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# Microdevice arrays with strain sensors for 3D mechanical stimulation and monitoring of engineered tissues

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ABSTRACT

Native and engineered tissue development are regulated by the integrative effects of multiple microenvironmental stimuli. Microfabricated bioreactor array platforms can efficiently dissect cue-response networks, and have recently integrated critical 2D and 3D mechanical stimulation for greater physiological relevance. However, a limitation of these approaches is that assessment of tissue functional properties is typically limited to end-point analyses. Here we report a new deformable membrane platform with integrated strain sensors that enables mechanical stretching or compression of 3D cell-hydrogel arrays and simultaneous measurement of hydrogel construct stiffness in situ. We tested the ability of the integrated strain sensors to measure the evolution of the stiffness of cell-hydrogel constructs for two cases. First, we demonstrated in situ stiffness monitoring of degradable poly (ethylene glycol)-norbornene (PEG-NB) hydrogels embedded with mesenchymal stromal cells (MSCs) and cultured with or without cyclic tensile stimulation for up to 15 days. Whereas statically-cultured hydrogels degraded and softened throughout the culture period, mechanically-stimulated gels initially softened and then recovered their stiffness corresponding to extensive cell network and collagen production. Second, we demonstrated in situ measurement of compressive stiffening of MSC-seeded PEG-NB gels cultured statically under osteogenic conditions, corresponding to increased mineralization and cellularization. This measurement technique can be generalized to other relevant bioreactor and organ-on-a-chip platforms to facilitate online, non-invasive, and high-throughput functional analysis, and to provide insights into the dynamics of engineered tissue development that are otherwise not available.
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## 1. Introduction

Tissue development, homeostasis and disease are influenced by the integration of multiple environmental cues, including biochemical stimuli, mechanical forces, and extracellular matrix material properties [1,2]. Cell-driven tissue responses to one type of stimulus can be modulated by other environmental cues, resulting in context-specific responses [3]. In vitro efforts to understand complex interactions between multiple microenvironmental stimuli have been limited by the low experimental throughput of traditional bioreactors. To address this need, microfabricated bioreactor arrays have been developed to combinatorially prescribe and probe multiple environmental stimuli to systematically define cue-response relationships [4–8]. For example, Figallo et al. developed a device with arrayed culture wells to enable systematic and precise variation of mass transport and hydrodynamic shear to study their effects on stem cell functions [6], and Gobaa et al. developed a platform with an array of soft hydrogel microwells that simultaneously probed substrate stiffness and combinations of adhesion proteins on stem cell fate [8].

Bioreactor array platforms have provided important insights into the regulatory roles of specific environmental stimuli and their interactions on cell fate and tissue development [9–11]. However, despite the critical regulating effects of 3D mechanical stimulation on cell and tissue function [12–14], there are few examples of

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bioreactor array platforms that include mechanical stimuli. To address this need, we and others have developed microfabricated platforms that apply 2D dynamic mechanical stretch to cells adhered to deformable membrane arrays [15,16]. These platforms have been used to study the mechanobiological responses of cells to combinations of matrix proteins, growth factors, and 2D dynamic stretch, with higher throughput than standard cell stretching systems [15]. Performing similar systematic, combinatorial experiments in 3D mechanically active environments is also of broad interest, particularly for tissue engineering applications in which microenvironmental stimuli can be used to guide cell function and tissue formation in vitro [5,17–19]. For some tissues, such as cartilage and intervertebral disc [20,21], tissue growth is predominantly modulated by compressive forces, whereas other tissues such as heart muscle, heart valves, blood vessels and bladder benefit from 3D loading that includes in-plane tension for tissue growth [22,23]. Thus, we have adapted our deformable membrane platforms to enable both 3D mechanical compression [24,25] and stretching [26] of arrays of cell-seeded hydrogel constructs, which enable combinatorial investigation of a variety of relevant mechanobiological stimuli on cell fate and function and optimization of culture conditions.

A limitation of these approaches, and indeed most bioreactor-based approaches for tissue engineering, is that assessment of tissue functional properties is limited to end-point analysis [27–29]. End-point analysis is inherently destructive and requires manual and offline assays with bulky analytical equipment. In contrast, continuous monitoring of the functional status of engineered tissues would provide information on dynamic changes in tissue development and enable feedback for appropriate control and optimization, akin to other bioprocesses. However, there are few examples of bioreactor array platforms [30] capable of quantitatively tracking the functional status of tissue development, with cellular analysis being the most common approach [6,31–33]. However, cellular markers are not necessarily reflective of tissue functions, particularly for load-bearing tissues in which tissue mechanical properties are among the gold standard function metrics.

To address this need, we report here a deformable membrane platform that enables mechanical stretching or compression of 3D cell-hydrogel arrays and simultaneously performs continuous stiffness measurement of the hydrogel constructs in situ with integrated strain sensors. We demonstrate that the integrated strain sensors are able to monitor hydrogel stiffness changes that correlate with matrix degradation, collagen synthesis, and mineralization. Our platform represents a promising approach to address the limitation of end-point analysis, enabling exploration of bioprocess control strategies and providing insights otherwise not available into the evolution of engineered tissues.

## 2. Materials and methods

### 2.1. Device fabrication

Building on our previous bulging membrane platforms [15,24,26], we have developed a new device array that applies dynamic 3D mechanical stimulation to soft biological tissues in the device and performs continuous on-chip tissue stiffness measurement (Fig. 1A). Specifically, we applied 3D mechanical stretching to the hydrogels by covalently bonding them to the bulging membranes, as shown in Fig. 1. Carbon nanotube (CNT)-based strain sensors, which exhibit strain-dependent electrical resistivity [24], were patterned and embedded in the deformable membranes to provide on-chip measurement of membrane deflection for estimating tissue stiffness. Membrane deflection is proportional to the sample tissue's stiffness, and thus the magnitude of sensor's resistive strain, $|\Delta R/R_0|$, vs. input pressure permitted the measurement of the samples' elastic moduli (Fig. 2).

To fabricate the bulging membrane platform with integrated strain sensors, off-stoichiometry thiol-ene based polydimethylsiloxane (OSTE-PDMS) was mixed at a composition ratio of 2:0.3:1.5:1.5:1.5 and poured onto an aluminum master mold. The mold contained features of pressure chambers and channel at a height of 0.25 mm. OSTE-PDMS was casted against the mold to a final height of 1 mm. OSTE-PDMS was cured with 365 nm UV light for two minutes at an average dose of 6.3 mW/cm$^2$, peeled off the aluminum mold and cut into strips having $3 \times 8$ membranes with each column connected by underlying single channels. These OSTE-PDMS strips were then bonded to glass substrates by first spin coating a ~10 μm-thick mortar layer of OSTE-PDMS on the glass, then placing the cured OSTE-PDMS device layer onto the coated glass slide and curing with UV for one minute. Then connection ports were punched with biopsy punches and connected to tubing.

Strain sensors were fabricated by screen printing strips of uncured CNT: OSTE-PDMS blends (mixed at 1:12 ratio in weight) over the OSTE-PDMS membranes and curing with UV for two minutes. The sensor strips typically had 50 μm thickness, 300 μm width and 1.4 cm length. One sensor strip was placed through the middle of each circular deformable membrane, which was of 5 mm diameter and spaced by 9 mm center to center from other deformable membranes. Electrical connectors were then bonded to the glass slide using cyanoacrylate super glue and connected to the strain sensor strips by applying additional uncured CNT: OSTE-PDMS blends, which were then cured with UV again for two minutes. The sensors and sensor-electrode interconnects were then passivated by spin coating ~100 μm-thick layer of OSTE-PDMS (Fig. 1C). Membrane deflection magnitude and strain sensor signals were calibrated against applied actuation pressure. Membrane bulging heights were experimentally measured using a telescopic lens (Fig. 1D). After calibration, the culture chamber pre-made of regular Sylgard PDMS was bonded to the device layer. Fig. 1B shows a completed device containing culture medium for applying 3D mechanical stretching.

### 2.2. Cell and tissue culture

Cryopreserved human bone marrow-derived MSCs were obtained from the Texas A&M Health Science Center College of Medicine Institute for Regenerative Medicine at Scott &White through a grant from NCRR of the NIH, Grant # P40RR017447. Passage 5 MSCs and complete culture medium containing 81.7% α-MEM with L-glutamine, 16.3% fetal bovine serum, 1% additional L-glutamine and 1% penicillin/streptomycin were used for all experiments. MSCs-seeded hydrogel constructs were cultured and mechanically stimulated with 5% nominal tensile strain at 0.1 Hz for 8 h/day for 15 days (Fig. S1). Culture medium supplemented with 100 μM ascorbic acid was changed every day. For static conditions, the gels were cultured without mechanical stimulation except during the measurement cycles, which were limited to 20–30 min duration applied every two to three days to minimize the effects of mechanical loading on cells. The cell-laden hydrogel arrays were cultured in 150 mm Petri dishes and maintained in a humidified 37 °C incubator with 5% $CO_2$.

### 2.3. PEG-NB hydrogel model system

OSTE-PDMS membranes were used to covalently bind poly (ethylene glycol)-norbornene (PEG-NB) hydrogels, a model biomaterial with tunable adhesion peptide identities and densities, elasticity, and degradability. PEG-NB was synthesized and

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