Macrophage Polarization in Response to Self-Assembling Peptides

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Statement of Purpose: Macrophages play diverse roles in inflammation, angiogenesis, and wound healing. In part this is accomplished through the macrophage's ability to maintain a dynamic phenotype across a spectrum of polarizations. In one characterization of this spectrum, classically activated macrophages (M1) are noted for their pro-inflammatory and phagocytic properties while alternatively activated macrophages (M2) are noted for their anti-inflammatory, pro-healing phenotypes. Both ends of this spectrum are relevant for biomaterials used as wound repair materials and as vaccines, both of which our group is studying. The objective of the work reported here was to determine the effects of self-assembling peptides, specifically Q11 (QQKFQFQFEQQ), on macrophage polarization in vitro. This peptide, which forms immunoactive nanofibers and gels, is under investigation both as a matrix for wound repair/cell delivery and as a novel vaccine platform. We additionally sought to understand how physical properties such as surface charge modulate macrophage activation, in an effort to adjust M1/M2 polarization systematically.

Methods: The J774.a1 murine monocyte macrophage cell line was used to study the polarization of macrophages in response to Q11 nanofibers. Macrophage differentiation was determined by functional assays for each phenotype. M1 function was determined using the Griess assay to measure nitric oxide production, whereas M2 function was determined by measuring arginase activity. These cells were first stimulated with varying concentrations of Q11 fibers alone with controls (IFNγ/LPS to stimulate M1 phenotype and IL-4 for M2 phenotype). Next macrophage polarization was studied with Q11 fibers in the presence of either IL-4 or IFNγ/LPS. Then to study the effects of altering the charge of Q11 fibers, we synthesized positively charged Q11 fibers by adding a lysine residue (KQ11) and negatively charged Q11 fibers by adding a glutamic acid residue (EQ11). Fiber morphology and assembly was verified with TEM. The macrophages were then stimulated with these charged fibers as described above.

Results: When stimulated with the Q11 fibers alone, we saw increased NO production (M1) in a dose-dependent and time-dependent manner (Figure1). However, arginase activity (M2) was not significantly altered regardless of Q11 nanofiber concentration or how long the macrophages were exposed to the nanofibers (not shown). When macrophages were incubated with IL-4 in the presence of Q11 fibers the arginase activity/M2 was significantly decreased as compared to the high arginase activity observed with IL-4 alone (Figure 1). Although IL-4 with Q11 fibers resulted in decreased arginase activity/M2, we did not observe any parallel increase in nitric oxide production or M1. Additionally IFNγ/LPS in the presence of Q11 fibers exhibited no difference in polarization towards M1 or M2.

Interestingly, when the charge of Q11 fibers was altered, the negatively charged EQ11 resulted in significantly lower NO levels then both the neutral Q11 fibers and positively charged KQ11 fibers ($p<0.05$). No difference was seen in arginase activity between any groups. Additionally the macrophage polarization did not significantly differ between Q11 and positively charged KQ11 fibers.

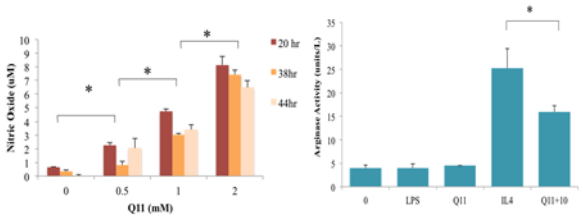

Figure 1. *Left*: M1 polarization/ NO production increased in a dose and time-dependent manner. *Right:* M2 polarization after stimulation by Q11 or IL4 with and without Q11 fibers present. *$p<0.05$

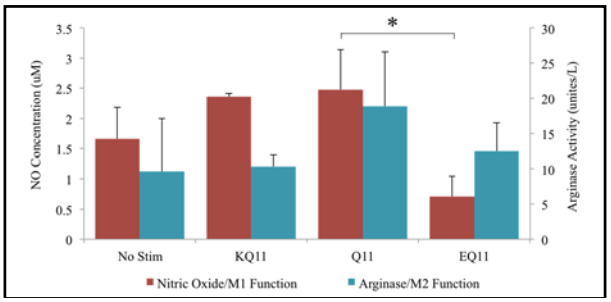

Figure 2. Macrophage polarization when stimulated with fibers of different charge. *$p<0.05$

Conclusions: Q11 fibers polarize macrophages in a dose- and time-dependent manner toward the M1 phenotype, but this effect can be reduced with negative surface charge on the nanofibers. Q11 nanofibers generally promoted M1. Even in the presence of M2-polarizing cytokines, the effect of Q11 nanofibers was to diminish M2. Interestingly, we saw that by providing negative (but not positive) charge on the nanofibers, polarization towards M1 could be reduced. We conclude that Q11 fibers appear to have some ability to influence specific macrophage polarization. The reasons for this influence of charge and the underlying will be exploited in designs of vaccines and wound healing materials.

References:

1. Chen, J. Biomaterials 2013, 34 (34), 8776-85.