Synthesis, Characterization and Biomedical Potential of Peptide-Gold Nanoparticle Hydrogels

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Abstract

Background: Nanotechnology has revolutionized fields like medicine and biotechnology by enabling the manipulation of materials at the atomic level. Gold nanoparticles (AuNPs) are particularly valued for their unique optical and chemical properties, making them ideal for biomedical applications. Hydrogels, known for their biocompatibility and water retention capabilities, are key materials in biomedical engineering. This study explores the synthesis and characterization of peptide-gold nanoparticle hybrid hydrogels, combining the benefits of AuNPs and peptide-based hydrogels for potential biomedical applications. Methods: Peptides were synthesized using solid-phase peptide synthesis (SPPS) and characterized through high-performance liquid chromatography (HPLC) and mass spectrometry. Gold nanoparticles were produced via the citrate reduction method and functionalized with peptides through Au–S bonds. These functionalized peptides self-assembled into hydrogels, which were then analyzed using transmission electron microscopy (TEM), UV-Vis spectroscopy, and dynamic mechanical analysis (DMA) to evaluate their structural, optical, and mechanical properties. Results: The peptides showed high purity and accurate molecular weights. AuNPs were successfully cryo-TEM imaging confirmed nanoparticle incorporation, and rheological analysis demonstrated enhanced mechanical strength and shear-thinning behavior in the hybrid hydrogels. Conclusion: The developed peptide-gold nanoparticle hybrid hydrogels show great potential as multifunctional biomaterials, suitable for various biomedical applications such as drug delivery and tissue engineering.

Keywords: Nanotechnology, Gold Nanoparticles, Peptide Hydrogels, Biomedical Engineering, Drug Delivery

Significance | This study demonstrated multifunctional hybrid hydrogels with potential for biomedical applications, combining enhanced mechanical properties and tunable optical characteristics.

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Introduction

Nanotechnology, a multidisciplinary field that operates at the nanoscale, has revolutionized various scientific domains, including materials science, medicine, and biotechnology (Bhushan, 2010). The concept of manipulating materials at the atomic or molecular level was first proposed by Richard Feynman in 1959, who emphasized the vast potential for innovation at these scales (Feynman, 1959). Over the years, nanotechnology has evolved into a robust scientific field, enabling the development of novel materials and devices with applications ranging from drug delivery to environmental monitoring (Whitesides & Grzybowski, 2002).

Among the various nanomaterials, gold nanoparticles (AuNPs) have garnered significant attention due to their unique optical, electronic, and chemical properties (Daniel & Astruc, 2004). AuNPs are particularly notable for their size-dependent optical properties, which arise from localized surface plasmon resonance (LSPR), a phenomenon where conduction electrons on the nanoparticle's surface oscillate in resonance with incident light (Jain et al., 2006). This property makes AuNPs ideal candidates for applications in

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imaging, diagnostics, and therapeutics, especially in fields such as cancer treatment, where targeted delivery and controlled release are critical (Murphy et al., 2008).

Hydrogels, which are three-dimensional polymeric networks capable of retaining large amounts of water, have also emerged as crucial materials in biomedical engineering due to their biocompatibility, tunable mechanical properties, and ability to mimic the extracellular matrix (ECM) (Peppas et al., 2000). The versatility of hydrogels stems from their ability to undergo physical or chemical cross-linking, leading to the formation of stable networks that can be tailored for specific applications, including tissue scaffolding and controlled drug release (Hoffman, 2002).

In recent years, the self-assembly of short peptide sequences has been extensively studied for the creation of peptide-based hydrogels. These hydrogels exhibit favorable properties such as biocompatibility, biodegradability, and the ability to form under physiological conditions (Holmes et al., 2000; Zhang et al., 2002). The incorporation of aromatic capping groups, such as the Fmoc group, facilitates the self-assembly process through non-covalent interactions like π–π stacking, hydrogen bonding, and hydrophobic interactions (Ghosh et al., 2007; Yan et al., 2010).

This study explores the synthesis and characterization of peptide-gold nanoparticle hybrid hydrogels, which combine the advantages of both peptide-based hydrogels and AuNPs. These hybrid materials hold significant potential for various biomedical applications, including drug delivery, tissue engineering, and photothermal therapy. The study aims to design peptides that can self-assemble into hydrogels and conjugate with gold nanoparticles to create multifunctional hybrid systems.

Materials and Methods

Peptide Synthesis

The peptides were synthesized using the solid-phase peptide synthesis (SPPS) method, a technique that provides precise control over peptide sequence and structure (Fields & Noble, 1990). The synthesis involved the stepwise addition of amino acids to a growing peptide chain anchored on a resin, followed by purification using high-performance liquid chromatography (HPLC) (Carpino, 1993). The final peptides were lyophilized and stored at -20°C until further use (Figure 1).

Peptide Characterization

Following synthesis, the peptides were characterized using High-Performance Liquid Chromatography (HPLC) to assess their purity and mass spectrometry to confirm their molecular weights (Gausepohl et al., 1992). These analytical techniques ensured that the peptides were of sufficient quality for subsequent functionalization with gold nanoparticles. HPLC is particularly valuable in determining the purity of peptides by separating and quantifying the individual components in the mixture (Figure 2), while mass spectrometry provides precise molecular weight information, crucial for confirming the successful synthesis of the desired peptides (Figure 3).

Gold Nanoparticle Synthesis and Functionalization

Gold nanoparticles were synthesized using the citrate reduction method, a classic approach that produces monodisperse AuNPs with controlled sizes (Turkevich et al., 1951). In this method, gold chloride is reduced by sodium citrate, leading to the formation of AuNPs. The size and shape of the nanoparticles can be controlled by adjusting the reaction parameters, such as the citrate concentration and temperature (Frens, 1973). The synthesized AuNPs had an average diameter of 13 nm, as determined by UV-Vis spectroscopy and TEM.

For functionalization, the gold nanoparticles were conjugated with the synthesized peptides through Au–S bonds, which form between the gold surface and the thiol groups of cysteine residues in the peptides (Love et al., 2005). This functionalization process was confirmed by a shift in the surface plasmon resonance (SPR) peak observed in the UV-Vis spectra of the functionalized nanoparticles, indicating successful peptide attachment (Haiss et al., 2007).

Hydrogel Formation

The functionalized peptides were dissolved in water and subjected to conditions that promote self-assembly into hydrogels. The presence of the Fmoc group and aromatic amino acids in the peptide sequences facilitated the formation of a stable hydrogel network through non-covalent interactions, including π–π stacking and hydrogen bonding (Capito et al., 2008). The self-assembly process was optimized by adjusting the pH and peptide concentration, ensuring the formation of a robust hydrogel matrix capable of incorporating the gold nanoparticles.

Characterization of Hydrogels

The peptide-gold nanoparticle hydrogels were characterized using various techniques to assess their structural and optical properties. Transmission Electron Microscopy (TEM) and Cryo-TEM were employed to examine the morphology of the hydrogel and the distribution of AuNPs within the matrix (Schoenfisch & Pemberton, 1998). These techniques provided high-resolution images that revealed the formation of a well-organized hydrogel network with evenly distributed nanoparticles.

UV-Vis spectroscopy was used to monitor the optical properties of the hydrogels, particularly the SPR peak of the gold nanoparticles, which provides information on the aggregation state and environment of the AuNPs within the hydrogel (Figure 4). The rheological properties of the hydrogels, such as their mechanical strength and viscoelastic behavior, were evaluated using dynamic mechanical analysis (DMA), which measures the material's response to oscillatory stress (Li & Mooney, 2016).

Results

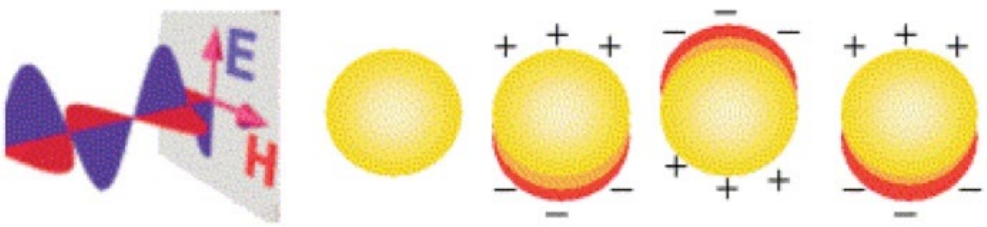

Figure.1. Schematic drawing of the interaction of an electromagnetic radiation with a metal nanosphere. A dipole is induced, which oscillates in phase with the electric field of the incoming light.

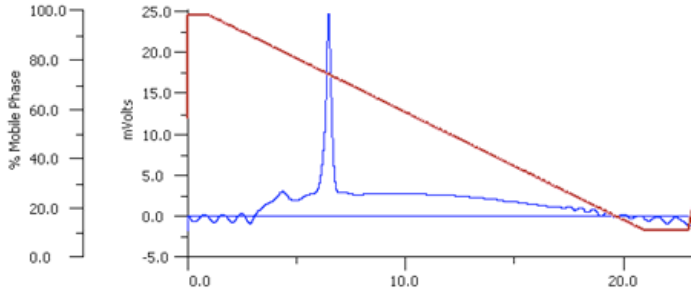

Figure 2. Only one compound is presented by the single peak detected by HPLC for peptide Fmoc-Cys-Gly-Gly.

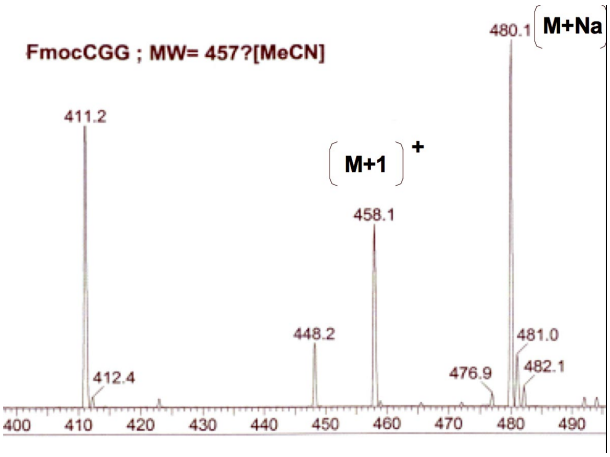

Figure 3. Mass Spectroscopy spectrum of synthesized peptide FmocCGG

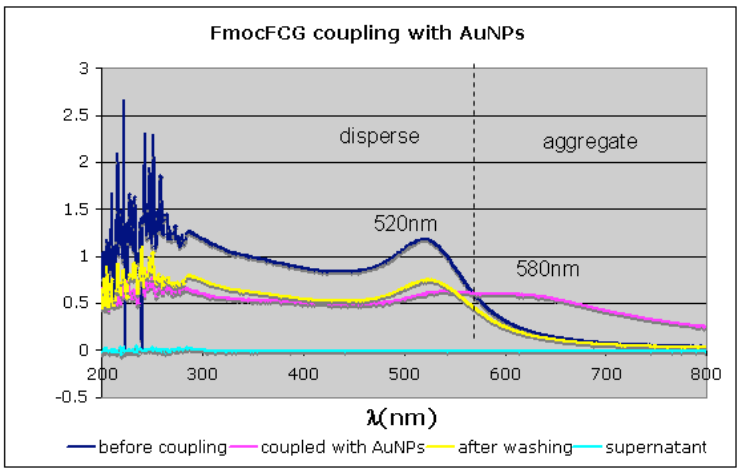

Figure 4. UV-Vis tests for AuNPs coupling with peptide FmocFCG at different stages.

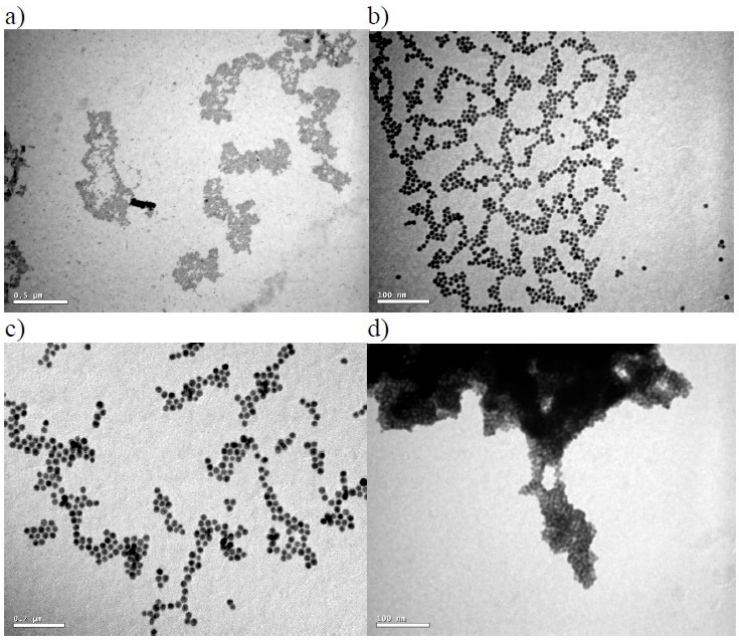

Figure 5. Example of TEM images of one peptide FmocCGG-AuNPs sample after gelation form. (a) under 40kv, 0.5μm scale, AuNPs were aggregated into groups.(b) under 100kv, with 100nm, AuNPs aggregated pattern in one group. (c) under 400kv, 0.2μm close up viewing for particles. (d) under 200kv, 100nm, AuNPs were in gel.

Peptide and Nanoparticle Characterization

The peptides synthesized via SPPS were confirmed to be of high purity through HPLC analysis, and their molecular weights were verified using mass spectrometry. The gold nanoparticles exhibited a characteristic SPR peak around 520 nm, confirming their successful synthesis. TEM analysis revealed spherical nanoparticles with a uniform size distribution (Figure 5 a-d).

Hybrid Hydrogel Characterization

The hybrid hydrogels were successfully synthesized with AuNPs uniformly dispersed throughout the peptide matrix, as confirmed by TEM and cryo-TEM imaging. The UV-Vis spectra of the hybrid hydrogels showed a red-shift in the SPR peak, indicative of nanoparticle incorporation into the hydrogel matrix (Figure 6). Rheological testing demonstrated a significant increase in the storage modulus (G') of the hybrid hydrogels compared to the peptide-only hydrogels, suggesting enhanced mechanical properties.

Rheological analysis revealed that the hybrid hydrogels exhibited shear-thinning behavior, a desirable property for injectable biomaterials. The storage modulus (G') was significantly higher than the loss modulus (G''), indicating that the hydrogels possessed a solid-like structure with sufficient mechanical strength for potential biomedical applications.

Discussion

The successful synthesis and characterization of peptide-gold nanoparticle hybrid hydrogels demonstrate their potential as multifunctional biomaterials. The combination of peptide self-assembly with gold nanoparticle incorporation results in materials with enhanced mechanical properties, tunable optical characteristics, and potential responsiveness to external stimuli such as light.

The ability of the hybrid hydrogels to incorporate AuNPs without significant aggregation is particularly noteworthy, as it suggests that the hydrogels can maintain the nanoparticles' unique optical properties, which are critical for applications such as photothermal therapy (Jain et al., 2006). Additionally, the shear-thinning behavior observed in the rheological studies suggests that these materials could be used in injectable formulations, facilitating minimally invasive delivery in therapeutic settings (Hoare & Kohane, 2008).

Future work will focus on exploring the biomedical applications of these hybrid hydrogels, particularly in drug delivery and tissue engineering. The tunable properties of the hydrogels make them ideal candidates for controlled drug release, where the release profile can be adjusted by modifying the hydrogel composition or the peptide sequence (Peppas et al., 2000). Furthermore, the integration of gold nanoparticles opens up possibilities for developing responsive materials that can be activated by light or other external stimuli, providing a means for targeted therapy with minimal side effects (Murphy et al., 2008).

Conclusion

This study has successfully developed peptide-gold nanoparticle hybrid hydrogels, demonstrating their potential as advanced biomaterials for biomedical applications. The hydrogels combine the favorable properties of both peptides and gold nanoparticles, resulting in materials with enhanced mechanical strength, tunable optical characteristics, and potential for responsive behavior. The findings of this study pave the way for further exploration of these materials in drug delivery, tissue engineering, and other therapeutic applications.

Author contributions

I.U.K. conceptualized and supervised the study, analyzed the data, and finalized the manuscript. All authors have read and approved the final version of the paper.

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Competing financial interests

The authors have no conflict of interest.

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