Development of a Fully Reversible In Vitro Platform to Spatiotemporally Control Multiple Bioactive Peptides Using DNA Handles

Fallon M. Fumasi[1], Tara MacCulloch[2,3], Nicholas Stephanopoulos[2,3], Julianne L. Holloway[1,2]
[1]Chemical Engineering, School for Engineering of Matter, Transport, and Energy; [2]Biodesign Center for Molecular Design and Biomimetics; [3]School of Molecular Sciences; Arizona State University, Tempe, AZ 85287

Statement of Purpose: Musculoskeletal injuries and diseases are a significant health concern in the United States with autographs as the primary standard; however, some disadvantages include donor site injury and morbidity, chronic pain, and a limited tissue supply. A potential solution is to use natural polymers, like hyaluronic acid (HA), combined with bioactive peptides to create tissue-engineered scaffolds to promote osteogenesis. However, during bone regeneration, there are complex molecular signaling cascades where multiple soluble factors are expressed at precise times and locations [1]. This makes the design of biomaterials that are capable of recapitulating the natural spatiotemporal signaling cascade challenging, but essential for promoting functional tissue regeneration. Due to DNA's high specificity, we combined the use of complementary single stranded DNA (ssDNA) for temporal control of bioactive peptide presentation with a photosensitive HA hydrogel for spatial control to develop an in vitro platform that can independently and reversibly control the spatiotemporal display of multiple bioactive peptides.

Methods: Norbornene-modified HA (NorHA) hydrogels were synthesized by dissolving NorHA in a photoinitiator and crosslinked with non-degradeable DL-dithiothreitol (DTT) when exposed to UV light. The total molar equivalence with DTT was set to 20% leaving the remaining 80% of the reaction sites available for tethering with ssDNA. The hydrogels were soaked in a ssDNA/photoinitiator solution followed by photoconjugation using a photomask for spatial control and rinsed. A complementary fluorescently labeled biomolecule DNA (cB) strand was added, rinsed, and imaged. For temporal control, the cB strands and the complementary displacement DNA (cD) strands were designed with a toe-hold region allowing for the removal of the cB strands from the hydrogel surface. Using this approach, DNA handles were used to spatiotemporally tether peptides of interest, including RGD for cell-matrix adhesion, HAVDI for cell-cell adhesion, and OGP for osteogenesis. Cell behavior on these hydrogel surfaces were assessed using a phalloidin stain to analyze cell morphology and alkaline phosphatase staining as an early marker of osteogenesis.

Results: To spatiotemporally control bioactive peptide delivery for improved control over cell behavior, ssDNA was bound to the surface (S) of NorHA hydrogels through photoconjugation using a photomask with 200 μm thick lines. When cB strands were added, fluorescent lines were observed demonstrating that DNA was spatially bound to the surface. After adding cD strands, no fluorescent lines were detected demonstrating complete removal of cB from the surface. Mismatched biomolecule (mB) strands, mismatched displacement (mD) strands, and having no DNA bound to the hydrogel surface served as controls and resulted in no fluorescent activity, Figure 1a. To demonstrate full reversibility, cB and cD strands were used over 5 full cycles with no decrease in efficacy, Figure 1b. Hydrogels were also made with two different ssDNA handles photopatterned to the surface resulting in two different cB strands spatially arranged in a crosshatch pattern, Figure 1c. Both cB strands were removed and re-added to show precise temporal control. This platform has been used for preliminary cell work where surfaces were modified with RGD, HAVDI, or OGP-DNA and results indicate similar bioactivity compared to tethered peptides without DNA.

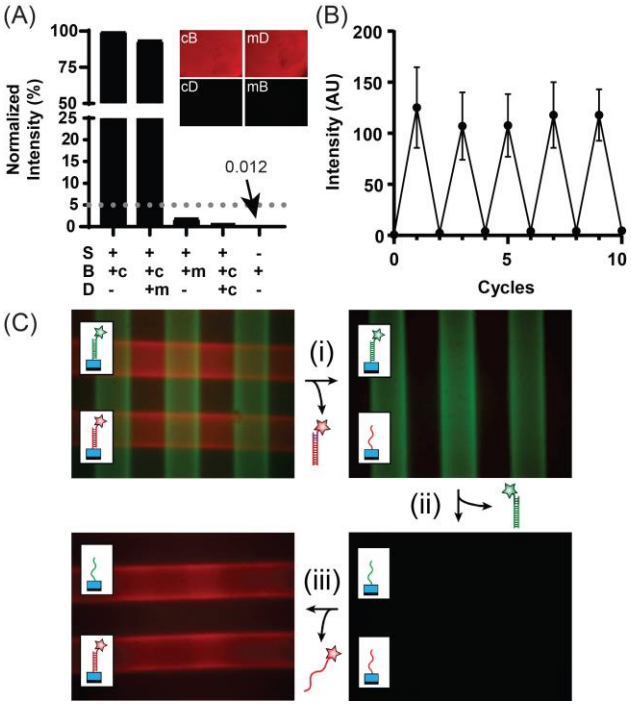

Figure 1: (A) Demonstration of temporal control using complementary DNA handles that can be (B) reversibly controlled over multiple cycles. (C) Independent spatiotemporal control of two different ssDNA handles: Initial crosshatch pattern showing cB1 (horizontal) and cB2 (vertical) conjugated to the surface, followed by (i) removal of cB1 and (ii) removal of cB2 using the corresponding cD stands, and (iii) regeneration of cB1.

Conclusion: This work demonstrates that using photoresponsive NorHA as the platform allows for spatial control while the correct DNA sequence on the biomolecule and displacement strands is required for proper temporal control. Fluorescent molecules were used as a model while ongoing work is using complementary peptide-conjugated ssDNA strands to evaluate the synergistic interplay between cell adhesion (both cell-cell and cell-matrix) and osteogenesis.

References: [1] Gao, C., et. al. Bone Res. 2017; 5: 17059.