4D Tissue Engineering via Cell Traction Force-Mediated Shape Morphing in Single Layer Degradable Hydrogel Scaffolds

Kaelyn Gasvoda, Aixiang Ding, David S. Cleveland, Eben Alsberg
Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL 60612

Statement of Purpose: Four-dimensional (4D) shape change has been previously reported in tissue engineering applications to model morphodynamic tissue development. There have been a wide range of methods used, such as controllable swelling ratio in a bi-/tri-layer system [1], infrared light [2], and temperature differences [3]. However, these methods of 4D shape change depend on external stimuli, which neglects cell participation in shape evolution that occurs during development and healing processes. Cell traction forces (CTF) have been explored as a strategy for 4D shape change that would not depend upon external stimuli. While CTF-based 4D transformation strategies using, for example, DNA Velcro [4], lithography [4], and microplates [5], have been reported, they require complicated fabrication processes and/or non-cytocompatible materials, which may limit their widespread use and *in vivo* application. Here, we report a cell-laden rapidly degradable hydrogel system where the hydrogel degrades so quickly that a predominantly cell-only construct results by 4 days and is capable of CTF-driven 4D shape-morphing transformations.

Methods: Oxidized methacrylate alginate microgels (OMA) and gelatin methacrylate (GelMA) were synthesized using already established protocols. OMA was mixed homogeneously with GelMA to make the bulk polymer composition of the bioink. NIH 3T3 cells (ATCC, Manassas, VA) were then added at 100×10^6 cells/mL polymer. The cell-laden bioink was printed into sheets that were then photocrosslinked and punched into discs with various initial outer diameters (d_0) (Figure 1). At various timepoints, images were collected and the outer diameter of the samples were measured (d_t) to determine shape changes.

Results: CTF-based tissue architectural transformation is a morphogenic driving force *in vivo* and supports long term 4D tissue formation. Figure 1B depicts the shape change of initially flat, circular constructs over 14 days of culture. In the image series, a darker border of the constructs starts to appear at early timepoints due to the edges of the constructs folding over themselves. At D7 and D14, the edges are substantially more defined and the constructs take on a "bowl" shape, as depicted in the schematic in Figure 1A. The change in outer diameter from the initial geometry was then quantified (Figure 1C). While the final construct size post-culture was dependent upon the initial construct outer diameter, all constructs exhibited the same phenomenon of curling inwards along the edges to form a bowl. Control samples with dead cells did not change shape (data not shown), providing evidence that the shape change in the experimental groups is caused directly by encapsulated cell CTFs. Other shapes were also investigated (data not shown).

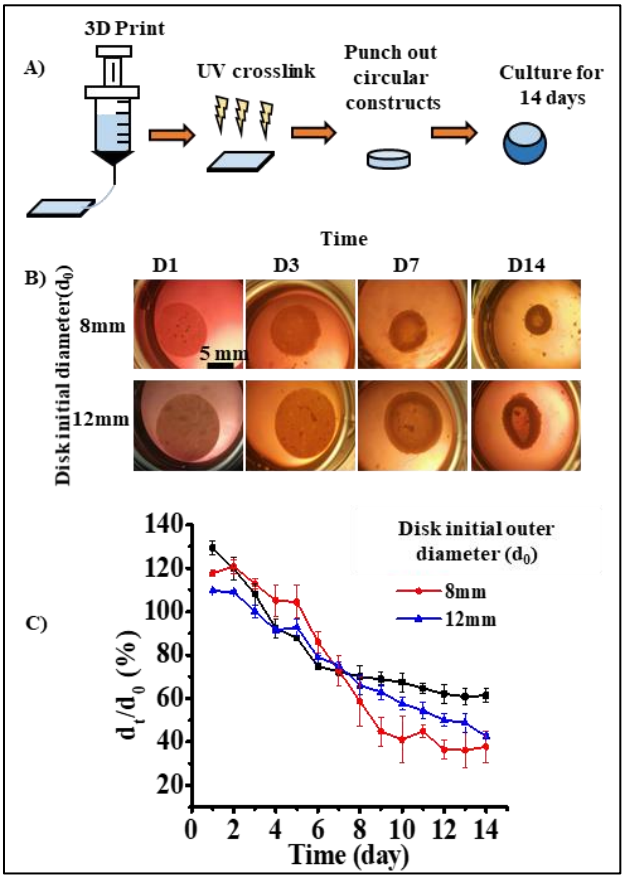

Figure 1: 4D shape change due to cell traction forces. A) Overall schematic of sample production. B) Time lapse photomicrographs highlighting the substantial changes in construct shape from two different starting diameters (d_0). C) Graphical analysis of initial outer diameter shrinkage of the constructs over 14 days.

Conclusions: Here, we have presented a 4D shape morphing structure that rapidly becomes a cell-only condensation and maintains a curved structure. The final shape that is observed from CTF generated forces can be controlled through the initial designed shape. This simple fabrication strategy, along with the cytocompatible and degradable biopolymers used, may allow for widespread use to study developmental processes and in tissue engineering applications.

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References:

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