THREE-DIMENSIONAL MECHANICAL COMPRESSION OF BIOMATERIALS IN A MICROFABRICATED BIOREACTOR WITH ON-CHIP STRAIN SENSORS

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ABSTRACT

This paper reports a microfabricated mechanical testing platform, its use for biomaterial stiffness measurements, and its preparation for dynamic mechanical stimulation of arrayed tissue samples. We present measured structural and mechanical properties of hydrogel materials and demonstrate that human mesenchymal stem cells (hMSC) can be cultured in 3D gels within the platform. By integrating microfluidics and dynamic mechanical environments, this platform provides suitable culture conditions for arrayed load-bearing tissue constructs.

KEYWORDS

Micro device, mechanical loading, biomaterial, hydrogel, mesenchymal stem cell, bioreactor

INTRODUCTION

Traditional 2D cell culture platforms do not reconstitute the *in vivo* cellular microenvironment and preclude long-term cell culture and tissue growth. 3D platforms that better mimic the microstructure, dynamic mechanical properties, and biochemical functionalities seen *in vivo* are therefore increasingly used to analyze complex cell behaviors and tissue growth in a more physiologically relevant context [1]. Cell fate is governed by many factors that include solutes such as growth factors and cytokines, mechanical and biochemical properties of the extracellular matrix, cell type, and applied physical forces such as mechanical stresses [2]. To study the interplay of these factors and to optimize *ex vivo* tissue regeneration, array-based microfluidic culture platforms provide combined advantages of biomimicry and increased experimental throughput [3]. In this work, we present a microfluidic platform for culture of arrayed tissue constructs under conditions that include dynamic mechanical loading. The integrated elastomeric strain sensors in our platforms are used to measure the sample's mechanical properties.

MATERIALS AND METHODS

As shown in Fig. 1, the platform is based on deformable (bulging) membranes that compress samples when pressure is supplied through an underlying channel network. Input pressure was controlled using a programmable pressure regulator (Marsh Bellofram model 3420) and membrane deflection amplitude was monitored by carbon nanotube (CNT) strain sensors (gauges) that were integrated in the membranes. Blends of CNT and PDMS are known to exhibit strain-dependent resistivity [4]. Membrane deflection was reduced in proportion to the sample's stiffness and the magnitude of sensor's resistive strain, $|\Delta R/R_0|$, versus input pressure therefore permitted measurement of the samples' compressive moduli. Deformable membranes were fabricated using standard soft-lithography and embedded strain sensors were based on multiwalled carbon nanotubes that were blended with PDMS to form an elastomeric material that exhibits strain dependent electrical conductivity. Strain sensors were patterned by screen-printing and were embedded in the membranes. To minimize perturbations of membrane bulging caused by embedded strain sensors, we measured tensile properties of various CNT:PDMS blends to select the materials that matched the tensile properties of PDMS.

For synthetic tissue constructs, we used a photo-polymerizable gelatin-based hydrogel, gelatin methacrylate (GelMA) [5, 6], and we varied several recipes of GelMA to support human mesenchymal stem cells (hMSCs) in 3D cultures. Gelatin was mixed in phosphate buffered saline (PBS) using various weight to volume (w/v) ratios (5%, 10%, and 15% GelMA), and environmental SEM images showed (w/v)-ratio-dependent porosity (Fig. 2A). To measure sample stiffness, we applied cyclic driving pressure and measured the corresponding peak-to-peak resistive strain signal. To construct synthetic tissues, we suspended hMSCs in PBS at moderate cell densities (~10^5 cells/mL), which were mixed with equal volumes of 10% GelMA + 0.5% photo initiator. The resulting hMSC:GelMA solution was then cast in cylindrical wells (radius = height = 2.5 mm) and polymerized by exposure to UV light for 30 seconds (final GelMA w/v = 5%). The resulting cylindrical tissue constructs were loaded in the micro device and cultured for up to 21 days in osteogenic culture media (DMEM + 16.5% FBS + 10 mM β-glycerophosphate + 50 µM ascorbate + 10 nM dexamethasone). During culture, we applied cyclic compressive loads to measure tissue elastic moduli: ~10% strain was applied for 30 minutes every third day using a driving frequency of 0.1 Hz. "Live/Dead" (calcein AM/ethidium homodimer) staining was used to assess cell viability and we stained for α-smooth muscle actin (αSMA) because hMSC contractility is correlated with α-SMA content [7] and increased α-SMA expression in hMSCs is known to result from mechanical compression [8].

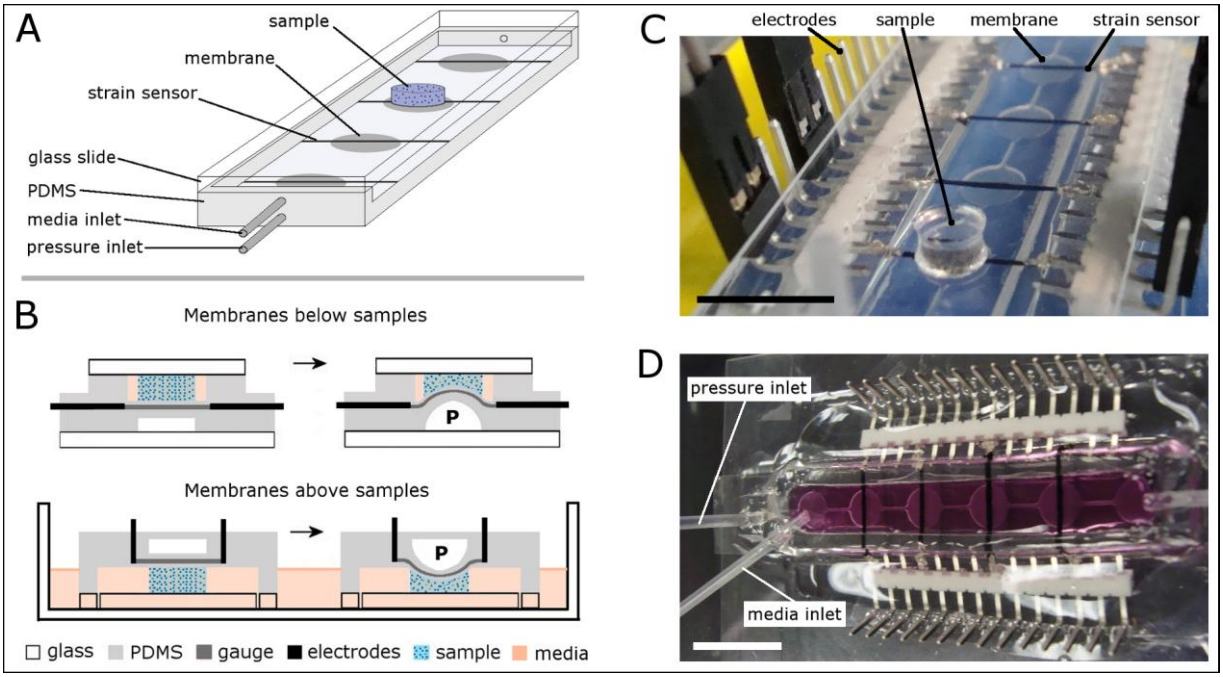

Figure 1. Micro device for biomaterial mechanical compression. (A) Schematic of the microdevice. Samples are confined between a rigid glass coverslide and deformable elastomeric membranes that are deflected (bulging) using pressure supplied through a single inlet. Nanotube-based strain sensors provide continuous readout of membrane deflection amplitude. Perfusion is achieved using a (fluid) media inlet; (B) two configurations of the microdevice: Samples are either cultured in the device (top) or on glass coverslides that are loaded in the device for stimulation or measurement (bottom); P = pressure; (C) Micro device with a single sample; electrode leads are embedded in PDMS for electrical insulation; scale bar = 1 cm; (D) Micro device with perfused cell culture media; membrane elements with embedded strain sensors are actuated by pressure and perfused with media as described in (B); scale bar = 1 cm.

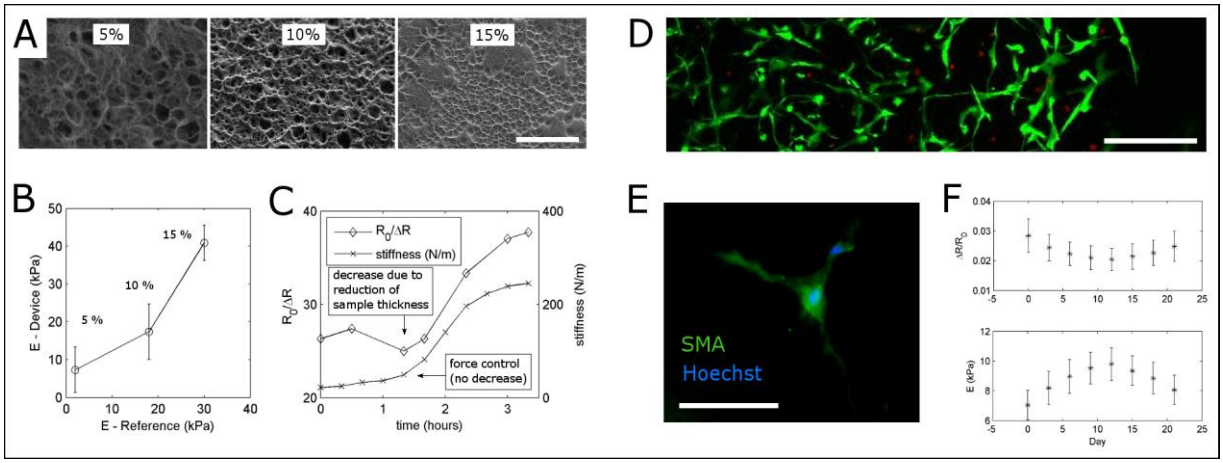

Figure 2. Structural and mechanical characterization of biomaterials. (A) Scanning electron microscope images of GelMA with indicated percent weight to volume ratios (w/v %) of gelatin to PBS; scale bar = 100 µm; (B) Elastic moduli, E, of three GelMA formulations (w/v % indicated) measured using the micro device and reference platforms; data are mean ± SD (N = 3); (C) Inverse resistive strain, $R_0/\Delta R$, and stiffness of GelMA drying in air (w/v = 5%);(D-F) hMSCs embedded in GelMA (w/v = 5%) (D) Calcein AM/Ethidium homodimer viability staining of hMSCs embedded in a GelMA cylinder (height= radius =2.5 mm, w/v = 5%) and cultured in the micro device for 1 week. Live or dead cells appear green or red, respectively; the image is in the XY-plane near the gel center (z = 1.25 mm); scale bar = 200 µm; (E) αSMA expression in hMSCs: αSMA (green) and nuclei (blue) are shown; scale bar = 100 µm; (F) time-dependent resistive strain, $\Delta R/R_0$ (top panel), and elastic modulus, E (bottom panel), of hMSC:GelMA tissue constructs during three weeks of culture in the micro device; cell proliferation and gel contracture resulted in increased E-values during the first 12 days, followed by decreased E-values that resulted from gel decomposition; data are mean ± SD (N = 5).

RESULTS AND DISCUSSION

Elastic moduli, E, of three different GelMA formulations that were measured using our micro device and a commercially available reference platform (TestResources 840 series) were in good agreement (Fig. 2B). To demonstrate time dependent changes to GelMA mechanical properties, we measured drying-induced GelMA stiffening (Fig. 2C). These measurements proved that our device can be used to measure biomaterial stiffening within a range of elastic moduli that is relevant to the onset of tissue stiffening during cell-based matrix remodelling.

We observed hMSC spreading and proliferation in GelMA (w/v = 5%) during the first week of culture. Cell viability assays and confocal imaging revealed high cell viability (~80%, see Fig. 2D) throughout the gel volumes (number of gels analyzed = 6) and expressed αSMA (Fig. 2E). In contrast, αSMA expression was insignificant in hMSCs that were grown on 2D GelMA substrates under otherwise identical culture conditions (data not shown). Cell morphology is known to depend on matrix dimensionality but feedback between cell-based matrix remodelling and cell phenotype is only beginning to be studied [9].

After roughly two weeks in culture, gels decomposed and released cells into the culture medium. On-chip measurement of the tissue's elastic moduli captured this behavior: E increased during the first 12 days and subsequently decreased (Fig. 2F). Our present work focuses on (i) improving gel formulations for long-term culture and (ii) quantifying tissue stiffness changes during osteogenic and chondrogenic hMSC-based matrix mineralization. Long-term tissue culture in our micro device will permit systematic studies of multivariate factors that contribute to *ex vivo* regeneration of load-bearing tissues.

CONCLUSIONS

We demonstrated that arrayed mm-sized tissue constructs can be cultured in our microfabricated bioreactor. Mechanical stimulation is a key regulatory factor that many culture platforms lack, and integrated strain sensors permit online monitoring of tissue stiffness. This platform will permit the screening of soluble factors such as growth factors for their efficacy in tissue regeneration protocols.

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