Identifying Peptides with Cell-Specific Degradation

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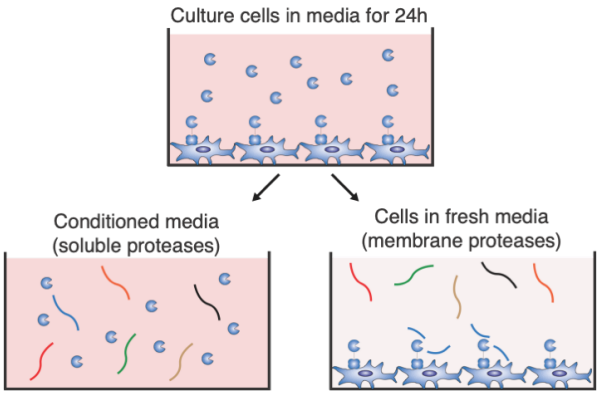
Statement of Purpose: Peptides are widely used in hydrogels and other biomaterials due to their ease of synthesis and ability to improve the biological properties of synthetic matrices. Peptides are natural substrates for cell-secreted proteases, making them ideal for enabling matrices to be modified by encapsulated cells. Protease activity varies by cell type, and proteases can either be attached to cell membranes (membrane-type proteases) or can be soluble proteases released into the media surrounding the cells. This makes it likely that there are peptide sequences which are cleaved by some cell types and not others, and that membrane-type proteases can be targeted to limit peptide cleavage to the immediate vicinity of the cell membrane.

In this work we have used a proteomics screen to identify new protease-substrate peptide sequences and quantified their degradation against six different cell types. We seek to identify peptides that are degraded by only one type of cell and not others. To control spatial degradation, we also are looking for that are only degraded by membrane-type proteases and not soluble proteases. Identifying cell-specific peptide degradation will enable the design of hydrogels containing multiple cell types but having controlled cell-specific microenvironments.

Methods: Peptides were synthesized on a microwave peptide synthesizer. The peptides were then purified via high performance liquid chromatography, lyophilized, and dissolved in ultrapure water. After being filter sterilized, the peptides were incubated with the six types of cells or with media that previously contained the cells. Samples were then taken at 0 hours and 24 hours. Peptide degradation was quantified using liquid chromatography-mass spectrometry (LCMS) to compare degradation of different peptides.

Results: We have developed a functional approach to quantifying peptide degradation by entire cell types. The peptides were incubated for 24 hours with human umbilical vein endothelial cells (hUVECs), human mesenchymal stem cells (hMSCs), and three types of macrophages (M0, M1, M2). To separate out whether these are being degraded by soluble or membrane-type proteases, we incubated peptide libraries with either the cell types of interest, or conditioned media that did not contain cells (Figure 1, Top). Our data indicates that we were able to identify peptides that were more than 10% cleaved by either hUVECs or hMSCs, but not the other cell types.



		Broadly Degraded	hUVEC Specific	hMSC specific
		Fraction of peptide remaining after 24h		
hMSC	Cells	0.35	0.98	0.50
hUVEC		0.52	0.61	0.95
M0		0.76	0.97	1.01
M1		0.75	0.93	1.04
M2		0.76	0.92	0.95
hMSC	Media	0.72	0.96	0.90
hUVEC		0.73	0.93	1.02
M0		0.73	0.98	0.94
M1		1.06	0.95	1.04
M2		1.05	0.96	1.00

Figure 1. (Top) A functional peptide degradation assay was designed in which cells are either cultured with soluble proteases (Media) or in conditions which contained membrane-type proteases (Cells). (Bottom) Peptides were identified which are either broadly degraded, cleaved specifically by membrane-type proteases on hUVECs or hMSCs.

Conclusions: A methodology was developed to identify and quantify peptide degradation by membrane-type proteases on specific cell types. These peptides can be incorporated into biomaterials containing multiple cell types to enable the generation cell-specific microenvironments to better recapitulate the heterogeneity found within biological tissues.