WHAT COLLAGEN HYDROGEL FOR OPTIMAL MECHANOBIOLOGY OF 3D ENCAPSULLATED SMOOTH MUSCLE CELLS?

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Introduction

Vascular smooth muscle cells (SMCs) normally regulate mechanobiological homeostasis in arteries when they express a contractile phenotype [1]. Traction Force Microscopy (TFM) is a technique to assess *in vitro* traction forces between the cell and the extracellular matrix [2]. Unlike previously performed SMC cultures on a hydrogel surface, 3D models of embedded SMCs offer a more realistic model of the *in situ* cell-matrix interactions [3], [4]. Because the matrix itself can induce SMCs phenotypic transitions, 3D TFM requires the use of *in vitro* hydrogels where SMCs keep their contractile phenotype. This study aims to 1/ assess the behaviour of embedded SMCs embedded in hydrogels of varying composition and mechanical properties and then 2/ design a collagen hydrogel suitable for future TFM investigations.

Methods

Twelve chemically different hydrogels were derived from a 10 mg/ml Type I collagen solution (TeloCol-10, Advanced Biomatrix) varying the collagen solution dilution (from 2.5 to 10 mg/ml) and the pH (from 7.4 to 8 to allow cell viability). Hydrogels were prepared on ice to prevent early gelation. Each hydrogel solution was derived into both acellular and seeded hydrogels.

Primary aortic SMCs (AoSMC, Lonza) were cultured in growth medium (SmGM-2, Lonza) at 37°C with 5% CO_2. Cells at passage P10 were gently mixed with the hydrogel solutions at three cellular densities (from 50 000 to 150 000 cells/ml) resulting in 16 different seeded hydrogels. Both acellular and seeded hydrogels were cured at 37°C for 1h before immersion in medium. Cell nuclei and actin fibres were imaged in fluorescence with confocal microscope (Axio Observer Z1 station, Zeiss) after a 5 days differentiation. Each cell population was quantified and characterized by cell shape (elongated/round) and length (> or <100µm).

The acellular hydrogel viscoelastic behaviour was characterized with a stress-controlled rheometer (Discovery HR 2, TA Instruments) with 15mm plate-plate geometry at 37°C in oscillatory uniaxial compression and shear at frequencies in the range 0.1-10.0 Hz.

Results and Discussion

High fractions of SMC contractile phenotype (Figure 1, (a)) were found in low collagen concentration hydrogels (p=0.001, ANOVA) and for sufficient initial cellular densities (p=0.008, ANOVA). Only the initial cell density seemed to significantly affect their average length (p=0.011, ANOVA). For TFM applications, an optimal initial cellular density maximizing the fractions of contractile SMCs while avoiding cell-cell contact phenotype (Figure 1, (b)) was obtained with a collagen hydrogel concentrated at 2.5 mg/ml at pH=8, and seeded with 100000 cells/ml.

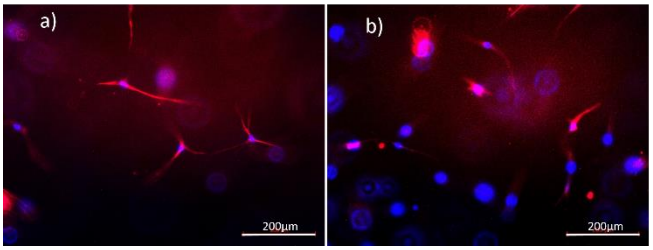

Figure 1: SMC populations in two different hydrogels. (a) Typical desired contractile phenotype. (b) A too high cell density limiting using TFM. Nuclei (blue) and actin fibers (red) were imaged by fluorescence.

The acellular hydrogels exhibited different viscoelastic behaviours in shear and compression, with storage and loss moduli intersecting in compression. This could suggest that hydrogel microstructure present an anisotropy of organization or in cross-link point density along the axial axis. Because the SMC basal tone and contractibility adapt to the mechanical behaviour of the extracellular matrix [5], the SMCs traction force study requires 3D TFM on embedded cells in order to assess potential force heterogeneity.

Conclusion

A hydrogel was designed for studying *in vitro* embedded SMC mechanobiology. Future work will include the quantification of SMCs basal tone and assessment of their mechanobiological response to matrix loading.

References

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