Self-Assembling MMP-2 Cleavable Hydrogel Drug Delivery Systems

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Introduction: Peptides are the fastest growing segment of the pharmaceutical industry, and are generally considered the ideal therapeutic: specific, potent, small enough for diffusion, etc. That said, peptide therapeutics suffer from a major drawback, they are easily cleaved by circulating proteases and are thus short lived; this factor makes them almost impossible to effectively deliver *in vivo*. Self-assembling peptide $(RADA)_4$ nanofibers (NFs) are a novel class of biocompatible peptides that, upon injection, assemble into a 3D hydrogel matrix which is capable of storing water and large molecules. Moreover, it is thought that synthesizing peptide therapeutics to self-assembling domains thatsubsequently form a hydrogel will afford protection from circulating proteases. Utilizing the spatiotemporal profile of tissue resident enzymes affords an 'on-demand' release strategy. Understanding the matrix self-assembly and the release of bound peptide therapeutics are vital for successful and reproducible clinical treatment. To this end, NF morphology and fractal dimension will be studied using transmission electron microscopy (TEM) and degradation of $(RADA)_4$ based hydrogels with matrix metalloproteinase-2 (MMP-2) cleavable bonds will be observed using matrix assisted laser desorption/ionization mass spectrometry (MADLI).[(1) (2)]

Methods: The peptides made were $(RADA)_4$-GG-GPQG+IASQ (CS1) and $(RADA)_4$-GG-GPQG+PAGQ (CS2), known for their high and low MMP-2 sensitivity, respectively.[(3)] The '+' denotes the scissile bond. These were synthesized using Fmoc chemistry with 95+% purity. To assure NF formation in the reaction conditions used for enzymatic digestion, TEM was performed onsystems containing an overall 0.5%wt/v concentration of 25%, 50%, and 75% $(RADA)_4$; the remainder being either CS1 or CS2 peptides. TEM analysis of NF matrix developmentfor 0, 1, 2, 4, 6 and 24 hr time periods was used to determine morphology of the self-assembly as well as Hausdorff Fractal analysis. HausdorffFractal analysis was performed on these images using Matlab®. MALDI mass spectrometry was used to enzymatic activity through product formation upon incubating the peptide matrices with 40nM active MMP-2 at 37°C for three weeks. A control without MMP-2 was also performed.

Preliminary Results: NFs were present in all samples, where Figure 1 illustrates this for 24 hr growth. NFs (all ~5-10nm) form bundles of thickness: (a) $(RADA)_4$, 15-200nm for both (b) CS1, and CS2. CS1 doped with (d) 25%, (e) 50%, and (f) 75% $(RADA)_4$ has NF bundles 10-50nm, 10-50nm, and 10-200nm, respectively. At 25% and 50% doping, these NFs are similar to $(RADA)_4$, while at 75% doping these fibers are bundle into matted curved shapes. CS2 doped

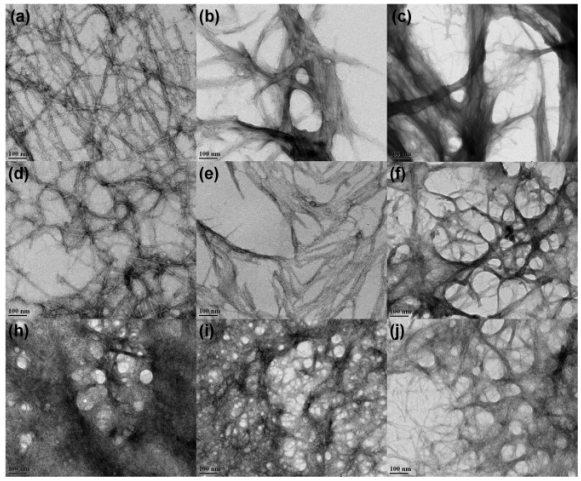

Figure 1.TEM images at 110,000X magnification of (a) $(RADA)_4$, (b) CS1, (c) CS2, CS1 doped with (d) 25%, (e) 50% , (f) 75% $(RADA)_4$, and CS2 doped with (h) 25%, (i) 50%, (j) 75% $(RADA)_4$.

with (d) 25%, (e) 50%, and (f) 75% $(RADA)_4$ has NF bundles 10-1000nm, 10-500nm, and 10-200nm, respectively. A matted network is present and is decreasingly thick and structured with addition of $(RADA)_4$. At 25% $(RADA)_4$ this network is more structured than any other group.

All growth periods preceding 24 hrs show NF growth with relatively less bundle development. This was characterized using Hausdorff fractal dimensions and is shown in Figure 2. All fractal dimensions increase as a function of time to their final values after 2 hours. The highest dimensions are calculated from the pure peptide groups, which are 1.8, 1.7, and 1.7 for $(RADA)_4$, CS1, and CS2, respectively. Other dimensions are lower ranging from 1.4-1.6.

MMP-2 cleavage of the NF matrix was observed with formation of the product $(RADA)_4$-GG-GPQG using MADLI and is shown in Figure 3. Peaks at 2525.2m/z for CS1 and 2478.8 m/z for CS2 groups. The peak 2125.1m/z is 80% intense as the CS1 substrate and 10% intense as the CS2 substrate.

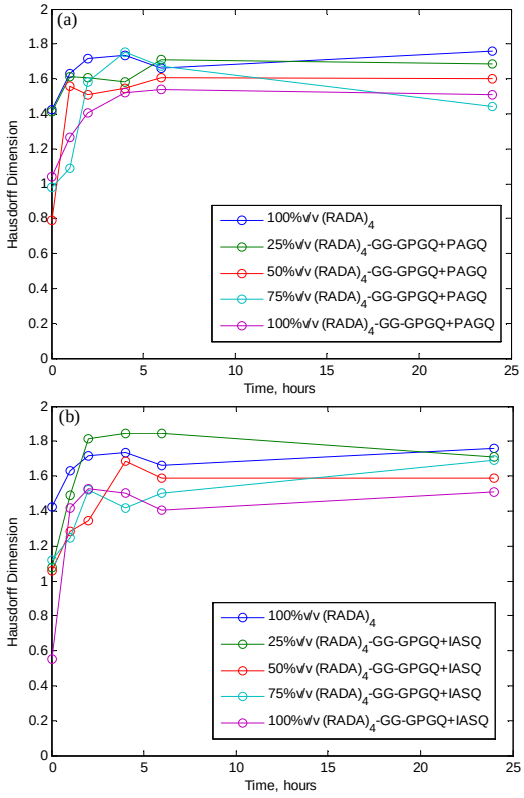

Figure 2. Hausdorff fractal dimension as a function of time of (a) CS1 doped with 25%, 50%, and 75%$(RADA)_4$, and (b) CS2 doped with 25%, 50%, and 75%$(RADA)_4$.

Discussion:

NF matrices were present in all samples. Only after 2 hrs did apparent fractal growth cease for all mixtures, which is when growth stability may occur. The 75% CS2 was an intense matted network that may allow for extended drug release.These NFs form diverse morphologies, but there is no evidence to suggest that some peptides are being excluded from NF formation. Despite this difference in morphologies, preliminary results show expected product formation upon MMP-2 digestion.Quantitative analysis of product formation needs to be conducted.

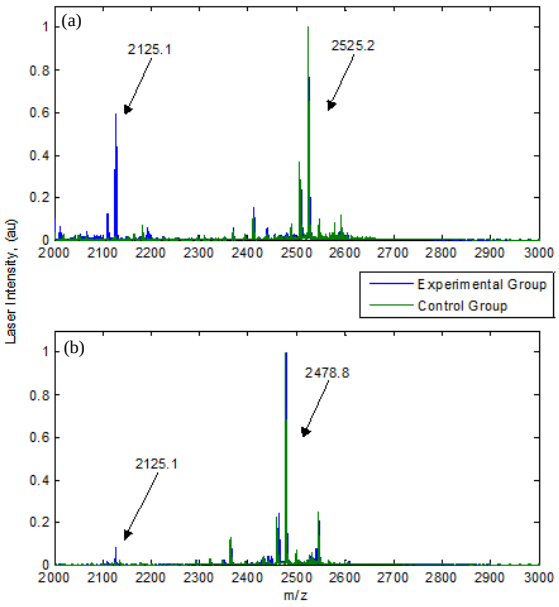

Figure 3.MALDI MS of (a) CS1 and (b) CS2 compared to a control without MMP-2

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References:

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