THE INFLUENCE OF MECHANICAL BOUNDARY CONDITIONS ON CONTRACTION FORCE IN 3D MACROPOROUS SCAFFOLDS

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Introduction

Tissues like bone, tendon and muscle fulfil specific mechanical functions depending on their structural organisation. Mechanical boundary conditions influence this cellular structuring. The details of this linkage between cellular matrix organization and extrinsic mechanical constrains is unclear. Hypothesis of this work is that extrinsic tissue resistance drives cellular and tissue self-organization.

Methods

To analyse matrix remodelling, we established a novel tissue culture system, which allows live monitoring of tissue contraction against different mechanical resistances (differently shaped spring wires). Experiments were performed over 14 days. Macroporous collagen-I scaffolds (1.2±0.4 kPa; rectangular shape 1.5x5x13mm; initial clamp distance 6mm) were seeded with primary human dermal fibroblasts in a density of $1.5 \cdot 10^4$ cells/µl. Scaffold contraction was monitored over 14 days. Cell and ECM formation and organization inside the scaffold pores were analysed at day 14 (actin cytoskeleton, fibronectin, fibrillar collagen structures by second harmonic generation microscopy).

Results

Scaffold contraction differed across spring resistances with $\Delta l/l$=5.0% at 6.6N/m (M) and 12.3% at 1.4N/m (S) while free floating samples (ff) led to 26.9% contraction (fig. 1a). Final contraction force F_c was 1.2 times higher in the stiffer spring wire (1.27mN±0.4) compared to the softer one (1.04mN±0.1) (fig.1b). Surprisingly, scaffold contraction showed three distinct phases: (1) a delay phase with moderate increase of Δl and F_c, (2) a subsequent force build-up/pronounced contraction phase and (3) a saturation of contraction phase. Cell behaviour changed from phase 1 to 2 from a direct cell micro-environment interaction (organization within a scaffold pore) to a coordinated contraction of the cells beyond the scale of single cells. The later phase led to distinct collagen I network orientations – scaffolds switched from isotropy to anisotropy over time but dependent on mechanical resistance.

Discussion

The results highlight the role of macroscopic mechanical boundary conditions in cell-scaffold interaction, generated contraction forces and cell organization towards tissue. The gained knowledge helps to better understand the dynamics of tissue growth and the cellular processes leading to tissue orientation. This might help to design mechanical environments for improved endogenous tissue regeneration.

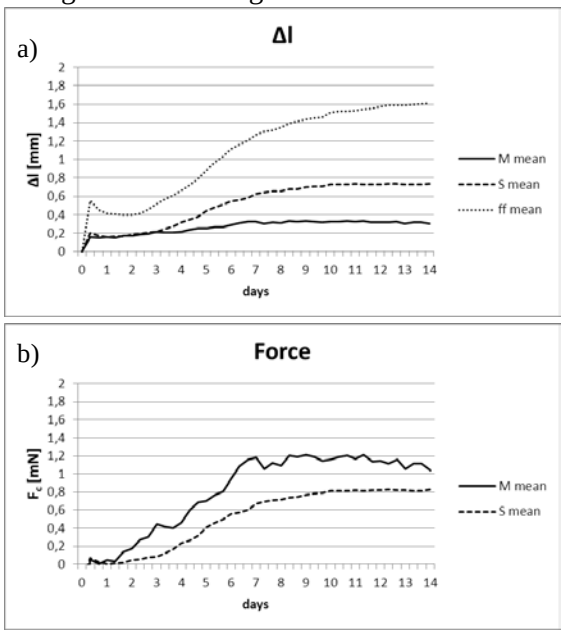

Figure 1(a) *scaffold contraction over 14 days under different mechanical resistances (M=stiff, S=soft, ff=free floating)(mean value of three measurements);* ***(b)****contraction force calculated from the contraction data and the spring constants of the spring wires*

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