Development of a pH-responsive Peptide-based Hydrogel for Effective Eradication of MRSA Biofilm

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Introduction

- Methicillin-resistant *Staphylococcus aureas* is a biofilm forming bacteria, well-known for causing nosocomial infections. Biofilms hinder wound healing by protecting bacteria from the immune system and antibiotics.
- Hydrogels in combination with anti-microbial peptides offer a good alternative to conventional antibiotics to treat infected wounds. Hydrogels mimic the extracellular matrix, supporting tissue regeneration and healing while eradicating biofilms.
- Our lab has developed a self-healing, injectable peptide-polymer hydrogel that can encapsulate antimicrobial agents for the treatment of chronic bacterial infected wounds. The gel strength is derived from the peptide self-assemblies and covalent bonding with the polymer.
- The low pH in chronic wounds often make targeted delivery of antimicrobial agents difficult. Current hydrogels release antimicrobials minimally, requiring high concentrations, which may cause cytotoxicity.
- Therefore, we aimed to create a pH-responsive peptide-polymer hydrogel for controlled antimicrobial release at infection sites. The hydrogel disassembles at low pH, releasing antimicrobials more efficiently and reducing cytotoxicity.
- We have used multi-domain peptides (MDPs) with a novel non-natural ionic amino acid for the pH-sensitive disassembly. The polymer component provides structural support while peptides trigger disassembly in acidic environments.
- We have structurally characterised the hydrogel and tested its anti-microbial activity against MRSA. We have also assessed its cytotoxicity towards a mammalian cell line.

Experimental Section

Synthesis and purification of peptides

⬇

Preparation of pH responsive hydrogel

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Structural characterization of peptides and hydrogel

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Evaluation of pH-responsive activity of hydrogel

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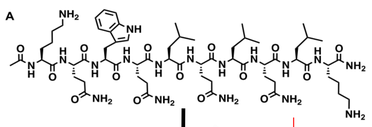
Evaluation of bioactivity

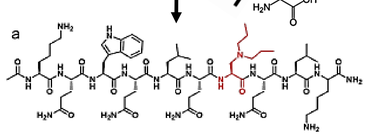
Results

Design and Synthesis of Peptides

Peptide 141



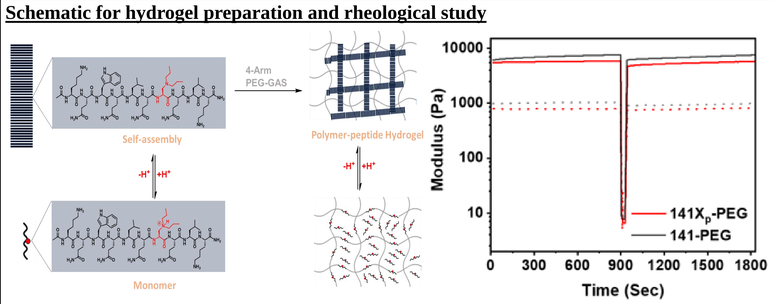
Peptide 141 Xp



- The 141Xp peptide was synthesized by substituting one of the hydrophobic leucine residues with the Fmoc Xp amino acid, a non-natural amino acid synthesized in our lab.
- This substitution brings the transition pH of the peptide to a biologically relevant neutral or weakly acidic range
- Peptides were synthesized on a Prelude® peptide synthesizer following standard solid phase synthesis protocol.

Hydrogel Preparation and Characterization

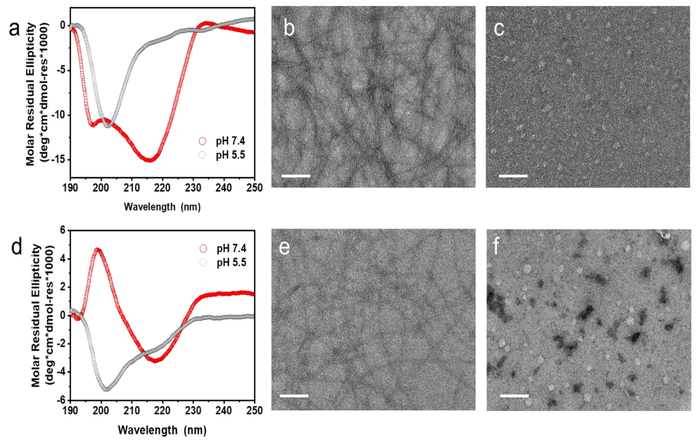
Schematic for hydrogel preparation and rheological study



- The hydrogel is based upon two levels of interaction; (1) the self-assembly of the peptide 141Xp which forms a sandwich-like beta sheet structure due to the presence of an alternating hydrophobic-hydrophilic domain and (2) an NHS-activated PEG polymer, where the NHS group reacts with the amine side chains of the peptide, establishing the second network in the peptide hydrogel.
- Rheological studies depicted good storage modulus and self-repair ability of the hydrogel after a 1000% shearing strain, alluding to its injectability.

Structural Characterization of Peptides and Hydrogel

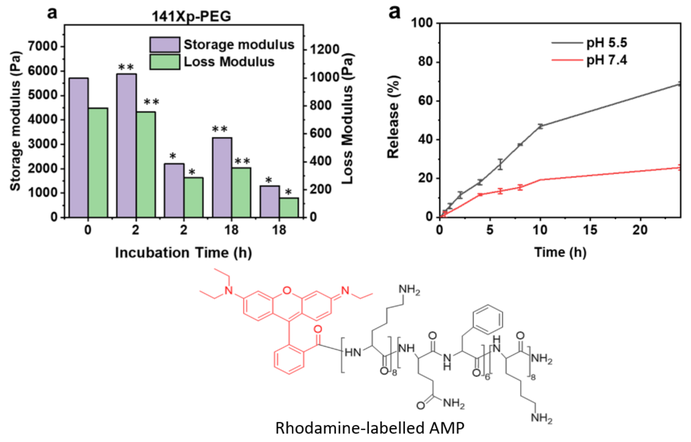
CD and TEM



- The pH responsiveness of 141Xp was characterized by CD spectroscopy and TEM studies. The peptides formed beta sheet structure at the physiological pH of 7.4.However, upon lowering the pH to 5.5, due to protonation of the tertiary amine in Xp amino acid, the peptide dis-assembled.
- Similarly, the same pattern of supramolecular assembly and dis-assembly was observed for the 141Xp-polymer (PEG) hydrogel.

pH Responsive Activity of Hydrogel

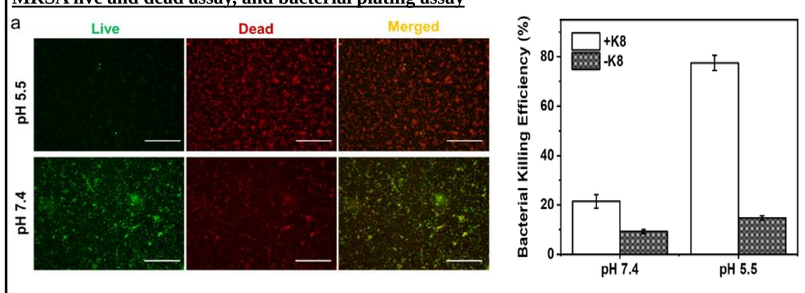
Rheological study and release assay with hydrogel



- pH-responsiveness was further monitored in a rheological assay where the gel was incubated in different pH buffers. Expectantly, the storage modulus was much higher for the gel incubated at pH 7.4.
- This property has been exploited for the encapsulation and pH-dependent release of a cationic rhodamine-labelled antimicrobial peptide (AMP) in the hydrogel. A much lower release rate was observed at the physiological pH.

Bioactivity of The Hydrogel

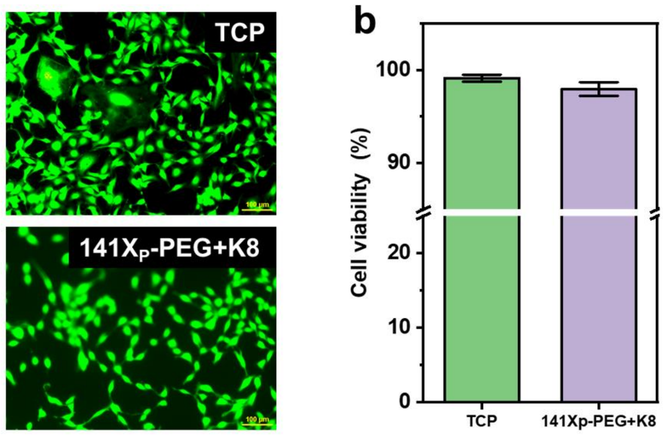
MRSA live and dead assay, and bacterial plating assay



- Antimicrobial abilities of the hydrogel was assessed using a live and dead assay on MRSA biofilm. At pH 7.4, similar numbers of both live and dead bacteria were observed, likely due to the diffusion of the AMP or, its presence on the surface of the biofilm. However, at pH 5.5, improved bacterial killing was observed, as the gel disassembled and released the AMP
- To further assess the bacterial killing, bacterial (MRSA) plating assay was done. The test samples included hydrogels with and without the AMP while a control set was taken where the hydrogel was absent against biofilms. Interestingly, the hydrogel alone also displayed a certain amount of killing efficiency though it was much lower than that of the hydrogel encapsulating AMP. Moreover, the killing efficiency was higher for the set-up maintained at a lower pH of 5.5 compared to those maintained at the physiological pH of 7.4

Mammalian Cell Cytotoxicity Assay

Live and dead assay, cell viability assay using HDFa cells



- The cytotoxicity of the hydrogel at pH 7.4 was evaluated using HDFa cells through a Live/Dead staining assay. The results indicated that most cells remained viable after 24 hrs of incubation with the hydrogels.
- Furthermore, qualitative analysis demonstrated that the hydrogel exhibited good cytocompatibility with HDFa cells with an average cell viability of approximately 97%.

Conclusion

- We have demonstrated the synthesis of a pH-responsive double network hydrogel using self-assembly of peptide-PEG conjugates.
- The peptide sequence consists of a newly designed non-natural ionic amino acid, which adjusts the transition pH of the peptide in the weakly acidic range.
- While these peptides have been studied in solutions before, this work extends their use to the development of bulk hydrogel materials.
- The combination of pH-responsive peptide self-assembly with covalent synthetic polymers allows for the dynamic control over the assembly, structure and rheological properties of the hydrogel.
- This hydrogel approach leverages antimicrobial peptides, self-assembling nanostructures, and pH-triggered release mechanisms.
- The innovative hydrogel system could offer a more targeted, effective, and safer treatment option for patients with biofilm-associated infections.

Future Work

- Extending this work on to in vivo studies to examine the hydrogel's efficacy in the physiological environment.
- Expanding the library of the pH-responsive hydrogel forming MDPs containing non-natural amino acid Xp.
- Exploring further applications of hydrogel in encapsulation and pH-triggered release of enzymes and drugs to ameliorate different disease conditions such as diabetes.

Acknowledgement

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