In-Situ Synthesis and Self-assembly of Peptide-PEG Conjugates: A facile Method for the Construction of Fibrous Hydrogels

Haritha Asokan Sheeja, He Dong*

Department of Chemistry and Biochemistry, The University of Texas at Arlington, TX 76013.

E-mail: hxa8189@mavs.uta.edu

Abstract

Peptide-based hydrogels have garnered attention in biomedical fields due to their unique combination of biocompatibility, biodegradability, and tunable hydrophilicity/hydrophobicity. However, many self-assembling peptides lack ability to form robust hydrogel required for biomedical applications. In this study, we present a novel approach involving the synthesis of peptide-PEG conjugates to address this challenge and investigate their hydrogel formation properties. The hydrogel comprises dual networks: the first network arises from peptide self-assembly into a β-sheet secondary structure, while the second network forms through covalent bonding between peptides and a 4-arm PEG utilizing N-hydroxysuccinimide chemistry. Our investigation highlights the efficacy of this methodology with lysine-rich peptide sequences. Additionally, upon incorporation of antimicrobial peptides, the hydrogel exhibits potent bacterial killing capabilities with minimal cytotoxicity to mammalian cells. This innovative approach holds promise for the development of advanced peptide-polymer hydrogel materials, offering enhanced performance in various biomedical applications.

Introduction

- Hydrogels are cross-linked polymeric networks capable of retaining large amounts of water and maintaining 3D hierarchical structures
- Biocompatible, Biodegradable, Injectable, tunable mechanical stability
- Peptide hydrogels are commonly assembled through intricate supramolecular interactions, unfolding hierarchically in a concentration-dependent manner.
- Despite their effectiveness in promoting hydrogel formation, most of the peptide hydrogels exhibit relatively low storage moduli.
- Our goal is to create a new hydrogels utilize a double network comprising both covalent and non-covalent interactions, resulting in significantly enhanced storage moduli.

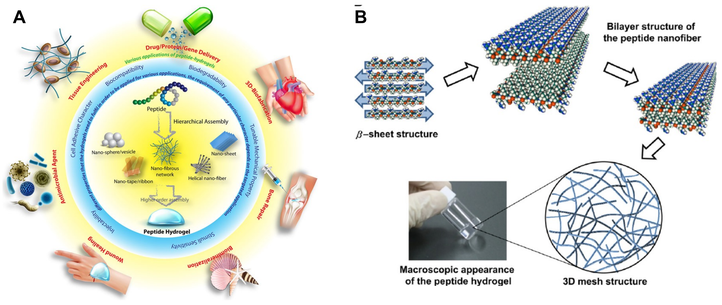
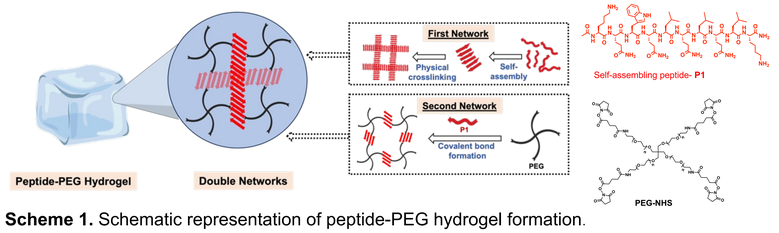


Figure 1. (A) Applications of peptide hydrogels.[1] (B) A schematic diagram of the formation of the hydrogel from the peptide monomer.[2]

Design of Peptide-PEG hydrogels



Scheme 1. Schematic representation of peptide-PEG hydrogel formation.

- The formation of the Peptide-PEG hybrid hydrogel entailed an NHS-amine reaction between a self-assembling peptide, P1, containing lysine, and a 4-arm PEG terminated with an NHS ester.
- First network, the self-assembly of peptides resulted in the formation of a weak hydrogel.
- Upon introduction of PEG, the hydrogel's rigidity markedly increased due to the formation of covalent bonds, constituting the second network.

Table 1. Peptide sequences used in this study.

Name	N-	Sequences	C-
P1	CH_3CO	K(QW)(QL)(QL)(QL)K	$CONH_2$
P2	CH_3CO	KK(QL)(QL)(QL)KK	$CONH_2$
P3	CH_3CO	KKK(QL)(QL)(QL)(QL)(QL)(QL)KK	$CONH_2$
P4	CH_3CO	KKKKKKKK(QF)(QF)(QF)(QF)(QF)(QF)KKKKKKKK	$CONH_2$

Characterization of Hydrogel Formation

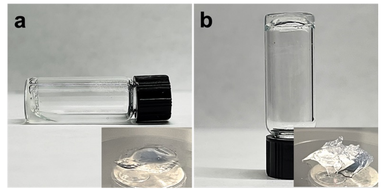


Figure 2. Photographs of (**a**) P1 and (**b**) P1-PEG

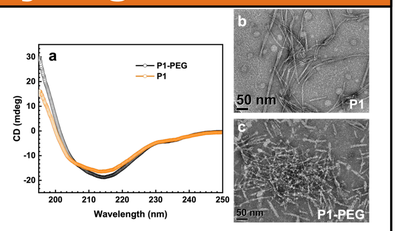


Figure 3. (**a**) CD and (**b-c**) TEM of P1 and P1-PEG

- P1 forms a viscous solution through self-assembly at higher concentrations.
- PEG increases hydrogel rigidity by forming covalent bonds through the reaction between amine and reactive NHS group.
- β-sheet secondary structure remains intact after PEG addition, indicating no interference with self-assembly.
- Nanofiber formation is observed in both P1 and P1-PEG.

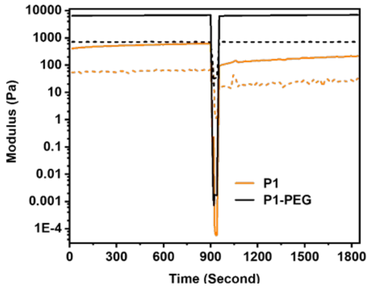


Figure 4. Time sweep measurements.

- Measurements at an angular frequency of 6 rad/s and 0.2% strain over a duration of 15 minutes.
- 1000% strain to disrupt the hydrogel.
- ~100% recovery after releasing the strain, suggesting its suitability as an injectable hydrogel for biological purposes.

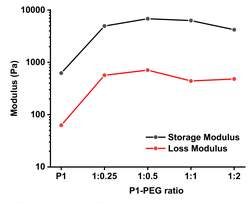
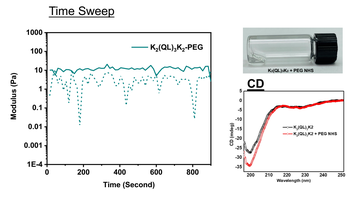
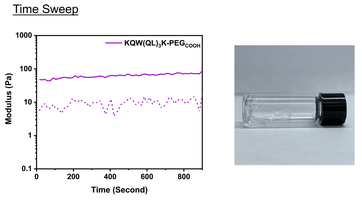


Figure 5. Rheology measurements at different P1-PEG ratios.

- The strongest hydrogel was formed by P1-PEG (1:0.5).



- PEG-NHS does not facilitate the formation of hydrogels with monomeric peptides P2.
- Self-assembly of peptide is very important for forming strong hydrogels.



- PEG-COOH does not enable the creation of hydrogels with self-assembled peptides
- Covalent bond formation between PEG-NHS and amine residues plays a crucial role in enhancing rigidity.

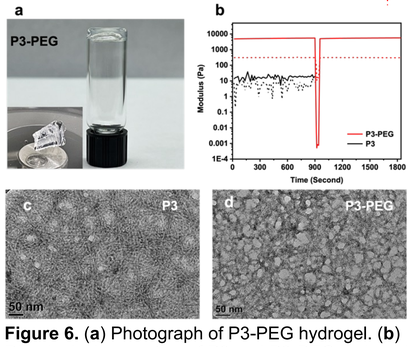


Figure 6. (**a**) Photograph of P3-PEG hydrogel. (**b**) Rheological properties of P3 and P3-PEG hydrogels. TEM images of (**c**) P3, and (**d**) P3-PEG reveals the presence of long nanofibers.

- P3 shows hydrogel formation upon the addition of PEG-NHS.
- Rheological investigations of P3-PEG reveal a notable enhancement in hydrogel rigidity upon PEG introduction.
- P3-PEG exhibits excellent injectability characteristics.
- TEM reveals the formation of long nanofibers both in the presence and absence of PEG-NHS.
- This approach for forming hydrogels is applicable to any self-assembling peptides with lysine side chains

Potential Application: Antimicrobial Therapy

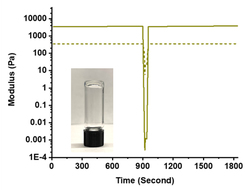


Figure 7. Time sweep measurements of P4P1-PEG

- Antibacterial evaluations of the P1-PEG hydrogel indicated a lack of notable antibacterial characteristics.
- To improve antibacterial efficacy, P4 (constituting 5% of the total weight) was incorporated into the hydrogel, forming P4P1-PEG.
- Due to the alternating hydrophobic and hydrophilic residue pattern, P4 is expected to have favorable interaction with P1
- P4P1-PEG demonstrates ~100% bacterial eradication efficiency and good cytocompatibility.

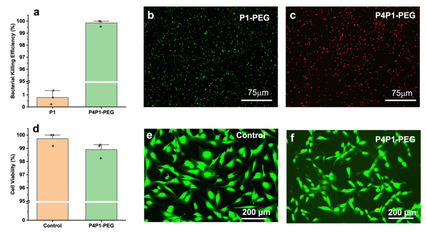


Figure 8. Top panel: (**a**) Bacterial killing efficiency assay of P1-PEG and P4P1-PEG hydrogels against *E. coli* . live and dead images of *E.coli* for (**b**) P1-PEG and (**c**) P4P1-PEG. Bottom panel: (**d**) Cell viability of HDFa cells in the presence and absence of P4P1-PEG gels . Fluorescence microscopic images of HDFa cells through live and dead assays for (**e**) Control and (**f**) incubated with P4P1-PEG after 24 h.

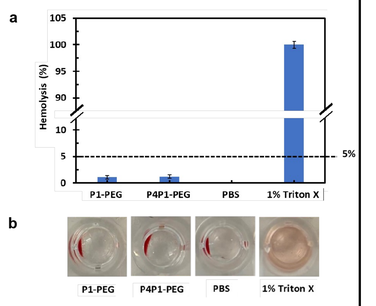


Figure 9. Hemolysis assay of P1-PEG and P4P1-PEG showing good hemocompatibility.

Summary and Future Direction

Summary of Key elements and Finding of Research

- We have successfully showcased a novel approach to synthesizing Peptide-PEG hydrogels.
- Hydrogels consist of dual networks: the initial network is established through self-assembly, while the secondary network is created via covalent bond formation.
- CD and TEM confirmed PEG introduction does not interfere with peptide's self-assembling property
- Peptide-PEG ratio 1:0.5 yields most rigid hydrogel formation
- P1-PEG alone is not antimicrobial. Antimicrobial peptides like P4 easily incorporated into P1-PEG gels
- P4P1-PEG exhibits high antimicrobial properties and low cytotoxicity

Future studies envisioned for the research

- Future efforts will be directed towards the design and synthesis of pH-responsive hydrogels for pH-dependent drug delivery applications.

Potential for Positive Impact on Communities

The current findings offer valuable insights into the fundamental understanding of Polymer-peptide hydrogel formation. This knowledge holds significant promise for transforming other self-assembling peptides into hydrogels for various biological applications.

References

1. Das, S., & Das, D. (2021). Rational design of peptide-based smart hydrogels for therapeutic applications. Frontiers in Chemistry, 9, 770102..
2. Nagai, Y., Yokoi, H., Kaihara, K., & Naruse, K. (2012). The mechanical stimulation of cells in 3D culture within a self-assembling peptide hydrogel. Biomaterials, 33(4), 1044-1051

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Dong Lab