Design and Synthesis of Self-Assembling Peptides for Hydrogel Formation

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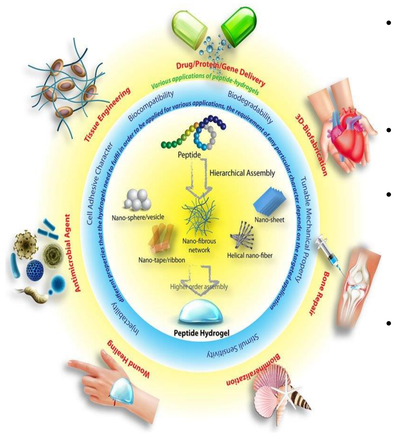
Abstract

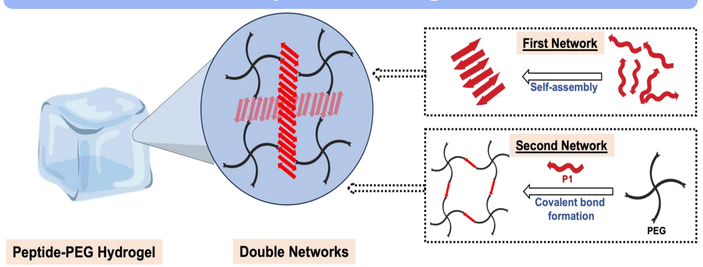
Peptides are adaptable structures that can be used to create a variety of nanostructures, which can then be used to create hydrogels and nanoparticles. The characteristics of peptide-based hydrogels are outstanding qualities such as tissue-like flexibility, biodegradability, high water absorption capacity, injectability, and mechanical stability. Peptide-based hydrogels are therefore the most intriguing for biological applications and diagnostic research, such as drug transport, cellular engineering, regenerative medicine, and biomedicine. Many self-assembling peptides, however, are unable to create strong hydrogels that are appropriate for use in biomedicine. In this work, we present the synthesis and design of a self-assembling peptide that can conjugate with PEG to generate a stable hydrogel. The peptide has an amphiphilic region that facilitates self-assembly into β-sheet nanofibers and contains lysine residues to improve solubility in aqueous solutions. A solid-phase peptide synthesizer was used to create the peptides, and HPLC was used to purify the final product. The β-sheet secondary structure of the peptide was revealed by CD spectroscopy, and MALDI was used to confirm the successful synthesis. These peptides employed in further studies to generate PEG-peptide conjugates, which are facilitated the production of strong hydrogels appropriate for a variety of biological uses.

Introduction



- Peptide-based hydrogels contain incredible properties: Biocompatibility, biodegradability, injectability, and tunable mechanical stability.
- Biomedical applications: Cellular engineering, drug transport, regenerative medicine, and more.
- Traditional peptide-based hydrogels relied on non-covalent networks at high concentrations but had low storage moduli, making them unsuitable for biomedical applications.
- Double network formation using N-hydroxysuccinimide (NHS) coupling to create peptide-PEG conjugates, improving hydrogel strength while maintaining biocompatibility.

Peptide Design



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.
- The alternating hydrophobic-hydrophilic pattern promotes the formation of sandwich-like nanofibers for self-assembly.
- Leucine and tryptophan embed within the hydrophobic core due to their hydrophobic properties, while glutamine remains on the outer surface, creating a balance between intermolecular hydrogen bonding and hydrophobic interactions among the repeating units.
- The second network involves the NHS-activated PEG polymer containing the NHS group that reacts with the lysine sidechains on the peptide sequence to generate the covalent N-hydroxysuccinimide (NHS) coupling reaction.

Peptide Design

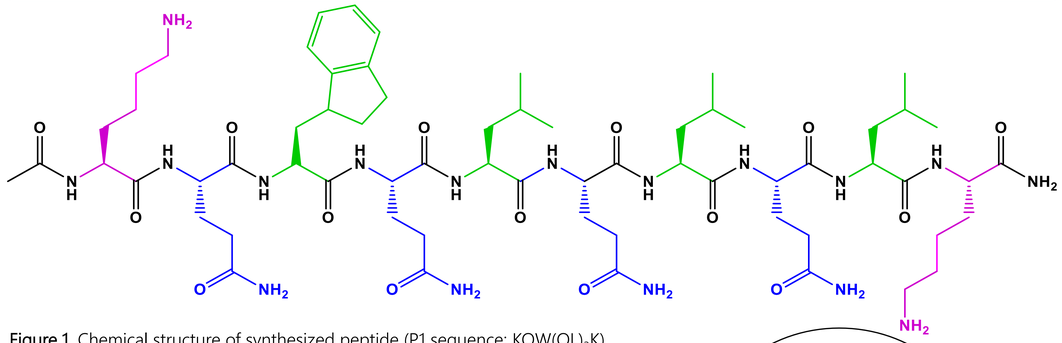


Figure 1. Chemical structure of synthesized peptide (P1 sequence: $KQW(QL)_3K$)

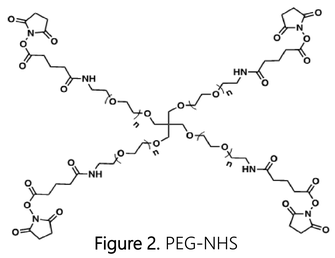


Figure 2. PEG-NHS

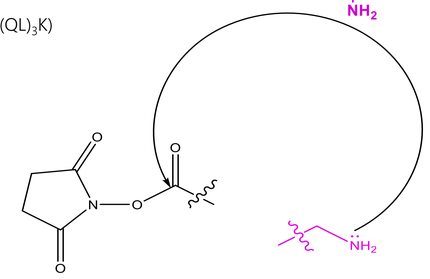


Figure 3. NHS-amine coupling reaction between PEG-NHS and lysine containing peptide sequence..

Synthesis and Purification

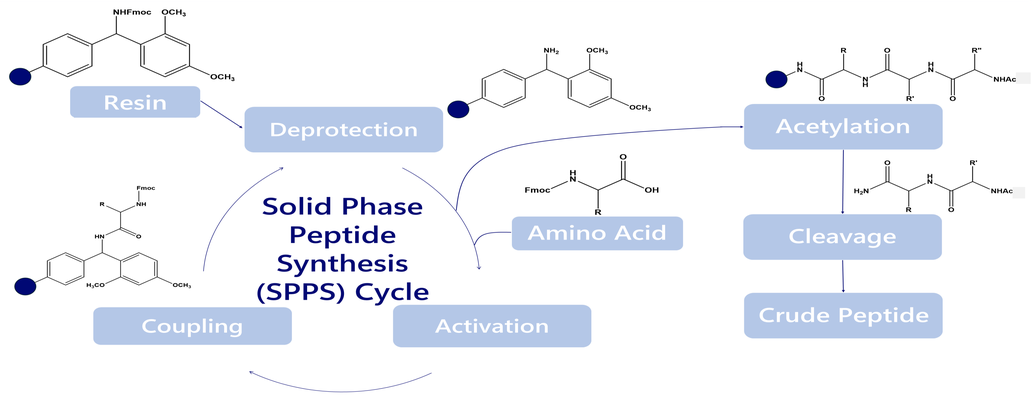
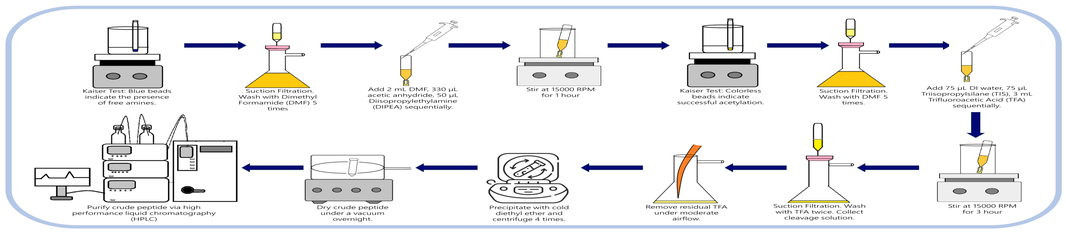


Figure 4. Standard Fmoc-Solid Phase Peptide Synthesis (SPPS) Cycle procedure used for this study.



Scheme 2. Detailed methodology containing acetylation, cleavage, and purification of synthesized peptide.

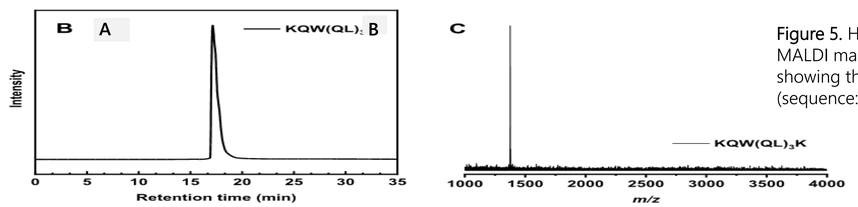


Figure 5. HPLC chromatogram and MALDI mass spectrophotometry showing the successful synthesis (sequence: $KQW(QL)_3K$).

Results

Figure 6. Vial inversion to assess hydrogel strength. **(a)** Synthesized peptide sequence in PBS 1x. **(b)** Synthesized peptide with PEG in PBS 1x.

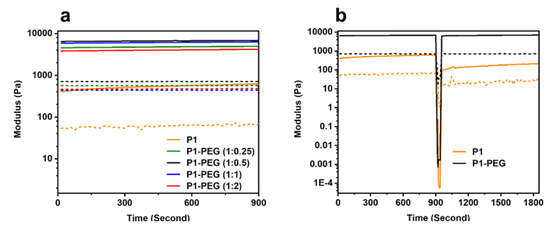


Figure 7. **(a)** Rheological properties of the peptide hydrogel (2 wt %) containing different ratios of PEG during time sweep (frequency: 6 rad/s, strain: 0.2%). **(b)** Dynamic time sweep of P1 and the strongest P1–PEG gel (1:0.5) over 900 s (frequency: 6 rad/s, strain: 0.2%).

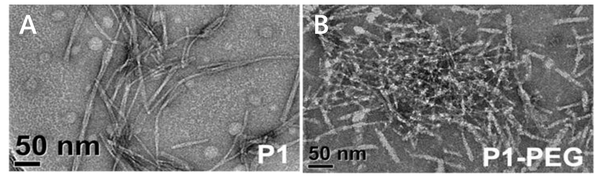


Figure 8. Transmission electron microscope (TEM) images to showcase **(a)** peptide nanofibers of synthesized protein and **(b)** double cross-linking between synthesized protein and PEG to form the hydrogel.

Conclusion

- We have effectively developed a self-assembling peptide sequence suitable for synthesizing peptide-based hydrogels.
- NHS-amine coupling chemistry is employed to fabricate a double network hydrogel, offering vast applications in various fields.
- Key Components for Hydrogel Formation:
  - Lysine-rich self-assembling peptide featuring an alternating hydrophobic-hydrophilic sequence to establish a sandwich-like β-sheet structure.
  - NHS-activated PEG polymer, which interacts with the amine side chains of the peptide.

Future Work

- Assessment of the strength and antimicrobial efficacy of the hydrogel for biological applications.
- Investigation of the potential for extending this method to create double network hydrogels using other amine-containing self-assembling peptides.

Acknowledgments

PREM
PARTNERSHIPS FOR RESEARCH AND EDUCATION IN MATERIALS

References

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