Stress-induced Response of 3D Bioprinted Cell Filaments Under Controlled Strain Rates

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Statement of Purpose: Cells spontaneously self-organize into three-dimensional (3D) aggregates during embryogenesis and organogenesis. This self-organization is influenced by various factors, including imposed deformation and mechanical stress. Mechanical stress not only induces deformation but also triggers morphological remodeling and functional changes within tissues. Over the past few decades, researchers have made extensive efforts to engineer tissues and study how they respond to mechanical stimuli. However, most existing studies have focused on cell spheroids and monolayers, which have a complicated internal stress state and a two-dimensional (2D) nature. This makes it difficult to gain precise insights into how mechanical stress affects the behavior of 3D tissues [1]. In addition, although recent studies have demonstrated that necking and rupture can occur in strip-like heart tissues formed between two micropillars or constraining boundaries, the tensile stress developed there is solely due to active contraction of cells [2]. A method that allows for precise control of external stress on tissues with predefined geometry and composition is still lacking. In particular, it is conceivable that the externally applied stress could trigger remodeling of the cytoskeleton and extracellular matrix (ECM), ultimately accelerating the development of active cell contraction and morphology change of the tissue. However, properly designed experiments and theoretical investigations are still needed to confirm this hypothesis. The present study aims to address these outstanding issues.

Methods: Cells were resuspended in a calcium chloride-containing dextran aqueous solution as bioink, while sodium alginate served as the matrix. This combination enabled the construction of centimeter-scale linear structures via embedded direct writing. After 24 hours of culture under standard conditions, the cells self-aggregated and reorganized into continuous, uniform, scaffold-free cellular filaments. A uniaxial stretching platform was fabricated using digital light 3D printing. Force-sensing pillars were created by crosslinking polydimethylsiloxane (PDMS) with a weight ratio of 20:1 for the polymer and curing agent. The whole setup was positioned on an inverted optical microscope, where controlled displacement was applied using a high-precision XYZ platform with a resolution of 0.2 μm. Different strain rates were applied to the cell filaments, and optical images and videos recorded real-time changes in fiber morphology, focusing on necking and fracture processes. The instantaneous force on the fibers was calculated from the displacement response of the micropillar, enabling characterization of stress changes over time due to external mechanical stimuli.

Results: Rheological analysis indicates that the bioink exhibits pronounced shear-thinning behavior, facilitating stable extrusion and continuous deposition, thereby enabling high-fidelity freeform fabrication. The self-assembled cellular filaments have uniform diameters (Fig. 1), providing standardized specimens for mechanical testing. The 3D-printed tensile platform delivers controllable, stable uniaxial tension (Fig. 2a), allowing uniform axial loading of the filaments. Optical imaging clearly reveals the morphological evolution of the filaments during tensile loading, including the onset of necking and its progression to tissue failure (Fig. 2b). By measuring the displacement of force-sensing micropillars along the loading direction and using their known effective stiffness, we can infer the load acting on the filaments and compute the tensile stress in real time. These results demonstrate that the developed mechanical platform enables quantitative characterization of the mechanical response and morphology changes of cell filaments under various strain rates.

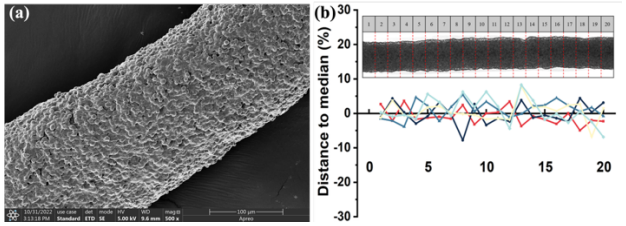

Figure 1. (a) SEM images and (b)variations in the average diameter of the cell filaments.

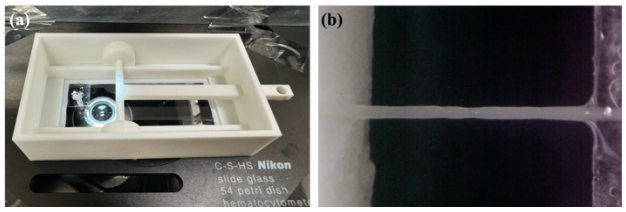

Figure 2. (a) Tensile platform incorporates force-sensing pillars. (b) Optical image of the necking phenomenon observed in cell filaments when stress is applied.

Conclusions: Embedded bioprinting enables the systematic production of uniform cellular filaments, which is crucial for investigating the mechanical properties of engineered 3D tissues at cellular and microtissue scales. The uniaxial tensile platform developed for this project provides precisely adjustable stress in both air and liquid environments, allowing for the systematic investigation of stress-induced responses, such as necking and fracture, across a range of microtissue sizes and cell types. This project will deepen our understanding of collective cellular functions and provide valuable insights for innovative regenerative medicine strategies. The data generated will also support the advancement of future theoretical models.

References:

[1] Harris A R, et al. *P Natl Acad Sci Usa*, 2012, 109(41): 16449-16454.

[2] Karakan M Ç, et al. *Lab on a Chip*, 2024, 24(6): 1685-1701.