during mechanical stimulation, which could be correlated with the spatial orientation of f-actin in the cytoskeleton. The response of the f-actin polymerization/depolymerization mechanism was depending on the stimulation frequency. During the exertion phase at 0.5 Hz f-actin fibers were mainly oriented parallel to the external strains whereas at low frequencies (0.01 Hz) the fibers were distributed more randomly. Unstimulated cells showed an orientation distribution close to uniform. These findings suggest that in the concept of tensional homeostasis⁴ the intended endogenous matrix tension is depending on the stimulation frequency.

The reason for the overall increase of stiffness has yet to be identified. Possible effects are an increase of cell mass or the deposition of ECM, e.g. collagen type I. A fibroblast cell line was chosen for these experiments because of its well defined phenotype. However, our next experiments will focus on primary tenocytes on partially degradable textile scaffolds to investigate the balance between ECM formation and scaffold degradation in combination with cyclic mechanical stimulation.

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