Self-assembled peptide hydrogels as scaffolds for wound healing

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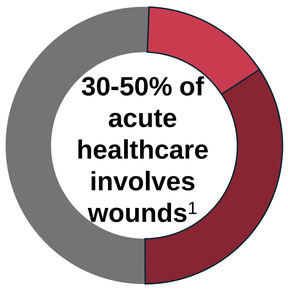
Introduction and objectives

1. In Canada, it is estimated that:



And that 1.1% of acute impatients in canadian hospitals and 7.9% of long-term care patients suffer from chronic wounds[2].

2. Chronic wounds greatly affect living conditions of patients and add to the burden on healthcare systems. Such wounds show[3]:

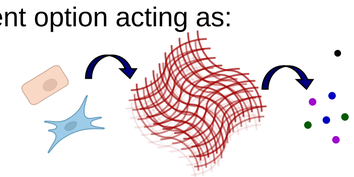
- Decreased re-epithelialization and cell proliferation.
- Decreased angiogenesis.
- Increased inflammation and risk of infection.

Various conditions alter the wound healing process, including[3]:

- Diabetes
- Obesity
- Age
- Chemotherapy

3. Self-assembled protein and peptide-based matrices are an interesting treatment option acting as:

- Scaffold for cell proliferation
- Delivery system for therapeutic molecules



Such matrices mimic the extracellular matrix in the skin and are biocompatible, degradable, and robust.
Synthetic peptides also allow production of matrices with greater purity, control over the composition and lower immunogenicity[4,5].

Objectives: Produce fully synthetic peptide-based matrices functionalized with bioactive sequences to improve wound healing.

Methodology

Peptide sequences:

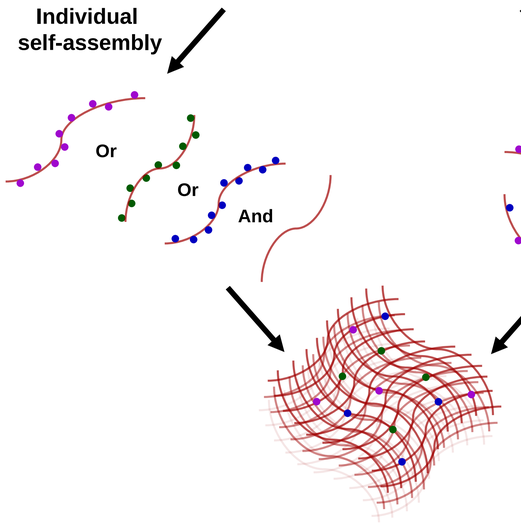
Flexible spacer

KI10-COOH: KGSGSSNNFGAILSS-COOH
PRa-I10: PHSRNSGSGSGSGSGRGDSPGSGSSNNFGAILSS-NH_2
FGF2-I10: YRSRKYSSWYVALKRGSGSSNNFGAILSS-NH_2
IG19-I10: IGKEFKRIVQRIKDFLRNLGSGSSNNFGAILSS-NH_2

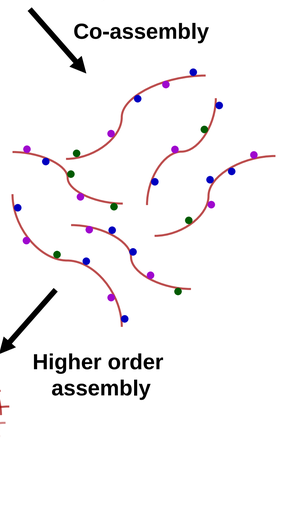
Bioactive sequences:
PRa – Cell adhesion
FGF2 – Growth factor
IG19 - Antimicrobial

Self-assembly peptide:
I10 – Synthetic β-peptide
Added charges (Lysine and COOH) for higher order assembly.

Individual self-assembly

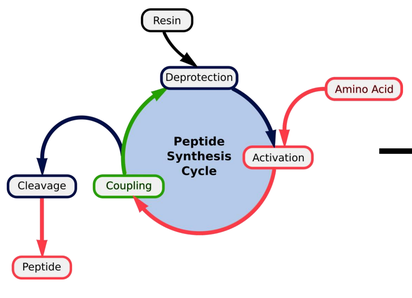


Co-assembly



Higher order assembly

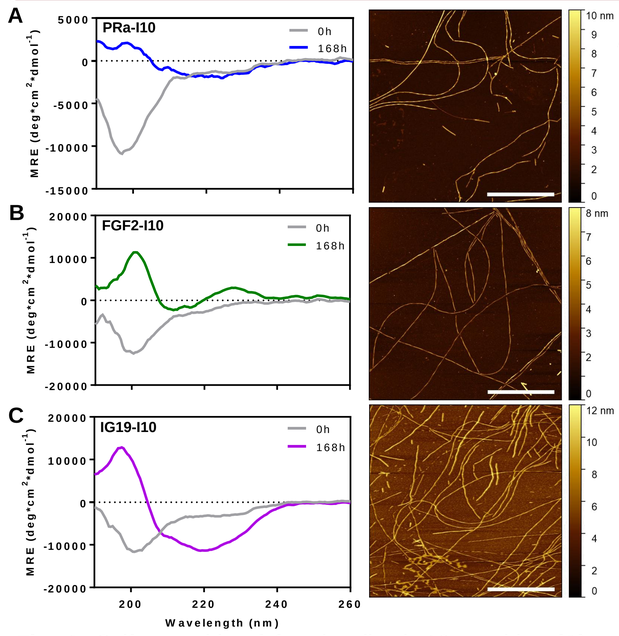
Solid phase peptide synthesis[6]

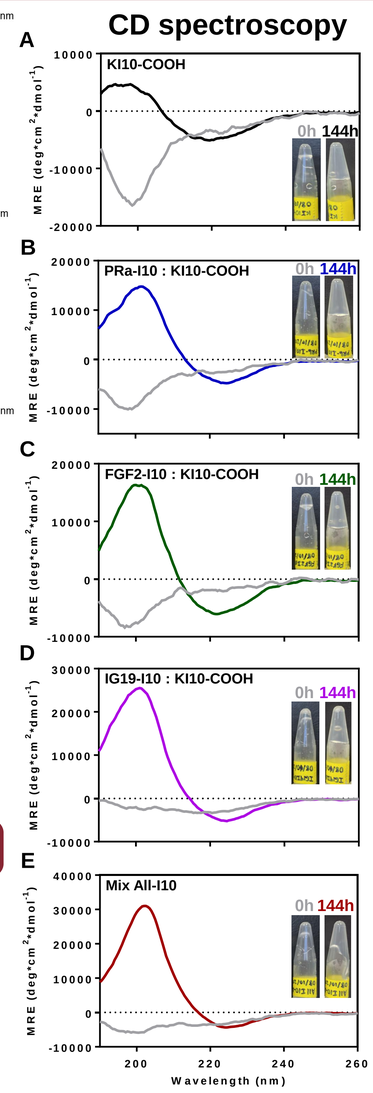


- **Purification by reverse phase HPLC**
- **Analysis by mass spectrometry (ESI-TOF)**

Results

A) Peptide self-assembly into β-sheet-rich fibrils and hydrogels





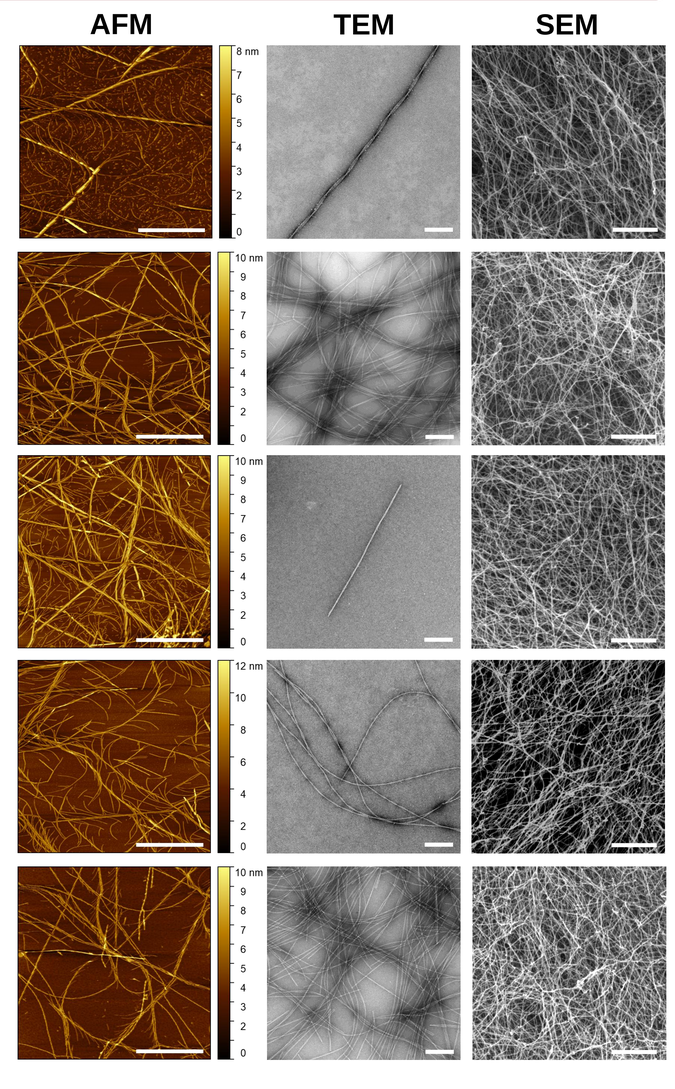


Fig. 1. Self-assembly of functionalized I10 peptides **(A)** PRa-I10, **(B)** FGF2-I10 and **(C)** IG19-I10 into β-sheet-rich fibrils as observed by circular dichroism spectroscopy (left) and atomic force microscopy (right). Peptides were assembled at a concentration of 4 mM in 20 mM Tris-HCl buffer, pH 7.4 at **(A, B)** 37ºC without agitation or at **(C)** 25ºC with agitation. Scale bar is 1 µm.

Fig. 2. Assembly and coassembly of the KI10-COOH hydrogelator peptide with functionalized I10 peptides into β-sheet-rich hydrogels as observed by circular dichroism (CD) spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). **(A)** Assembly of KI10-COOH, **(B-E)** Coassembly of KI10-COOH with **(B)** PRa-I10 2 mM, **(C)** FGF2-I10 20 µM, **(D)** IG19-I10 400 µM or **(E)** All I10 peptides (PRa-I10 2 mM, FGF2-I10 20 µM and IG19-I10 400 µM). Peptides were assembled at a total concentration of 14 mM in 20 mM Tris-HCl buffer, pH 7.4 at 37ºC without agitation. Scale bar is 1 µm for AFM and SEM, and 200 nm for TEM.

B) Thermal stability of fibrils

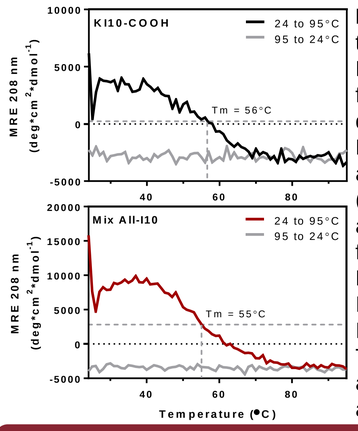


Fig. 3. Determination of the melting point of KI10-COOH and All-I10 fibrils by circular dichroism spectroscopy. KI10-COOH was (top) assembled at 14 mM or (bottom) coassembled at 11,58 mM with functionalized peptides PRa-I10 (2 mM), FGF2-I10 (20 µM) and IG19-I10 (400 µM) in 20 mM Tris-HCl buffer, pH 7.4 at 37ºC without agitation for 144h.

C) Cell adhesion to hydrogels

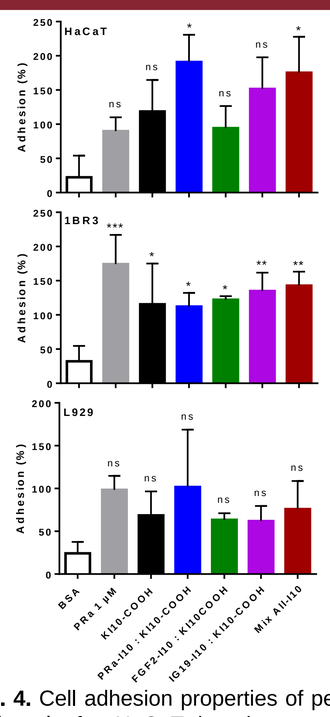


Fig. 4. Cell adhesion properties of peptide hydrogels for HaCaT keratinocytes, and 1BR3 and L929 fibroblasts. Cells were cultivated for 1h on KI10-COOH (co)-assemblies diluted at 7 µM, then washed and incubated with rezazurin. Adhesion is expressed as % metabolic activity relative to adhesion on tissue culture-treated plates. n = 2. ns - non significant;
*p < 0.05; **p < 0.01; ***p < 0.001.

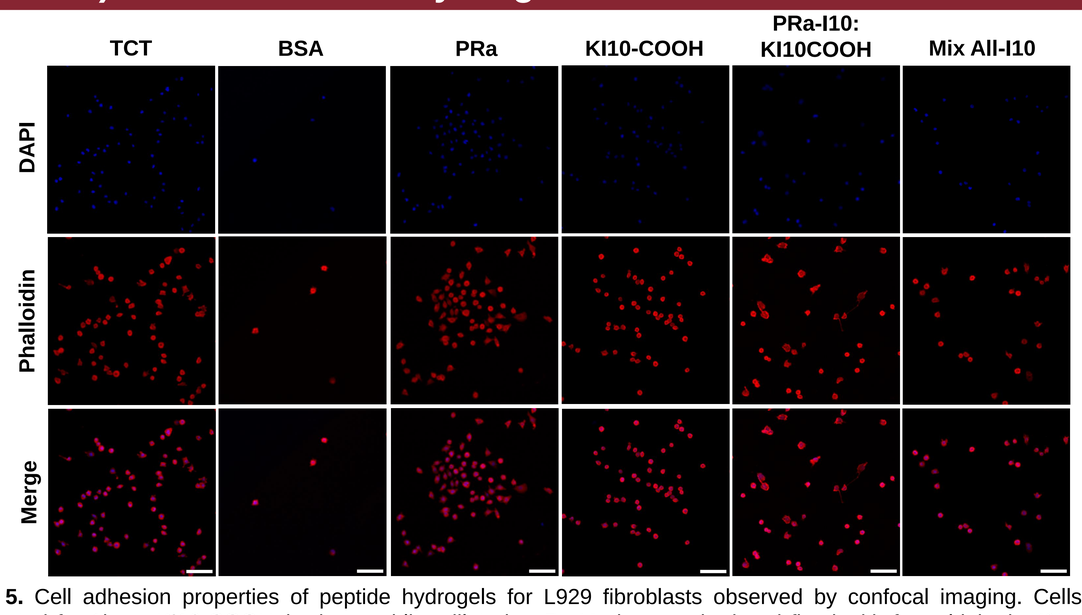


Fig. 5. Cell adhesion properties of peptide hydrogels for L929 fibroblasts observed by confocal imaging. Cells were cultivated for 1h on KI10-COOH (co-)assemblies diluted at 7 µM, then washed and fixed with formaldehyde 4%. Nuclei were stained with DAPI and actin was stained with phalloidin-Texas Red. TCT: Tissue-culture treated. Scale bar is 100 µm.

Conclusions and future work

Conclusions

- The I10 β-peptide and its functionalized versions self-assemble into β-sheet-rich nanofibrils.
- The charged version of the I10, KI10-COOH, is a potent hydrogelator.
- I10-based assemblies show adhesive properties for keratinocytes and fibroblasts.

Future work

- Cell migration and atimicrobial assays
- Wound healing assays in mouse model

References

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