Chemical Engineering Doctoral Defense

An In Vitro Platform to Spatiotemporally Control Multiple Bioactive Peptides Using Reversible DNA Handles

School for Engineering of Matter, Transport and Energy

Fallon Fumasi
Advisor: Julianne Holloway

Abstract

The natural healing process for bone has multiple signaling cascades where several soluble factors are expressed at specific times to encourage regeneration. Human mesenchymal stromal cells (hMSCs) have three stages of osteogenic differentiation: an increase in cell number (day 1-4), early cell differentiation showing alkaline phosphatase (ALP) expression (day 5-14), and deposition of calcium and phosphate (day 14-28). The first two stages are of particular interest since cell adhesion peptides have been shown to have biological significance during bone regeneration. However, far less is known about the temporal dependence of these signals.

To mimic these complex systems, developing dynamic biomaterials has become a popular research area over the past decade. Advances in chemistry, material science, and manufacturing have enabled the development of complex biomaterials that can mimic dynamic cues in the extracellular matrix. One specific area of interest is spatiotemporal control of multiple biomolecules; however, this has generally required diverse chemical approaches making the process difficult. To circumvent these issues, I developed a novel method that combines a photoresponsive hydrogel with single-stranded DNA to spatiotemporally control multiple biomolecules using a single conjugation scheme.

Here, I describe a detailed protocol to manufacture a fully reversible, spatiotemporal platform using DNA handles. Norbornene-modified hyaluronic acid hydrogels were used to spatially control biomolecule presentation while single-stranded DNA was used to temporally control biomolecule presentation via toehold-mediated strand displacement. This platform was used to orthogonally control the presentation of multiple biomolecules with simple and complex spatial patterning, as well as control the cell morphology of hMSCs by changing the presentation of the cell adhesion peptide RGDS.

Then, this system was applied to study the temporal presentation of cell adhesion peptides and their effect on early osteogenic differentiation of hMSCs in vitro. The peptides used were RGDS, HAVDI, and OGP. OGP alone expressed higher ALP when presented from day 7-14 than day 0-7 or 0-14. When RGDS, HAVDI, and OGP were combined, there was an increase in ALP activity when HAVDI was presented from day 0-3 indicating that HAVDI plays a role at earlier time points during osteogenic differentiation.

October 29, 2021; 2 PM;
Zoom Link: https://asu.zoom.us/j/85216527038