

## A self-assembling peptide-based scaffold to support cell growth

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**INTRODUCTION:** For tissue engineering (TE) purposes, self-assembling scaffolds are desirable. Many current strategies for such scaffolds concentrate on carbohydrate-based or peptidic materials; several groups are pursuing the construction and use of  $\beta$ -amyloid-like structures in the area<sup>1</sup>. By far the most abundant natural protein-assembly motif, however, is the  $\alpha$ -helical coiled coil. Indeed, many of the proteins of the extracellular matrix (ECM) have coiled coils (laminins, matrilins, thrombospondins, fibrinogen/fibrin)<sup>2</sup>. Sequence-to-structure “rules” for the folding and assembly of coiled coils are now available, and this understanding permits the rational design of coiled-coil peptides and structures. One such design from our own laboratory is the SAF (Self-Assembling Fibrillar) system<sup>3</sup>. This comprises two complementary peptides that, when mixed, combine to form a “sticky ended” building block for fibres. The resulting structures are tens of nanometres thick and tens of microns long. One obstacle to using the SAFs in TE is stability. The work described here addresses the issue of assembling the SAFs in physiological conditions of pH, temperature and salt.

**METHODS:** Natural coiled-coil assemblies are “blunt-ended”; that is the helices associate side-to-side in register. To promote end-to-end association in the SAF system, the two SAF peptides were design to assemble offset, leaving complementary “sticky ends”, Fig. 1. We make the SAF peptides by peptide synthesis, and characterise them using a combination of spectroscopy and microscopy.

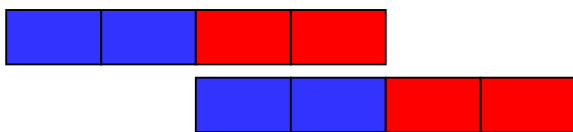


Fig. 1: Basic principle of “sticky ended” assembly, where red and blue blocks represent oppositely charged regions of peptide sequence.

**RESULTS:** A typical transmission electron micrograph for a SAF preparation is shown in Fig 2. We used this method to assay the assembly of several iterations of SAF designs engineered for increasing stability. The third-generation design

both folds into  $\alpha$ -helical structures and assembles into supramolecular fibres under physiological conditions and in cell-culture media. We are currently testing these fibres in culture with cells.

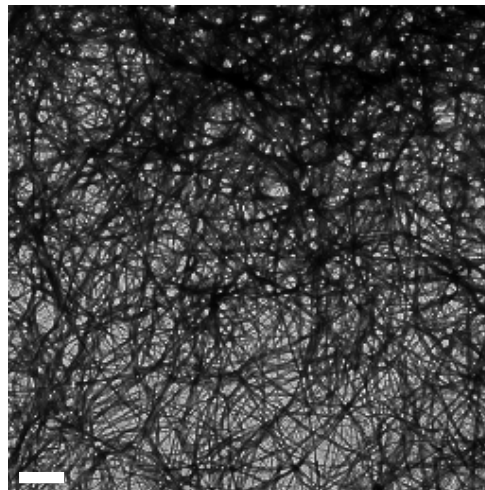


Fig. 2: Electron micrograph of SAFs in phosphate-buffered saline at 37°C. Scalebar 2 $\mu$ m.

**DISCUSSION & CONCLUSIONS:** The ECM is composed of a complex of proteins and carbohydrates. Many current scaffolds for 3D cell culture use polymers and carbohydrates as scaffold material. Peptide-based strategies for making scaffolds include  $\beta$ -sheet based peptides<sup>1</sup>, which, whilst robust and straightforward to construct, have the disadvantage of being linked to amyloid disorders. The SAF system presents an alternative candidate for peptide-based scaffolds. In addition to straightforward fibres, it is possible to design additional features, such as branching and networks of fibres, and to decorate them with recognition motifs<sup>4</sup>. Thus, systems like this are becoming real alternative to synthetic polymers and ex vivo scaffolds.

**REFERENCES:** <sup>1</sup> Zhang, S., et al. (2002). *Curr. Op. Chem. Biol.* **6**: 865-871. <sup>2</sup> Engel, J. (2004) *Internat. J. Biochem. & Cell Biol.* **36**: 997-1004. <sup>3</sup> Pandya, M.J., et al. (2000). *Biochemistry* **39**: 8728-8734. <sup>4</sup> Ryadnov, M.G & Woolfson, D.N. (2004) *JACS* **126**: 7454-7455.

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