Multimerizing Peptides Inspired by the Coiled Coil Domain of Fibrin for Constructing Self-Assembled Biomaterials

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Statement of Purpose: Our long-term goal is to design novel, synthetic, chemically defined, and tailorable extracellular matrix (ECM)-mimicking scaffolds for use within wound healing and soft tissue regeneration applications. To this end, self-assembling biomaterials formulated from peptides, peptide derivatives, and peptidomimetics are receiving active attention because they can be designed and engineered with nanoscale precision to achieve controllable fibrillar architectures and specifiable bioactivity.[1-2] The coiled coil motif is a useful module for constructing these hierarchical materials because its folding is well understood and because important functions such as cell binding and enzyme-specific degradability naturally reside within the coiled coil domains of native ECM proteins. Here, we sought to design a reliably multimerizing series of peptides inspired by the coiled coil domain of fibrin, which is an important constituent of the provisional scaffold in wound healing. The primary objectives were to understand the design requirements for maximizing coiled coil multimerization and to screen the cytotoxicity of the designed peptides.

Methods: *Peptide Design and Synthesis:* Using the prediction software MULTICOIL,[3] we identified peptides near the N-terminus of fibrin's triple helical coiled coil domain with a high probability of forming stable coiled coil multimers. Using this region as inspiration, we designed a family of six peptides (Table 1), including the

Table 1: Peptide helix positions, sequences, and substitutions

Position:	efgabcdefgabcdefgabcdefgabcdefgabcdefgab
$\alpha_{68\text{-}102}$	CRMKGLIDEVNQDFTNRINKLKNSLFEYQKNNKDS
$\beta_{110\text{-}144}$	CQLQEALLQQERPIRNSVDELNNNVEAVSQTSSSS
$\gamma_{49\text{-}83}$	CGIADFLSTYQTKVDKDLQSLEDILHQVENKTSEV
$\gamma_{49\text{-}83}$QQK	**QQ**IADFLSTYQTKVDK**K**LQSLEDILHQVENKTSEV
$\gamma_{52\text{-}85}$KQ	ADFLSTYQTKVDK**K**LQSLEDILHQVENKTS**Q**VKQ
$\gamma_{52\text{-}88}$KI	**I**DF**I**STY**I**TK**I**DK**KI**QS**I**EDI**I**HQ**I**ENK**I**SE**I**KQL**I**K

three native peptides from this region ($\alpha_{68\text{-}102}$, $\beta_{110\text{-}144}$, and $\gamma_{49\text{-}83}$), a γ-peptide with three amino acid substitutions designed to eliminate a repulsive electrostatic charge pair between the putative multimerized peptides ($\gamma_{49\text{-}83}$QQK), a second similar γ-peptide with a neutral isoelectric point ($\gamma_{52\text{-}85}$KQ), and a third γ-peptide with an additional trimer-specifying isoleucine hydrophobic core ($\gamma_{52\text{-}88}$KI). All peptides were produced with a CS Bio 136 peptide synthesizer using conventional methods. Identity, purity, and concentrations were determined with ESI mass spectrometry, HPLC, and UV sepctroscopy. *Circular Dichroism:* Using an AVIV 215, we analyzed secondary structure in PBS at peptide concentrations from 10-500mM and at pH values of pH 3-8.5. *Analytical Ultracentrifugation (AUC):* Multimerization was evaluated by sedimentation velocity and equilibrium sedimentation with a Beckman Optima XL-A and the software package SEDFIT.[4] *Cytotoxicity:* Cytotoxicity was evaluated for peptide concentrations up to 1mg/mL in cultures of human umbilical vein endothelial cells (HUVEC) using an MTS-based proliferation assay.

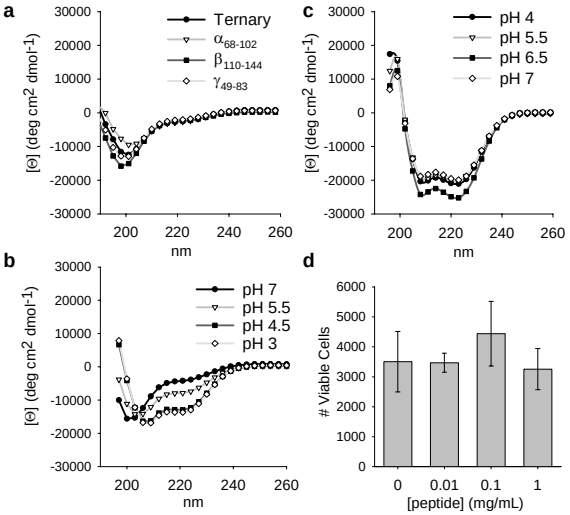

Figure 1: CD of 100mM $\alpha_{68\text{-}102}$, $\beta_{110\text{-}144}$, $\gamma_{49\text{-}83}$, and the ternary mixture (a), 100mM $\gamma_{49\text{-}83}$QQK (b), and 100mM $\gamma_{52\text{-}88}$KI (c). Cytotoxicity of $\gamma_{49\text{-}83}$QQK (d).

Results / Discussion: Native fibrin-derived peptides possessed a predominantly unfolded structure by CD even when mixed, regardless of pH (Figure 1a). Alpha helicity was improved, especially at low pH, by eliminating charge repulsions between coiled coil strands ($\gamma_{49\text{-}83}$QQK, Figure 1b). Helicity was then maximized with the introduction of an isoleucine hydrophobic core (*a* and *d* positions) in the peptide $\gamma_{52\text{-}88}$KI (Figure 1c). This peptide was largely insensitive to pH, illustrating the stability of the structure, and it demonstrated $[\theta]_{222}/[\theta]_{208}$ values of 1.04-1.06, close to the value of 1.1 that is diagnostic of coiled coil structure. AUC experiments demonstrated that $\gamma_{49\text{-}83}$QQK and $\gamma_{52\text{-}88}$KI additionally multimerized into bundles with oligomerization states ranging from dimers to tetramers. Peptide $\gamma_{49\text{-}83}$QQK was found to be non-cytotoxic in cultures of human endothelial cells (ANOVA, n=6, $p>0.05$) (Figure 1d).

Conclusions: With minimal, targeted changes in primary amino acid sequence, peptides from the coiled coil domain of fibrin can be designed to fold into multimeric coiled coil bundles. Moreover, the peptides were not found to be cytotoxic in cell culture. In our next experiments, we will utilize peptide $\gamma_{52\text{-}88}$KI as a basis for forming well-folded self-assembled biomaterials by producing multi-arm peptides and peptide polymers capable of forming networks. These peptides will serve as a basis for constructing three-dimensional self-assembled scaffolds and for tailoring the specific bioactivity of these scaffolds to achieve desirable cell and tissue responses.

References:

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