A PLATFORM FOR COMBINATORIAL MECHANOBIOLOGICAL STIMULATION OF ENGINEERED MICROTISSUES

Bogdan M. Beca[1], Christopher Moraes[1], Jason Nichol[2], Ali Khademhosseini[2], Yu Sun[1] and Craig A. Simmons[1]

[1]University of Toronto, Canada, [2]Massachusetts Institute of Technology, USA

ABSTRACT

This paper reports the design and use of a novel high-throughput mechanobioreactor system for multifactorial investigations of cell-seeded engineered microtissues. Combinatorial experiments were conducted to test the independent and combined effects of mechanical strain, matrix stiffness, and a biochemical factor on the expression of α-smooth muscle actin (α-SMA), a pathological marker, in porcine aortic valve interstitial cells (VICs). We found that mechanical tension, transforming growth factor-β1 (TGF-β1), and matrix stiffness, all significantly increased α-SMA levels independently ($p < 0.001$, $p < 0.001$, $p = 0.05$, respectively), as well as in a coupled manner.

KEYWORDS

Tensile bioreactor, high-throughput, valve interstitial cells, combinatorial, gelatin methacrylate

INTRODUCTION

In the aortic heart valve, the maintenance of homeostasis is a complex affair regulated in large part by valve interstitial cells (VICs), fibroblastic cells that remodel, repair and maintain the valve tissue during physiological low levels of injury. The function of VICs is modulated, at least in part, by biochemical stimuli like growth factors and cytokines, but also by mechanical factors, including intrinsic extracellular matrix properties and external mechanical loading [1]. Consequently, dysregulation of these interacting homeostatic stimuli can lead to disease initiation. For example, in calcific aortic valve disease, TGF-β1, abnormal mechanical loading, and matrix stiffness are believed to cause fibroblastic VICs to differentiate into contractile myofibroblasts [1] that express α-smooth muscle actin (α-SMA) and contribute to valve fibrosis, stiffening, and dysfunction. Recreating complex combinations of hypo- and hyper-physiological stimuli using tissue-engineered platforms is a powerful approach to study disease. While current bioreactor systems have made great strides towards this goal, they do not provide the throughput necessary to efficiently integrate and study the multiple stimuli that are required to accurately emulate the physiological microenvironment.

EXPERIMENT

The goal of this study was to develop a robust system with which cells and tissues could be subjected to a range of uniform tensile strains, with throughput that enabled combinatorial investigations of mechanical and non-mechanical stimuli. To do so, we designed a microfabricated substrate on which an array of cell-seeded hydrogels was patterned. The substrates were then subjected to tensile loading in a bioreactor. As described below, the design of the substrate and bioreactor enabled a range of mechanical, biomaterial, and biochemical stimuli to be investigated combinatorially for their integrated effects on cell and tissue response.

The substrate was designed to provide five different regions of strain along its length. This was achieved through a staircase geometry whereby successively decreasing cross sectional areas experienced larger strains for a fixed displacement. The nominal strains achieved were 2.5%, 5%, 10%, 15%, and 20% on cross sectional areas with thicknesses of 8 mm, 4 mm, 2 mm, 1.33 mm, and 1 mm, respectively (Figure 1).

The substrate was fabricated from polydimethylsiloxane (PDMS) through a combination of soft lithography and squeeze fabrication techniques previously demonstrated in our laboratory [2]. The staircase substrate was further patterned with dog-bone shaped microwells (seven per strain region – 35 in total per substrate) in which gelatin methacrylate (gelMA) hydrogels were moulded, and seeded with cells. The dog-bone profile was selected as it represents the standard geometry for tensile testing, and exhibits a uniform strain profile in its straight region. GelMA has been shown to support adhesion, proliferation and organization of cell cultures, exhibit excellent micropatterning properties, and be easily tailored to different stiffnesses [3], making it an attractive candidate for creating cell-seeded microtissues and a suitable choice for this study. The final hydrogel elastic moduli we selected corresponded to normal (6 kPa) and fibrotic (33 kPa) valve tissue [4].

The bioreactor vessel consisted of a sealed enclosure that contained four polycarbonate plates, each with two sub-compartments (eight sub-compartments total). The vessel was vented to atmosphere through polypropylene venting caps installed on the lid. In each sub-compartment, each substrate could be submerged in media and clamped at both ends by a pair of clamps consisting of a fixed clamp and a moving clamp. The moving clamps were bolted to moving transversal bars that were welded to the main actuating arm. The arm was correspondingly connected to an actuator rod. To actuate the main actuating arm, the actuator rod was part of a rack-and-pinion type mechanism, and was therefore connected to a gear mounted on a stepper motor, outside of the bioreactor vessel (Figure 2). The motor was connected to a personal computer and programmed for desired loading patterns through a programmable step motor driver.

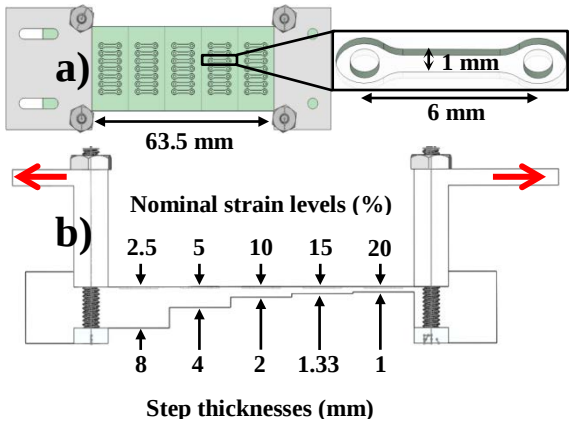

Figure 1: Schematic and dimensions of clamped substrate in top view (a), and side view (b) (inset depicts dog-bone microwell dimensions; red arrows denote direction of motion during stretch).

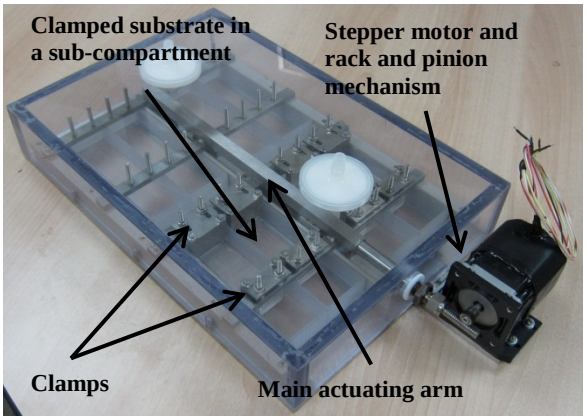

Figure 2: Setup of overall bioreactor system depicting the system components.

To characterize global and local longitudinal strains of the hydrogels, the substrates were stretched using a custom-designed single-substrate stretching device. The substrates were clamped in the device as in the bioreactor vessel, and were photographed in the unstretched and stretched positions. To track the strain of the gels, fiducial markers were applied at four different locations throughout the straight regions of the gels. Global strains were measured based on relative displacements of the markers at the edges; local strains were measured based on the relative change in length of the markers.

As expected, hydrogel strains on both stiffnesses increased with increasing substrate strain ($p < 0.001$) and were consistent between hydrogels in the same strain region (Figure 3; results for 33 kPa condition not shown, but similar). Similarly, local longitudinal strains increased with increasing substrate strain ($p < 0.001$) and were also consistent between hydrogels in the same strain region. Importantly, the strains of the four fiducial markers in each hydrogel were not statistically different, validating the use of the dog bone geometry and demonstrating strain uniformity in the straight regions of the gels (Figure 4).

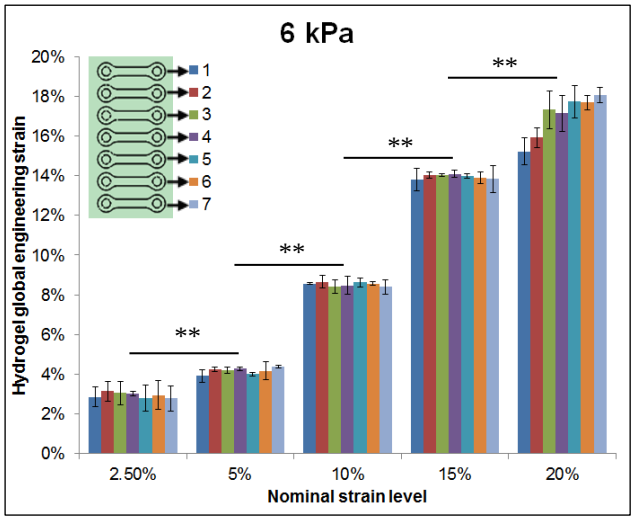

*Figure 3: Hydrogel global (post-to-post) longitudinal strains increased with increasing substrate nominal strains. Results for 33 kPa stiffness not shown but comparable to 6 kPa gels. Results presented as mean ± standard deviation (**p < 0.001).*

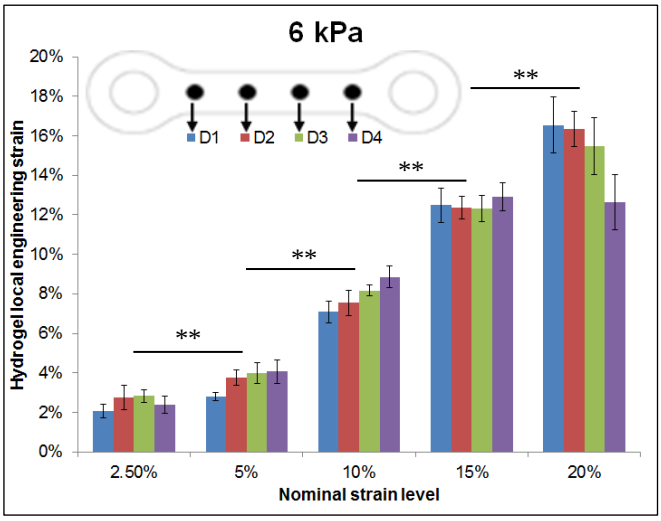

*Figure 4: Local strains within a hydrogel were not different within the same strain region but increased across strain regions. Results for the 33 kPa stiffness not shown but comparable to 6 kPa gels. Results presented as mean ± standard deviation (**p < 0.001).*

Finally, to demonstrate the utility of our platform, we applied the bioreactor to investigate the independent and combined effects of tensile strain (2.5-20%; 1 Hz), hydrogel stiffness (6 kPa or 33 kPa), and biochemical stimulation (0 or 5 ng/mL TGF-β1) on VIC myofibroblast differentiation (as evidenced by α-SMA-positive stress fibers). Accordingly, we found that α-SMA expression increased significantly ($p < 0.001$) on the 10% and 15% nominal strain magnitudes compared to lower strain magnitudes (static, 2.5% and 5%), suggesting a threshold effect (Figure 5). In addition, TGF-β1 and matrix stiffness were also shown to independently induce statistically higher α-SMA expression ($p < 0.001$ and $p =$

0.05, respectively). Interestingly, trends for interactions between strain magnitude and TGF-β1 treatment ($p = 0.076$) between stiffness and strain magnitude ($p = 0.127$) were observed, consistent with previous studies [5, 6] and emphasizing the utility of combinatorial investigations.

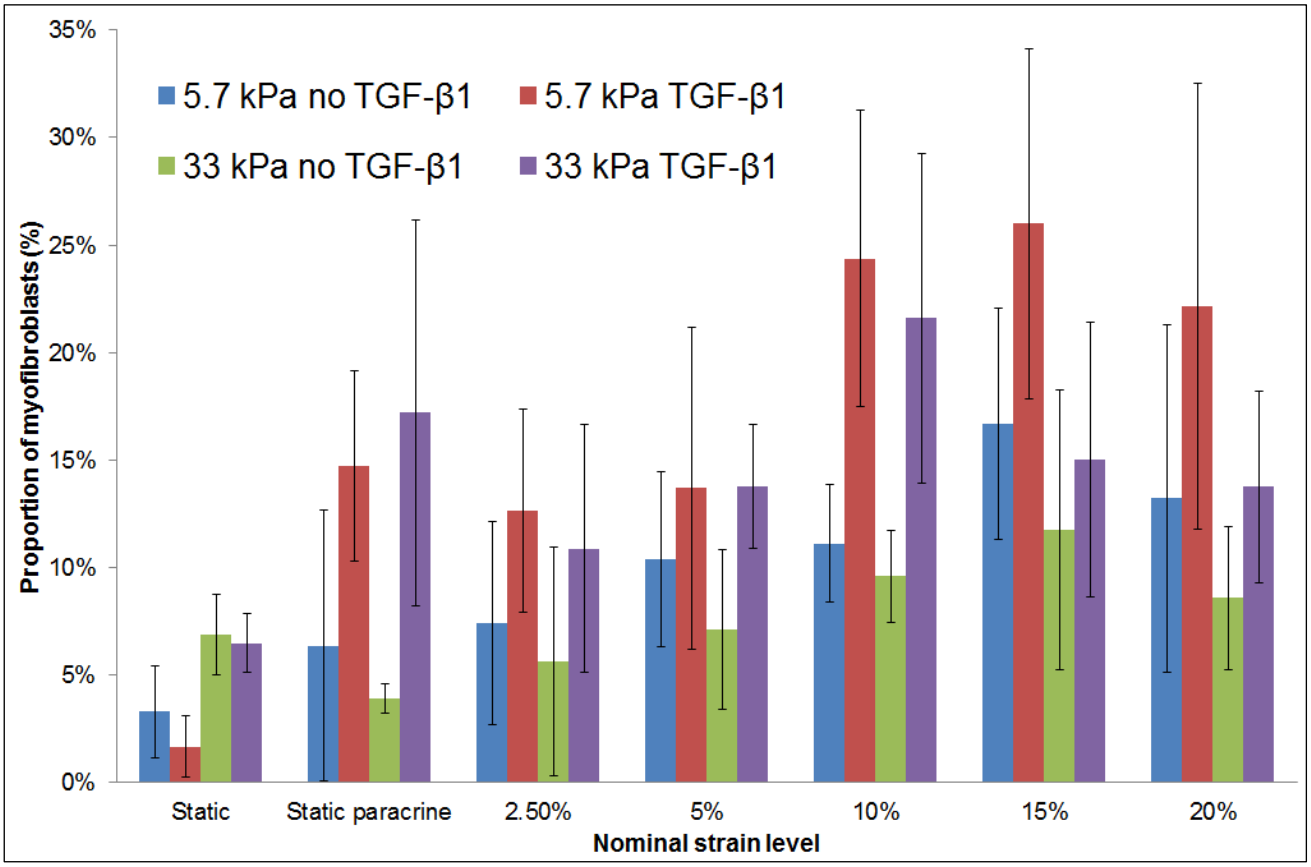

Figure 5: Myofibroblast differentiation was significantly affected by strain magnitude, presence of TGF-β1, and hydrogel stiffness ($p < 0.001$, $p < 0.001$, $p = 0.054$, respectively by ANOVA). Results presented as mean ± standard deviation.

In summary, we report the design and manufacture of a bioreactor system for high-throughput multifactorial screening of cell-seeded constructs or microtissues. It was found that strain magnitude, matrix stiffness, and biochemical stimulation significantly affected myofibroblast differentiation in a complex, non-linear manner. This underscores the importance of designing systems capable of probing multisignal regulation of cells and tissues in a high-throughput and combinatorial manner.

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CONTACT

Bogdan Beca b.beca@utoronto.ca