Controllably degradable self-assembling peptide materials for tissue engineering

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Introduction: Self-assembling peptide hydrogels are under development as promising materials for tissue engineering, vaccine development, and the delivery of drugs, growth factors, and cells. However, one limitation of these materials is that they are not easily degraded, owing to the extreme stability of fibrillized peptides, thus limiting one's ability to engineer the clearance or remodeling of these materials in vivo or in vitro. To address this challenge, we developed hydrolytically degradable self-assembling peptides by substituting key amide bonds within self-assembling peptides with hydrolytically susceptible ester bonds. Doing so required minimizing the disruptive nature of amide→ester substitution, allowing degradability while maintaining self-assembling behavior. We synthesized ester-containing analogs of the self-assembling peptide self-assembling peptide Q11 (QQKFQFQFEQQ) [1] and developed a family of "Depsi-Q11s" that formed fibers and hydrogels like their peptide analogs, but that degraded with kinetics that could be specified based on the amino acid residue placed next to the ester bond. This work represents the first instance, to our knowledge, of β-sheet fibrillar hydrogels degradable via non-enzymatic hydrolysis.

Methods: *Peptide synthesis*: Depsipeptides (containing both ester and amide bonds in their backbones) were synthesized with standard solid-phase peptide synthesis protocols, except that ester bond formation was accomplished with α-hydroxy acids and N,N'-diisopropylcarbodiimide chemistry catalyzed by 4-dimethylaminopyridine. Four α-hydroxy acids, glycolic acid, lactic acid, 2-hydroxycaproic acid and 3-phenyllactic acid (analogs of amino acids G, A, L and F) were used to generate Depsi-G, Depsi-A, Depsi-L and Depsi-F peptides. *Characterization*: TEM was employed to observe the formation and morphology of peptide fibers. Peptides were fibrillized, and HPLC was utilized to monitor peptide degradation over time for a range of pH, temperature, and peptide concentrations. Rheometry was used to evaluate the storage and loss moduli of peptide hydrogels. C3H10T1/2 multi-potent stem cells were encapsulated within Q11 and Depsi-Q11 hydrogels. MTS assay, calcein-AM, and ethidium homodimer staining were used to evaluate cell proliferation, morphology, survival, and death, respectively.

Results: The syntheses of Depsi-Q11s were successfully accomplished, as verified by MALDI mass spectrometry and HPLC. Depsi-Q11 peptides having ester substitutions between the fifth and sixth residues of the peptide retained their ability to form regular cross-β nanofibers when dissolved in water and neutralized with phosphate-buffered saline. Studies of degradation kinetics found that as expected, increasingly hydrophobic side chains proximal to the ester (Fig. 1) resulted in progressively slower degradation rates (Fig.2). Among the designed

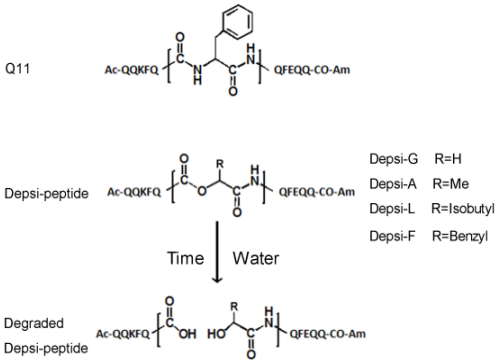

Fig.1 The sixth residue (Phe) in the peptide Q11 was replaced with α-hydroxy acids to generate Depsi-Q11s with variable hydrophobicity surrounding the ester.

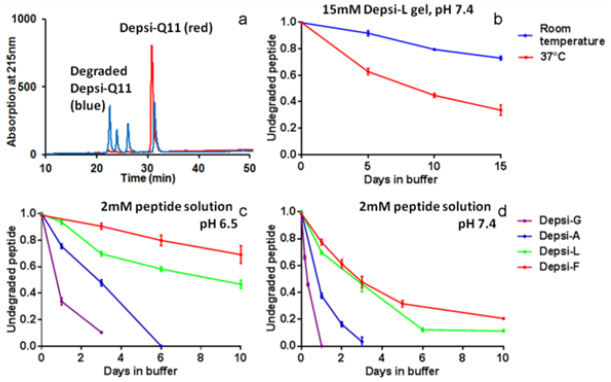

Fig. 2 Demonstrated by HPLC, Depsi-Q11s were hydrolyzed and formed predictable fragments (a). Faster degradations were observed in the conditions with higher temperature, higher pH and lower peptide concentration (b-d). Increasingly hydrophobic side chains of α-hydroxy acids resulted in progressively slower degradation rates.（c,d）

Depsi-Q11s, Depsi-L formed the most robust hydrogels, with initial storage moduli (G′) of 195kPa at 30mM peptide concentration, even higher than Q11's (30kPa). Moreover, gel stiffness decreased over the course of days, as the gels hydrolyzed. Finally, in 3D culture, C3H10T1/2 multipotent stem cells encapsulated in Depsi-L hydrogels exhibited considerably more spread morphologies and higher proliferation rates than in Q11, presumably owing to the ability of the material to be remodeled over time.

Conclusion: Depsipeptide analogs of self-assembling peptides retained the capacity to self-assemble into hydrogels and fibers, but they degraded via ester hydrolysis. The rate of degradation could be tuned by controlling the hydrophobic environment around the ester bond. Compared to non-degradable fibrillized peptides, they more readily supported the 3D expansion of multipotent stem cells.

Reference:1 Jung, JP. Biomaterials 2008; 29: 2143-2151