Self-Assembling MMP-2 Cleavable Hydrogel Drug Delivery Systems; Neural Tissue Biocompatibility and Response

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Statement of Purpose: In the event of neural tissue injury, neuroptrophic factor peptide analogues are capable of promoting healing by neurogenesis, promoting neuronal survival and attenuation of glial activation. Peptides have been considered as ideal therapeutic agents as they are: specific, potent, and small enough for diffusion. However, peptides are also digested and short lived, making their *in vivo* delivery difficult. Self-assembling peptide $(RADA)_4$ is a novel class of peptide that, upon injection, assemble into a 3D hydrogel nanofiber matrix, capable of storing water and molecules. Synthesizing peptide therapeutics onto $(RADA)_4$ can afford protection from circulating proteases and utilizing matrix metalloproteinase-2 (MMP-2) sequences allows an 'on-demand' and local tissue release strategy. Understanding self-assembly, controlled release of bound therapeutics, biocompatibility, and tissue response is vital for successful clinical application.

Methods: $(RADA)_4$-GG-GPQG+IASQ (CS1) and $(RADA)_4$-GG-GPQG+PAGQ (CS2), known for high and low MMP-2 sensitivity, respectively, were investigated.[1] The '+' denotes the scissile bond. Neuropeptides DGGL, a cilliary neurotropic factor analogue, and MVG, a brain-derived neurotrophic factor secretion stimulant, were tethered to the c-termini.[2,3] $(RADA)_4$-IKAV was used to promote glial attenuation and cell adherence.[4] Nanofiber matrix morphology of these peptides was studied using TEM. MALDI mass spectrometry was performed to observe product formation of CS1 and CS2 upon incubating the peptide hydrogels with 5 nM MMP-2 at 37°C for three days. These studies were performed with 0.5%w/v peptide. Microglia, primary cells associated with neural tissue host response, were seeded into PLL, and 1%w/v $(RADA)_4$ matrices with 0%, 10%, and 25% additions of $(RADA)_4$-IKAV. Inflammatory response was measured with morphology via confocal microscopy, NO_2 assay, and cytokines ELISAs for TNFα and IL1β, while viability was observed using MTT. Tissue culture was also performed with PC-12 cells, which are used to assay neuronal adhesion and neurogenesis. $(RADA)_4$-IKAV was mixed with $(RADA)_4$ at 10% increments to determined optimal adherence. Cells were counted with phase contrast microscopy and viability was measured by MTT assay. PC-12 cells were seeded into 1%w/v $(RADA)_4$ with 10% $(RADA)_4$-IKAV and 10% CS with tethered drugs and exposed to a 3 day treatment of 1nM MMP-2. Neurogenesis was observed by neurite extension using phase contrast microscopy and acetylcholine esterase signalling.

Results: Nanofibers were present in all samples observed by TEM, forming bundles that are 5-10 nm thick. MMP-2 cleavage of the nanofiber matrix was observed with formation of the product $(RADA)_4$-GG-GPQG using MADLI. Peaks at 2525.2 m/z for CS1, 2478.8 m/z for CS2, and 2125.1m/z for the product. Using an internal peptide standard, 31.6% product formation of CS1 and 8.6% product formation of CS2 were observed. Microglia morphology in $(RADA)_4$ was comparable to the PLL control and is shown in Figure 1. The thin and long cell processes noted are characteristic of ramified microglia. NO_2, TNFα and IL1β levels were the same in $(RADA)_4$ matrices and the PLL control, while MTT viability was higher in the $(RADA)_4$ groups.

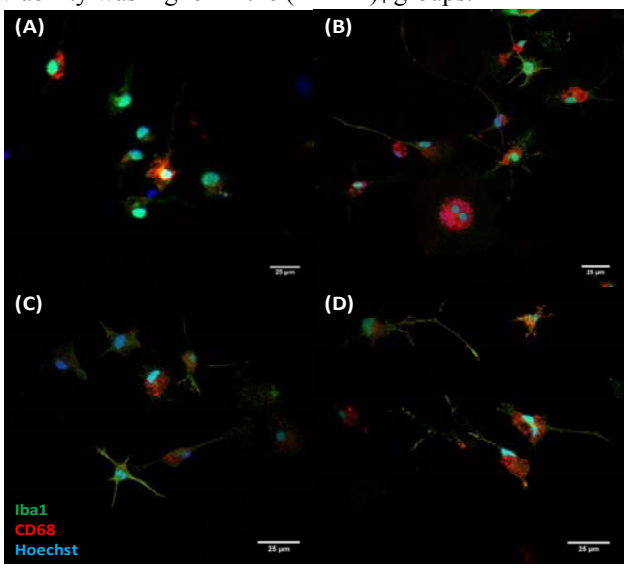

Figure 1. Confocal microscopy images of microglia seeded into (A) PLL, (B) 100% $(RADA)_4$, (C) 90% $(RADA)_4$: 10% $(RADA)_4$-IKVAV, (D) 75% $(RADA)_4$: 25% $(RADA)_4$-IKVAV, Labels include Iba1 (green), CD68 (red), and Hoechst (blue).

PC-12 cell adhesion occurs at 20-10% $(RADA)_4$-IKAV with 80-90% $(RADA)_4$, noted by cell count and MTT. Thin and long neurite extensions were noteworthy in each drug tethered CS mixture when exposed to MMP-2. Cellular acetylcholine esterase signalling showed a four-fold increase with DGGL release and a five-fold increase with MVG release after MMP-2 incubation, with negligible differences between CS1 and CS2.

Conclusions: Nanofiber matrices were present in all samples, suggesting drug delivery for all cases. MMP-2 digestion showed desired product peak intensities for the high activity CS1 and the low activity CS2. The microglia were ramified in any mixture of the $(RADA)_4$ and MMT may be due to higher microglia cell adhesion. The MMP-2 cleaved drug fragments promote neurogenesis in PC-12 cells, and these cells can be seeded into a $(RADA)_4$-IKAV/$(RADA)_4$ coating. Overall, the $(RADA)_4$ system has been demonstrated as a self-assembling tuneable drug release system with good neural tissue biocompatibility and response.

References:

1. Nagase H. Biopolymers. 1996;40:399-416.
2. Chohan MO. Neurobio of Aging. 2011;32:420-434
3. Shin MK. J Neurochem. 2011;116:205-216
4. Tysseling-Mattiace VM. J Neurosci. 2008;28:814-823.