Optimizing vascular morphogenisis in a self-assembling peptide hydrogel

Massachusetts Institute of Technology

John P. Casey; Vernella V. Vickerman-Kelly, M.S., Roger D. Kamm, Ph.D.
Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

LSU

INTRODUCTION

As biomedical laboratories pursue myriad approaches to tissue engineering, vascularization is a recurrent theme. It arises as both a potentially powerful method to limit the spread of cancer (through its inhibition) and a challenge which must be surmounted for tissue development. Currently, researchers study vascularization by culturing endothelial cells on any of a variety of soft hydrogels – such as collagen and Matrigel™ – which usually mimic the target tissue's extracellular matrix (ECM). In our experiments, we used a synthetic, ECM-mimetic, self-assembling peptide gel whose biomechanical characteristics can be finely tuned by environmental conditions.

The goal of the project was to characterize and optimize the culturing of endothelial cells on the peptide gel, and their network formation, within PDMS microfluidic devices. The devices allow for a greater degree of control over mechanical and chemical gradients than previous systems. The devices also provide the researcher with a better perspective for observing three-dimensional growth.

MATERIALS

The peptide gel we used, RAD16, is composed of alternating positive and negative amino acid residues, each separated by a [non-polar] alanine residue (Fig. 2a):

Primary Structure:
RAD16-I – RADARADARADARADA
RAD16-II – RARADADARARADADA
R = Arginine, A = Alanine, D = Aspartic Acid
Secondary Structure: continuous beta sheets
Tertiary Structure: elongated beta sheet bilayers
Quaternary Structure: strands of beta bilayers

When the pH or ionic strength of the peptide's environment reaches physiological levels, over ~pH 7, the solution will cure and form a gel.

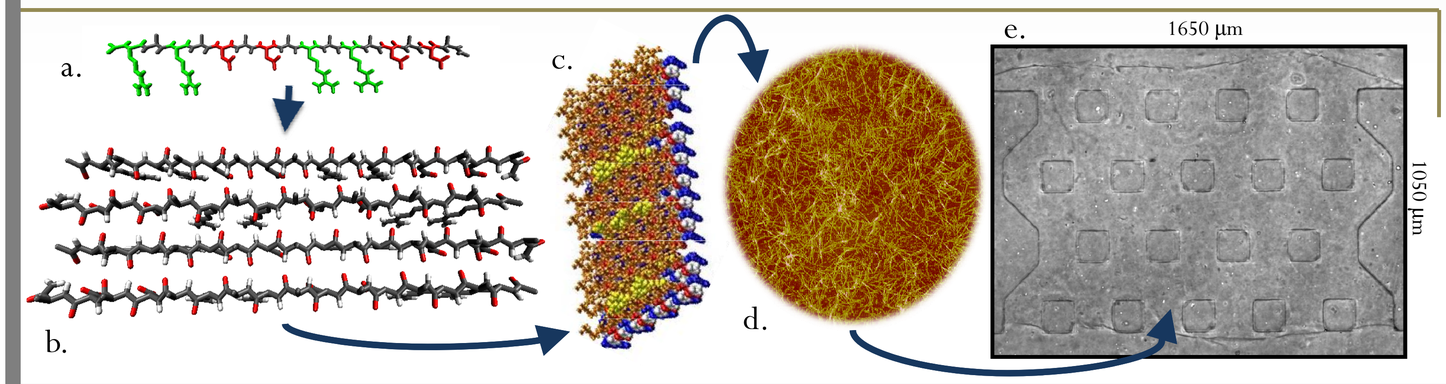

Figure 2: Schematic showing the use of a self-assembling peptide with microfluidic devices. Sixteen-residue strands of amino acids (a) form a bilayer of beta sheets (b, c), which then aggregate to form the fibers of a hydrogel (d). The hydrogel is injected into the gel region of a microfluidic device (e). All images except c. obtained from Nathan Hammond. Figure c. obtained from web.mit.edu/lms/www/research.htm.

METHODS

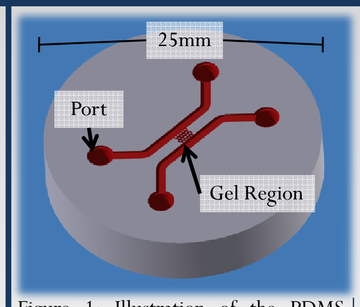

Figure 1: Illustration of the PDMS device.

Polydimethylsiloxane (PDMS) microfluidic devices were molded from patterns on silicon wafers. "Ports" were formed by punching 2mm holes from the patterned face of the PDMS through to the other side (Fig. 1). A solution of 50% RAD16-I (1% by weight in H_20), 46% dextran (20% solution), and 4% phosphate-buffered saline (PBS) was injected into the gel region of the device using a micro-injector. The patterned face was then covered with a glass coverslip.

Cell media (EBM-2 for microvascular endothelial cells) supplemented with vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and phorbol 12-myristate 13-acetate (PMA) was pulled in through the ports. The media, along with the PBS, cured the peptide to form a gel. Human microvascular endothelial cells (HMVECs, passage 4) were either suspended within the gel solution ($2x10^6$ cells/mL) at the time of injection or flowed through one of the channels 12 hours after curing ($1x10^6$ cells/mL) and left to proliferate within the channel. For those seeded on the gel, media supplemented with sphingosine-1-phosphate (S1P) was flowed through the channel opposite of the cells to generate a sphingosine gradient across the gel region. Media was refreshed every 24 hours. All samples were stored at 37°C in 5% carbon dioxide.

The samples seeded within the gel were fixed with paraformaldehyde and stained with a combination of 4',6-diamidino-2-phenylindole (DAPI) and phalloidin-rhodamine 4 days after seeding. The samples with cells seeded outside the gel were left to proliferate for 8 days before fixing and staining with DAPI and phalloidin-rhodamine.

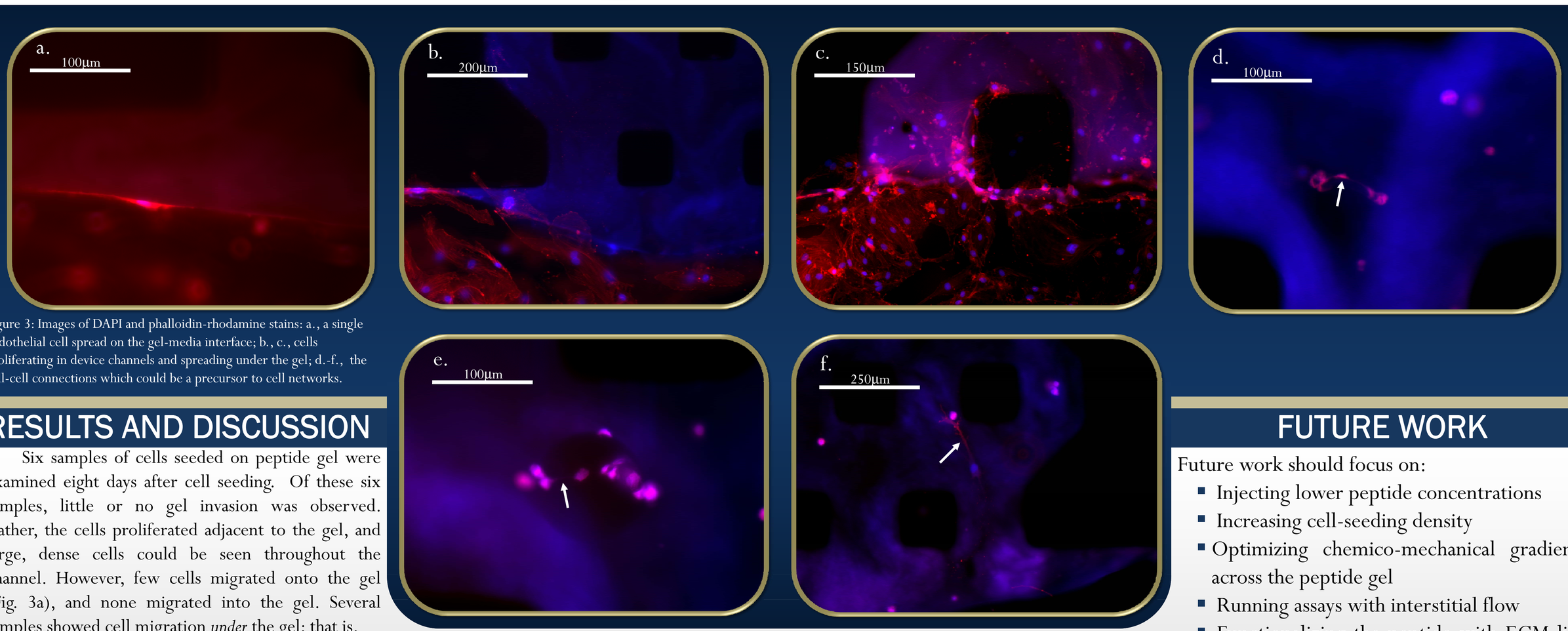

Figure 3: Images of DAPI and phalloidin-rhodamine stains: a., a single endothelial cell spread on the gel-media interface; b., c., cells proliferating in device channels and spreading under the gel; d.-f., the cell-cell connections which could be a precursor to cell networks.

RESULTS AND DISCUSSION

Six samples of cells seeded on peptide gel were examined eight days after cell seeding. Of these six samples, little or no gel invasion was observed. Rather, the cells proliferated adjacent to the gel, and large, dense cells could be seen throughout the channel. However, few cells migrated onto the gel (Fig. 3a), and none migrated into the gel. Several samples showed cell migration *under* the gel; that is, cells tended to squeeze between the coverslip and the gel region, as evidenced by the presence of actin in PDMS regions (Fig. 3b,c). Additionally, six samples of cells seeded in the peptide gel were examined four days after seeding. Of these, most of the cells present seemed to die either during the injection process or shortly thereafter, as they remained small and rounded in the gel. However, in several cases small connections or protrusions could be observed between two cells (Fig. 3d-f), indicating live cells which were able to communicate with each other.

The failure of the cells seeded outside of the gel to form networks within the peptide has several implications. The gel, with a terminal RAD16 concentration of 0.5% by weight, may have been too dense to encourage cell migration, invasion, and network formation. Also, the primary structure of the peptide seems to not have cell attachment sites which are favorable compared to the PDMS or glass. Finally, the S1P gradient may have been too low; a stronger gradient may have better encouraged migration through the gel.

The general stagnancy of cells seeded within the gel also indicates a few points. The procedure for suspending the cells in the peptide solution and injecting them into the gel region needs to be condensed and made gentler so that a higher proportion of the cells survive. This may require some chemical tweaking of the gel solution itself as well. Furthermore, the gel, again 0.5% RAD16-I, may have been too concentrated, and the cells too sparse for optimal network formation. However, examples of connected pairs of cells, a possible precursor of networks, encourage optimism for future assays.

FUTURE WORK

Future work should focus on:

- Injecting lower peptide concentrations
- Increasing cell-seeding density
- Optimizing chemico-mechanical gradients across the peptide gel
- Running assays with interstitial flow
- Functionalizing the peptide with ECM-like cell adhesion motifs

ACKNOWLEDGEMENTS

Principal Investigator:
Roger D. Kamm, Ph.D.
Kamm Lab Group (Kammsters)
Vernella V. Vickerman-Kelly, M.S.
Alisha Sieminski, Ph.D.
The Amgen Foundation
Michael Bergren
MIT Office of Undergraduate Advising and Academic Programming

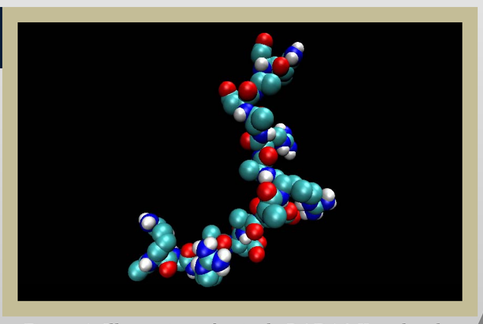

Figure 4: Illustration of a single RAD16-II molecule. Image obtained from Nathan Hammond.