Functionalization of hydrogels with a matrix metalloproteinase-sensitive fluorogenic substrate to measure cellular response to drug treatment

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Statement of Purpose: Inclusion of peptides sensitive to cellular secreted enzymes, such as matrix metalloproteinases (MMPs), in synthetic hydrogels is a common method to render these matrices degradable and responsive to cell-mediated remodeling. Degradation of these peptides is influenced by a number of factors, including pH, temperature, and expression and activity of cell secreted enzymes and endogenous inhibitors. Furthermore, the rate of degradation affects the material properties of the culture system, and material properties have been well documented to regulate cell function. Therefore, new methods are needed to characterize this degradation *in situ.* We have developed a functionalized material that utilizes a covalently coupled MMP sensitive fluorogenic substrate to provide a fluorescent read out of matrix degradation over time[1]. Here, we have applied this system to investigate how MMP activity of melanoma cells is affected by matrix stiffness and pharmacological inhibition of the BRAF signaling pathway, one of the most commonly mutated pathways in melanoma[2].

Methods: A quenched fluorigenic peptide based on a collagen derived sequence, GPQG↑IWGQ (where ↑ denotes the cleavage site), and a scrambled control were constructed using solid phase peptide synthesis. The peptide was labeled on either side of the cleavage site by a fluorophore, fluorescein, and a quencher, dabcyl. The peptide sequence also contained a cysteine residue at the C terminus for covalent incorporation into PEG-norbornene hydrogels using a thiol-ene reaction. Melanoma cells (A375s) were encapsulated in hydrogels with the fluorigenic peptide incorporated, and MMP activity, as indicated by increased fluorescence, was measured at 24 hrs. MMP activity was normalized to metabolic activity measured with alamarBlue (Life Technologies, Grand Island, NY). Cells were treated with 1 μM inhibitor for 24 hrs. Data presented are n=3+SEM, * $p<0.05$, *** $p<0.001$, as analyzed by a one-way ANOVA and Tukey's posttest.

Results: A fluorogenic sensor was constructed and characterized. For the backbone of the sensor, the peptide sequence GPQG↑IWGQ was utilized. This sequence has been previously demonstrated to be cleaved by a number of MMPs (MMP-1, 2, 3, 7, 8 and 9)[3]. Covalent coupling of the fluorigenic peptide to a PEG hydrogel dramatically reduced non-specific cleavage and enabled measurement of global MMP activity of encapsulated cells over time. Degradation of the sensor by cell secreted MMPs resulted in an over 8 fold increase in fluorescence, while a scrambled control peptide or treatment with a broad spectrum MMP inhibitor, GM6001, reduced the increase in fluorescence to background control levels. This functionalized hydrogel system was then used to investigate the regulation of MMP activity in melanoma cells. Increasing the crosslinking density of the PEG hydrogel increased the elastic modulus of the gel as measured by rheometry as well as MMP activity of encapsulated A375 cells (Fig. 1).

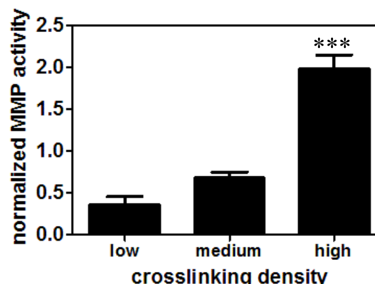

Figure 1. MMP activity of A375 cells encapsulated in PEG hydrogels with varied crosslinking density

We also investigated how pharmacological inhibition of the BRAF signaling pathway affects MMP activity. This pathway is known to up-regulate MMP activity, so we hypothesized that inhibition of the pathway would reduce MMP activity. Interestingly, we found BRAF or MEK inhibition resulted in increased MMP activity (Fig. 2).

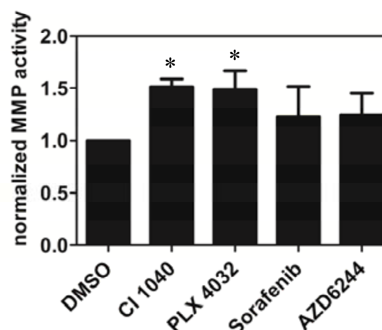

Figure 2. MMP activity of A375 cells treated with BRAF/MEK inhibitors

A similar trend was observed in two other melanoma cell lines, WM35 and WM239A, but no change with treatment was observed for WM115 cells. Current studies are investigating how increased MMP activity with drug treatment affects 3D cell migration.

Conclusions: Functionalization of PEG hydrogels with a fluorogenic sensor enabled *in situ* measurement of MMP activity. MMP activity of melanoma cells encapsulated in this system was regulated by material properties and pharmacological inhibition of the BRAF pathway. Future studies to investigate how increased MMP activity affects cell migration could have important consequences for melanoma metastasis.

References:

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