The Effects of Peptide Coated Surfaces on the Mesenchymal-Epithelial Transition in MCF-10A Cells

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Introduction:

A major problem in the medical devices industry is that implants are often rejected by the patient's body often causing the patient a lot of pain, discomfort and monetary cost [4]. To address this problem, we propose using implants coated with peptide sequences that resemble sections of the extracellular matrix (ECM) proteins. This will allow the implants to bypass the immune system and prevent this negative reaction and thus promote cell growth and adhesion. To use such peptide sequences, we need to know how those peptide sequences will effect cell behavior. In particular, Epithelial-mesenchymal transition (EMT) is known to play an important role in wound healing but also in the spread of cancer cells [2]. We used two peptide sequences: cyclic RGD (cRGD), and YIGSR, which are, respectively, associated with proteins involved in mesenchymal cell behavior and with epithelial behavior. These associations led us to hypothesize that surfaces with YISGR coatings will tend to make cells display epithelial phenotypes while cRGD would cause cells to show mesenchymal phenotypes.

Materials and Methods:

Our surfaces were prepared by mixing the peptides with ethylene glycol6 at dilution ratios of 1:100 and 1:10,000 and putting them on gold-coated glass slides. The solution on the gold was left over night and then was washed with methanol and seeded with MCF-10A cells. The cells were then incubated for 24 hours.

Migration videos were taken over 24 hours with each frame being taken every five minutes. Phase contrast imaging was done after 24 hours to give us insights into cell morphology. After phase contrast imaging, cells were prepared for immunofluorescent staining by using the following reagents: 4% paraformaldehyde, phosphate buffered saline (PBS), 5% glycine, 0.5 Triton X, and bovine serum albumin. Afterwards the primary antibodies for N-cadherin and E-cadherin were added to the cells and they were incubated for 24 hours. Next, the cells were washed twice with PBS and then incubated with Hoechst, and the secondary antibody for an hour. Finally, the cells were washed twice more in PBS and prepared for mounting using 50% glycerol and then imaged [1,3].

Results and Discussion:

From the migration studies, we observed that surfaces with cRGD displayed no group migration and that the cells had a round and evenly spread morphology. YIGSR surfaces tended to have clusters of cells with spindle morphology that migrated as a group. There was no apparent difference in single cell migration between the two peptide surfaces. Figures 1, (a) and (b) show the differences in morphology between the two surfaces.

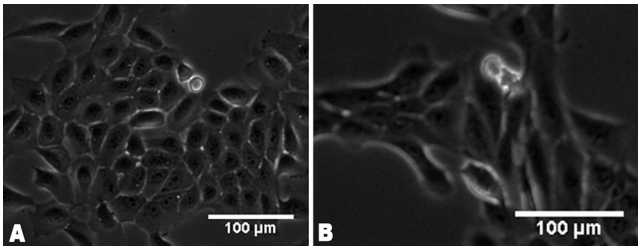

Figure 1: MCF-10A cells grown on a cRGD surface (a) and YIGSR surface (b).

E-cadherin staining (Figure 2) showed us that cells on a cRGD surface tend to display a higher percentage of E-cadherin membrane-positive cells than YIGSR surfaces (Figure 3). We also observe that the percentage of E-cadherin membrane-positive cells also decreases with decreasing peptide surface density.

While the higher surface density cRGD surface displayed higher levels of N-cadherin than the high surface density YIGSR surface, this trend switches for the low surface density samples (Figure 4). We also

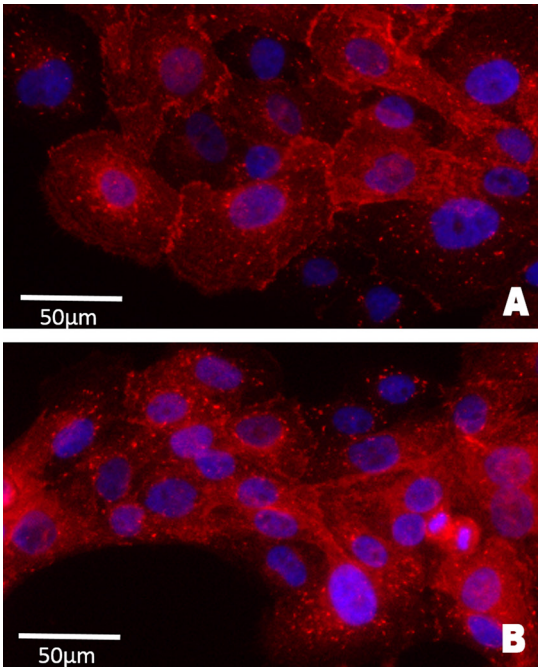

Figure 2: E-cadherin stained cells on cRGD surface (a), and YIGSR surface (b).

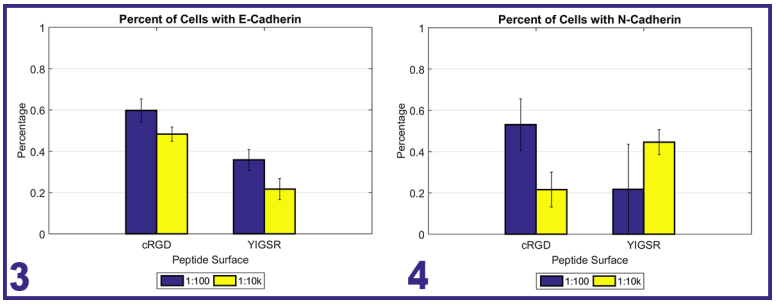

Figure 3, left: Percentage of E-cadherin membrane-positive cells on high and low surface density cRGD and YIGSR samples.

Figure 4, right: Percentage of N-cadherin membrane-positive cells on high and low surface density cRGD and YIGSR samples.

observe that N-cadherin decreases with decreasing surface density in the cRGD samples but N-cadherin increases with decreasing surface density in the YIGSR samples.

The changes in morphology and E-cadherin expression each suggest that YIGSR coated surfaces cause MCF-10A cells to display a more mesenchymal phenotype. The reduction of E-cadherin due to the decrease in peptide surface density allows us to conclude that decreasing peptide surface density causes cells to display a more mesenchymal phenotype. The N-cadherin results are more ambiguous. In the high density surfaces, the cRGD sample expresses more N-cadherin than the YIGSR sample. When looking at just the YIGSR samples, it once again appears as though decreasing peptide surface density increases the cells' mesenchymal phenotype. While this trend is not present in the cRGD samples, it is possible that another mesenchymal associated cadherin is acting instead of N-cadherin which might also explain why more N-cadherin is present in the high surface density cRGD samples than the high surface density YIGSR samples. In conclusion, cells on YIGSR coated surfaces tend to display a more mesenchymal phenotype than cells incubated on a cRGD coated surface. In addition, as peptide surface density decreases, cells on both peptide coated surfaces show a higher level of mesenchymal traits.

Future Work:

Going forward, it will be important to study the longer-term behavior of cells on these surfaces. The cells studied in this work were incubated on our surfaces for 24 hours. However, if transcription is required, changes in protein expression can take longer than 24 hours to occur. This work would also benefit from more quantitative experiments such as western blotting, and real time polymerase chain reaction (RT-PCR) which would give us a more quantitative measure of protein expression and mRNA present in the cells.

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