CELL ORIENTATION INFLUENCES THE MECHANICAL PROPERTIES OF FIBROBLAST POPULATED COLLAGEN VESSELS

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INTRODUCTION

Bio-artificial vessels composed of cells in a collagen matrix are being developed as replacements for damaged small caliber arteries. Most pure collagen-cell constructs lack the required strength to withstand *in vivo* conditions. The strength and stiffness can be altered by changing the microstructure of the construct such as the cell orientation, degree of collagen crosslinking and amount and/or type of matrix present. If the mechanical results of the microstructural changes could be predicted *a priori*, replacement vessels could be engineered to match specified design requirements.

Zahalak et al (2000) developed a model to correlate micro-structural parameters, such as cell number and orientation, to the mechanical behavior of collagen-cell constructs. The model validation included relaxation tests on constructs with randomly oriented cells. To investigate applications of this model, fibroblast populated collagen vessels (FPCVs) with two different cell orientations have been constructed and subjected to biaxial mechanical tests.

METHODS

FPCVs were constructed as described in Wakatsuki et al (2000). Primary chick embryo fibroblasts (1E6/mL) were combined with rat tail collagen type I (1 mg/mL) and standard culture media containing DMEM, antibiotics and 10% fetal bovine serum. The mixture was poured into a tubular Teflon mold with a central mandrel and placed in an incubator. After 6 hours each FPCV had contracted around the central mandrel and was removed to a tissue culture dish. L'Heureux et al (1993) showed that cell orientation could be influenced by controlling the contact of a bio-artificial vessel with the inner mandrel. The FPCVs were either allowed to maintain contact with the mandrel during incubation (= no-slip) or were dislodged twice per day using forceps to slip the FPCV along the mandrel (= slip).

The FPCVs were removed from the incubator after 4 days and small carbon markers were placed along each central length. The diameter and distance between the markers were measured to define the unloaded length and diameter. The FPCV was then removed from the mandrel and mounted in a biaxial test system. The test system can inflate and/or stretch an FPCV while recording the pressure, longitudinal force, diameter and length (for 1 pair of markers). Each FPCV was first preconditioned by overstretch. This consisted of 10 cycles at 5% over the maximum strain desired for testing. It has been determined that preconditioning by overstretch gives consistent stress-strain results that do not depend on the total number of stretches (Wagenseil et al, 2001). After preconditioning, each FPCV was subjected to a series of unidirectional stretches (i.e. either diameter or length was varied). The FPCV was immersed in HEPES-DMEM with 5% calf serum at pH 7.4 and 37°C throughout the protocols. Stresses and strains were calculated assuming the FPCV was an incompressible, thin-walled cylinder.

After mechanical testing, each FPCV was stained with a fluorescent cell membrane dye, fixed and imaged with a confocal microscope. The specimens were prepared so that the horizontal image axis (0°) corresponded with the longitudinal axis of the FPCV and the vertical axis (90°) corresponded with the circumferential axis. Cell orientation was measured in 5 confocal slices equally spaced through the specimen thickness. Round cells and cells that were cut off in the x-y image plane were not included. Cell angles were divided into 9 bins from -90 to 90° (no-slip) or 0 to 180° deg (slip).

RESULTS

The FPCVs demonstrated anisotropic mechanical properties. The slip FPCVs were stiffer in the circumferential direction, while the no-slip FPCVs were stiffer longitudinally (Fig. 1a and 1b). The slip FPCVs had to be stretched beyond their unloaded length to be inflated at all. If the slip FPCVs were not stretched, they would bend longitudinally instead of inflating. At these large longitudinal strains, the slip FPCVs could not be inflated beyond 0.2 circumferential strain without bursting. The no-slip FPCVs could be inflated at their unloaded length up to 0.4 circumferential strain. The no-slip FPCVs usually could not be stretched beyond their unloaded length without tearing at the ends.

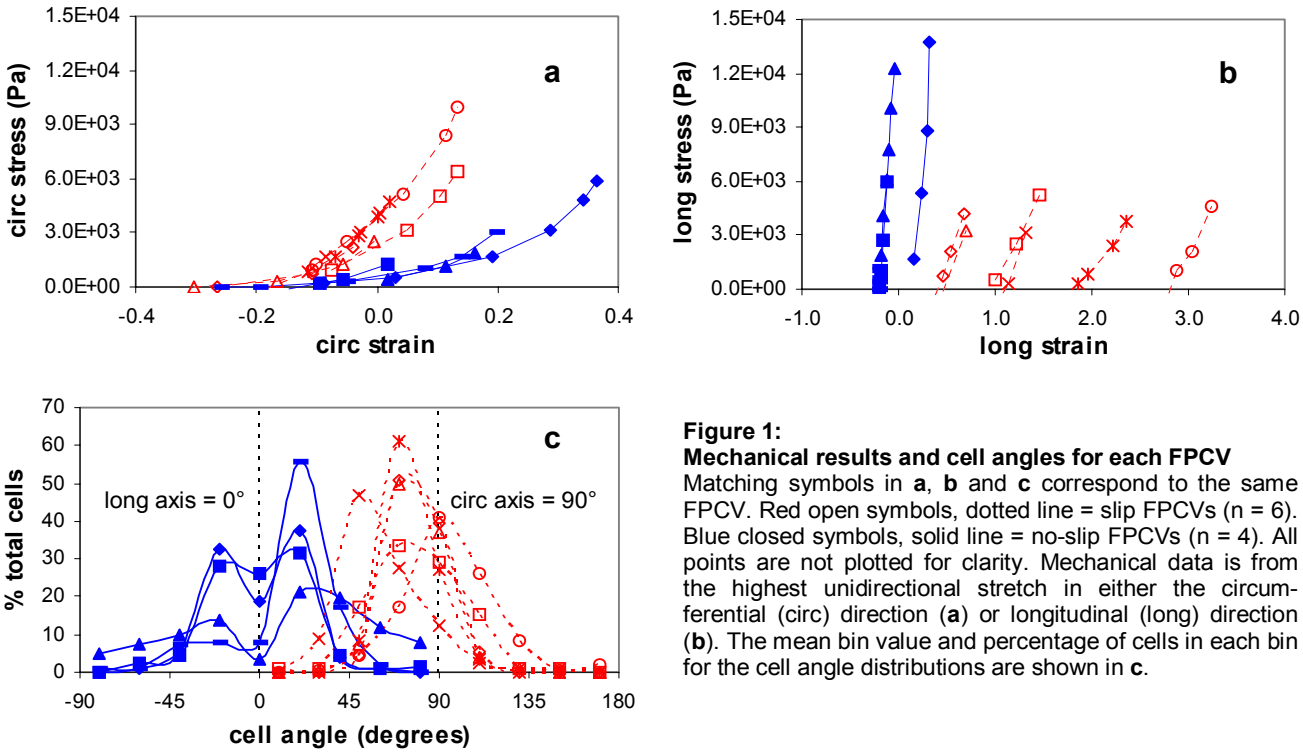

Figure 1:
Mechanical results and cell angles for each FPCV
Matching symbols in **a**, **b** and **c** correspond to the same FPCV. Red open symbols, dotted line = slip FPCVs (n = 6). Blue closed symbols, solid line = no-slip FPCVs (n = 4). All points are not plotted for clarity. Mechanical data is from the highest unidirectional stretch in either the circumferential (circ) direction (**a**) or longitudinal (long) direction (**b**). The mean bin value and percentage of cells in each bin for the cell angle distributions are shown in **c**.

The cell angle measurements (Fig. 1c) confirmed expectations that the slip and no-slip FPCVs would have orthogonal cell orientations. The slip FPCVs had a circumferential cell orientation, while the no-slip FPCVs had mostly longitudinally oriented cells. The slip FPCVs had a normal distribution of cell angles. The no-slip FPCVs often had a bimodal distribution with peaks around ±20°.

DISCUSSION

Each FPCV group was stiffest in the direction of cell orientation. While the mean cell angles were not precisely 0 or 90°, there was a distribution close to those angles. Small offsets were probably the result of misaligning the longitudinal and circumferential axes of the FPCV during preparation for imaging. The no-slip FPCVs were less consistently aligned than the slip FPCVs. The mandrels were made of Teflon and may have allowed some slippage to occur. The cells in the no-slip FPCVs may have been reorienting as the FPCV slowly detached from the mandrel over the incubation period.

It is difficult to quantitatively compare the mechanical results between the two groups because of the large difference in strain protocols. The material was assumed to be incompressible, so the stresses for a unidirectional stretch were influenced by the strain in all directions. The orthogonal strain was set to the minimum value used for the cyclic stretches in that direction (i.e. for a slip FPCV, one series of stretches from circ strain = -0.15 to +0.15 at long strain = 1.0 and one series from long strain = 1.0 to 1.3 at circ strain = -0.15). Due to the difference in stiffness between the slip and no-slip FPCVs, it was not possible to test them in the same strain range. The required difference in strain protocols alone is evidence that the cell orientation greatly influences the mechanical properties.

A constitutive equation is needed to compare such widely varying protocols. The constitutive equation presented in Zahalak et al (2000) was designed for cell-collagen constructs and includes cell orientation tensors as well as individual cell and matrix constants. The measured cell angle and mechanical data presented for the FPCVs will be used to test the validity of this constitutive equation. The model equations will be fit to the mechanical data to determine the cell and collagen constants. These constants can be used to predict the outcome of changing microstructural parameters such as cell number and matrix content. FPCVs could then be engineered to provide specific mechanical properties.

ACKNOWLEDGEMENTS

Dr. Frank Yin for equipment, Dr. George Zahalak for discussions and the Whitaker Foundation for funding (JEW, graduate fellowship).

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