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In situ synthesis of Ag/amino acid biopolymer hydrogels as mouldable wound dressing

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This manuscript introduces a one-pot fabrication for supramolecular hybrid hydrogels from low-cost commercially available natural products through a "green" strategy. In particular, the hybrid hydrogels which are developed with Fmoc-Glu-OMe, silver nanoparticles and chitosan exhibit outstanding antibacterial property and can be regarded as excellent mouldable wound healing biomaterials.

Wound infection and dehydration are key obstacles during the wound healing process. Supramolecular hydrogels are regarded as ideal wound treatment¹ and tissue engineering materials² since they can provide moist environments³ for wound healing and serve as protective barriers at wound interface.⁴ Besides, their biocompatibility, biodegradability⁵ and self-healing features⁶ make the removing process convenient and painless, reducing the chance of secondary trauma. In consequence, preparing mouldable and injectable hydrogels composite materials with excellent antibacterial activity and biocompatibility is of great significance to prevent infection and promote wound healing.⁷ Usually, the synthesis of hydrogelator requires a series of chemical treatment and laborious purification processes,⁸ and worse, some environmentally unfriendly reagents and solvents are inevitably involved during the operation. Therefore, natural products such as amino acids and biopolymers become favourable building blocks to develop self-assembly nanocomposite.⁹

Silver has been known as a novel antibacterial material no later than the era of Alexander the Great. It has been reported that silver exhibited broad-spectrum antibacterial effect against fungus, Gram-positive bacteria, Gram-negative bacteria¹⁰ and viruses.¹¹ Recently, a great number of studies have verified that the *in vivo* antibacterial activities of silver nanoparticles (AgNPs) are superior to other silver compounds.¹² Unfortunately, AgNPs are usually unstable in

bacterial-rich environments. It still remains a challenge to prepare and stabilize AgNPs in buffer solution.¹³ One particular solution is to prevent the self-aggregation of AgNPs by embedding Ag nanocomposites into hydrogels frameworks.¹⁴ Although a few antibacterial Ag nanocomposite/hydrogel materials have been reported, none of them is commercially available.¹⁵

In this communication, we demonstrate an *in situ* method to prepare AgNPs-containing hybrid hydrogels from low-cost commercial natural products. This work was inspired by the previous landmark achievements on natural gelators, which are derived from amino acids or peptides,¹⁶ especially diphenylalanine-based hydrogelators.¹⁷

We have screened a series of commercial amino acids products and found that several Fmoc(9-fluorenylmethyl) amino acids could spontaneously form hydrogels in a certain pH range. Among them, Fmoc-glutamic acid methyl ester (Fmoc-Glu-OMe) presents remarkable antibacterial activity and perfect gelation properties in silver nitrate (AgNO₃) solution. To our knowledge, Fmoc-Glu-OMe is one of the few but the cheapest gelation reagents, which can form stable hydrogels in AgNO₃ solution after enduring sonication stress for more than half an hour. Interestingly, upon sonication, AgNO₃ can be smoothly reduced to well-dispersed AgNPs in hydrogel network *in situ*, without extra reducing agents or stabilizers. To further improve the inferior mouldable performance of this hydrogel, chitosan, another natural hydrogelator with superior



Scheme 1 Illustration of the preparation strategy and applications of the supramolecular hybrid hydrogels.

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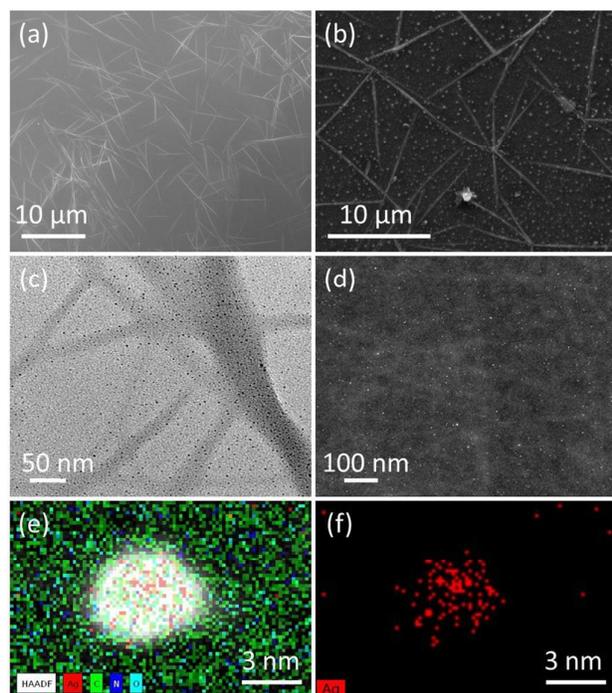


Fig. 1 (a-b) The SEM images of the hydrogels prepared from Fmoc-Glu-OMe (a), Fmoc-Glu-OMe/AgNPs (b), respectively. (c) Transmission electron microscopy (TEM) image of Fmoc-Glu-OMe/AgNPs hydrogels. (d) The STEM image of Fmoc-Glu-OMe/AgNPs/chitosan hydrogels. (e-f) The HAADF-STEM-EDS maps of Fmoc-Glu-OMe/AgNPs/chitosan hydrogels.

biocompatibility,¹⁸ biodegradability¹⁹ and antibiotic property,²⁰ was introduced into the Fmoc-Glu-OMe/AgNPs nanocomposite.²¹ As expected, monodispersed AgNPs were *in situ* synthesized within the stable and transparent hybrid hydrogels.

In mono-Fmoc-Glu-OMe hydrogels, the amine acid molecules self-assembled into well-dispersed fibrils with a diameter of around 10 nm and a length of more than 10 μm (Fig. 1a). Hearteningly, in the hybrid hydrogels, after sonication, silver ions could be spontaneously reduced to AgNPs while the gel state still kept integrated. It is observed that AgNPs were uniformly dispersed among Fmoc-Glu-OMe nanofibers, which avoided the self-aggregation of AgNPs (Fig. 1c). This could be further confirmed by the atomic force microscopy (AFM, Fig. S1b and 1c) and the scanning electron microscopy (SEM, Fig. 1b).

The high-angle annular dark field scanning TEM/energy-dispersive X-ray spectroscopy (HAADF-STEM-EDS) and X-ray photoelectron spectroscopy (XPS) were used to investigate the elemental compositions, morphologies, sizes, and dispersion of the composites. As displayed in Fig. S3, AgNPs with the uniform size of about 5 nm were monodispersed in the hybrid Fmoc-Glu-OMe/AgNPs/chitosan hydrogels. The Ag element was primarily distributed in the core of the nanoparticle, while the C, N, and O elements were widely scattered around the nanoparticle. The compositional linear scan profile with a higher spatial resolution across a single nanoparticle in the soft nanocomposite was illustrated in Fig. S4a. And it confirmed that the C, N and O elements were evenly distributed everywhere, while Ag element

was concentrated on the centre, which well corresponds to the preceding HAADF-STEM-EDS maps. The Ag valence of the hybrid hydrogels were further investigated by XPS (Fig. S4b). It can be seen that the two signal peaks appear at 374.5 eV and 368.4 eV, which coincide with the Ag 3d_{3/2} and Ag 3d_{5/2}, respectively, indicating the co-existing of AgNPs and silver ions.

The viscoelastic properties of these hydrogels were researched via rheology experiments. It is notable that the storage moduli (G') of the four composites were much larger than the loss moduli (G''), which reveals that they can be considered to be real gel materials (Fig. S4c). On the other hand, the Fourier transforming infrared spectra (FTIR) analyses were carried out to investigate the interaction forces in the various self-assembly hydrogels (Fig. S4d). Apparently, the adsorption peaks of those functional groups in the Fmoc-Glu-OMe/chitosan and Fmoc-Glu-OMe/AgNPs/chitosan hydrogels were both weaker than those in Fmoc-Glu-OMe hydrogels, suggesting that the existence of chitosan limited the vibration of those functional groups by involving extra intermolecular hydrogen bond interactions.

Intriguingly, the mechanical vibration could transform the stable and transparent hydrogels into turbid solution state, while the state could be easily restored to the previous gel state after stewing for a few minutes. The decent self-healing character of the hybrid hydrogels made it suitable for drug delivery, tissue engineering, etc. (Here, Rhodamine B was added to highlight the supramolecular hybrid hydrogels, Fig. 2a-c). Thus, Fig. 2d-e provide a solid evidence for the injectable ability and the mouldability of the hybrid hydrogels, respectively, and they could substantially ameliorate the functionality and extend the applications of the hydrogels.

The antibacterial activity of the supramolecular hybrid hydrogels was assessed by the *E. coli* strains (Gram-negative bacteria). The antibacterial performance of the Fmoc-Glu-OMe/AgNPs/chitosan hydrogels, Fmoc-Glu-OMe/AgNPs hydrogels, Fmoc-Glu-OMe/chitosan hydrogels, Fmoc-Glu-OMe hydrogels, and control group (untreated) was detected via shaking flask method, respectively. Fig. 3 is the optical microscope images for Fig. S5 under 50x magnification. It is obvious that Fmoc-Glu-OMe/AgNPs/chitosan hydrogels showed the highest antibacterial activity (Fig. 5a). The *E. coli* colonies appearing in the field of vision were

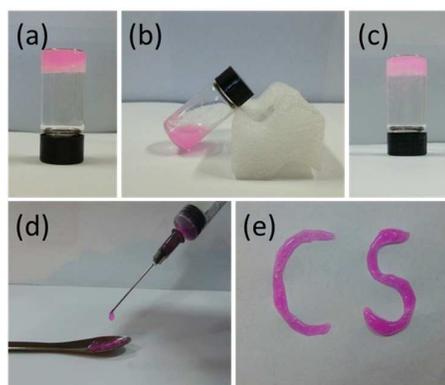


Fig. 2 (a-c) Photographs of the self-healing phenomena of the supramolecular hybrid hydrogels. The hydrogels were dyed by Rhodamine B. (d-e) Photographs of (d) the injectable character and (e) the mouldability of the hybrid hydrogels.

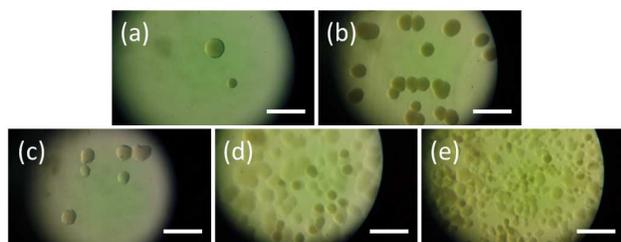


Fig. 3 The optical microscope images for photographs of bacterial colonies formed by *E. coli* strains treated with the hydrogels, which were prepared from (a) Fmoc-Glu-OMe/AgNPs/chitosan, (b) Fmoc-Glu-OMe/AgNPs, (c) Fmoc-Glu-OMe/chitosan, (d) Fmoc-Glu-OMe, and (e) the untreated bacteria were used as control, respectively (scale bar=0.2 mm).

the only bacteria in the whole disk (10 cm in diameter). Both of the Fmoc-Glu-OMe/AgNPs hydrogels and Fmoc-Glu-OMe/chitosan hydrogels exhibited decent antibacterial capability against *E. coli* colonies (Fig. 5b-c). The antibacterial ability of our Fmoc-Glu-OMe hydrogel was relatively poorer than the other three samples but still better than the untreated group (Fig. 5d and 5e). It seems that the synergistic effect of these three kinds of components causes the exceptional antibacterial ability of this hybrid nanocomposites.

We carried out *in vivo* experiments to test the wound healing feature of the hybrid hydrogel system. Fig. S6 shows the visual observations of wound healing process of four groups of rats, including control group I (Fmoc-Glu-OMe hydrogel), experimental group (hybrid nanocomposite), control group II (commercial antimicrobial), and the blank group (untreated). For the control group I and experimental group, the wound size reduced remarkably. Particularly, in the experimental group, the surface of the cut kept clear and clean during the whole observation period, alleging the outstanding wound healing ability of our nanocomposite. For the control group II, the cuts had no significant changes after 5-day treatments. As for the blank group, the cuts kept on decreasing but the zoom in Fig. S6 disclosed that there appeared various degrees of pus, flare and inflammation areas around the scar. These results demonstrated that this hybrid hydrogels could effectively accelerate wound healing and possess beneficial spreadability, facilitating it to serve as a promising candidate for wound caring applications such as antibacterial bandages and injectable wound paste materials.

In conclusion, we have successfully developed a facile method to fabricate AgNPs-containing nanocomposite through a one-pot green treatment with cost-effective and commercially available products. The outstanding antibacterial property of the as-prepared hybrid hydrogel provides a good chance to become excellent mouldable wound healing biomaterials. We believe that this supramolecular hybrid hydrogels will offer considerable potential in antibacterial agents and wound caring materials.

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