



# Dual-Functional Dextran-PEG Hydrogel as an Antimicrobial Biomedical Material

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Microbial infections continually present a major worldwide public healthcare threat, particularly in instances of impaired wound healing and biomedical implant fouling. The development of new materials with the desired antimicrobial property to avoid and treat wound infection is urgently needed in wound care management. This study reports a novel dual-functional biodegradable dextran-poly(ethylene glycol) (PEG) hydrogel covalently conjugated with antibacterial Polymyxin B and Vancomycin (Vanco). The hydrogel is designed as a specialized wound dressing that eradicates existing bacteria and inhibits further bacteria growth, while, ameliorating the side effects of antibiotics and accelerating tissue repair and regeneration. The hydrogel exhibits potent antibacterial activities against both gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) with no observable toxicity to mouse fibroblast cell line NIH 3T3. These results demonstrate the immense potential of dextran-PEG hydrogel as a wound dressing healthcare material in efficiently controlling bacteria growth in complex biological systems.

## 1. Introduction

Today, bacterial infections constitute one of the greatest global public healthcare challenges.<sup>[1–8]</sup> Specifically, wound infections, one of the most commonly acquired infections, are a leading cause of morbidity and mortality.<sup>[9,10]</sup> Extensive efforts have been made to develop an effective wound care device with antibacterial properties that would dress the wound, maintain a moist wound microenvironment, and prevent bacterial infection, thereby enhancing and accelerating the wound healing process.<sup>[11–13]</sup> Wound dressings made from biomaterials are often

used for protecting damaged skin or tissue from dehydration and microbial infections and are continually being developed further to address emerging challenges. Currently, commercial wound dressings include films, sponges, hydrocolloids, and hydrogels.<sup>[14–16]</sup> Among them, hydrogels offer numerous advantages for antibacterial wound healing, including high water content and soft consistency, which provide a moisturized local environment for wound healing and prevent secondary infections caused by the entry of microorganisms into the wound.<sup>[17–19]</sup>

Although a suitable moisturized wound environment can be easily achieved by using hydrogel dressings, other major concerns in the design of wound dressing, including effectively preventing and treating wound infection, still must be addressed and investigated further.<sup>[13,20]</sup>

Various strategies were utilized to encapsulate antibiotics into hydrogels to avoid bacterial infections at the affected area.<sup>[21–28]</sup> The depot provided by the hydrogel facilitates the loading, storage, and localized delivery of antibacterial and wound healing agents. Compared with systemic administration, the delivery of antibacterial drugs to local wound sites using dressing exemplifies superior benefits, including improved tissue compatibility, low occurrence of bacterial resistance, as well as reduced systemic toxicity.<sup>[13]</sup> However, noncovalently entrapping drugs may prompt rapid drug release, leading to an increased risk of side effects due to high local drug concentration and systemic toxicity of the released drug. Thus, an effective method for the incorporation of antibacterial drugs remains a challenge. Additionally, it is a common approach, as in initial empiric therapy, to use broad-spectrum antimicrobial agents for antimicrobial devices. Unfortunately, very few antibiotics are broad-spectrum and they may cause drug resistance.<sup>[29]</sup>

In an effort to overcome such undesirable consequences, we herein report an antibiotics conjugated hydrogel as an antimicrobial wound dressing with effective antibacterial activity against both gram-positive and gram-negative bacteria. Additionally, the hydrogel is biocompatible, biodegradable, and mechanically adjustable. The covalent immobilization of antibacterial drugs into the hydrogel can counteract the release and diffusion of high dose drugs and therefore, avoid systemic toxicity and other unintended side-effects, while protecting the wound against existing and future pathogens. The main components of the hydrogel are biocompatible polymer dextran

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(a natural glucose-containing polysaccharide) and poly(ethylene glycol) (PEG). Dextran is a complex, branched and hydrophilic polysaccharide composed of anhydroglucose rings. Due to its excellent biocompatibility and chemical functionality, dextrans have been extensively investigated as a safe biomaterial for the targeted and sustained delivery of drugs, proteins, enzymes, and imaging agents.<sup>[30–33]</sup> Dextran hydrogel is one of the increasingly popular healthcare materials, specifically as a wound dressing, because of its prominent performance in stimulating the wound healing process.<sup>[34]</sup> PEG is the most widely investigated synthetic polymer used in hydrogel preparation due to its unique properties, such as high hydrophilicity, nontoxicity, low protein adhesion, and nonimmunogenicity. In addition, both dextran and PEG, have been approved by the United States Food and Drug Administration (FDA) as biocompatible materials for various biomedical applications.<sup>[35–39]</sup>

In the study reported herein, two antibiotics polymyxin B (PMB) and vancomycin (Vanco), were covalently conjugated with 4-arm PEG with a thiol group at each chain end, followed by crosslinking with methacrylate functionalized dextran (MA-Dextran) to form a hydrogel dressing for wound healing, in which PEG acts as a bioinert molecule and resists protein adsorption, thus avoiding secondary damage and infections. PMB is an FDA approved cyclic polypeptide antibiotic against gram-negative bacteria that binds to the lipopolysaccharide of the bacterial outer membrane and ultimately causes cell death by increasing membrane permeation.<sup>[40,41]</sup> However, the clinical use of PMB has been largely limited due to its nephrotoxicity when used in its free molecular form.<sup>[42,43]</sup> Vanco, a glycopeptide antimicrobial drug also approved by the FDA, exhibits antibacterial activity against gram-positive pathogens by specifically binding to the cell wall,<sup>[44]</sup> but its clinical use was controversial due to systemic toxicity and increased bacterial resistance.<sup>[45–47]</sup> The PMB and Vanco conjugation strategy (two-in-one strategy) developed in this work provides a pragmatic and effective alternative to reduce the systemic side effects of these antibiotics by immobilizing them into the hydrogel matrix, while providing the hydrogel with dual-function to kill both gram-positive and gram-negative bacteria simultaneously. Mouse fibroblast cell line NIH 3T3 was used to assess cytotoxicity of the hydrogel and the antibacterial efficiency of the as-prepared hydrogel dressing against both gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) was evaluated via agar diffusion assay and time-kill test. Most notably, the gram-negative and gram-positive targeting antibiotics duality via covalent conjugation can potentially mitigate harmful effects to the normal microbiota in the body, prevents antibiotic resistance, and concurrently, sustains an optimal environment for wound healing. Indeed, this “two-in-one” dual-functional hydrogel forms the basis of a new strategy for generally improving antibacterial hydrogel efficacy and safety in wound healing applications.

## 2. Experimental Section

### 2.1. Materials

Dextran (Mw 40 000), LiCl, triethylamine, methacrylic anhydride, and 3-(maleimido)propionic acid N-hydroxysuccinimide

ester (BMPS) were purchased from Sigma. 4-arm PEG thiol (Mw 5000) was obtained from JenKem Technology. *E. coli* (ATCC 11775), *S. aureus* (ATCC 25923), and mouse fibroblast NIH 3T3 cell line were obtained from ATCC. Cell culture media Dulbecco's minimal essential medium (DMEM) was purchased from GIBCO. All other chemicals were purchased from Sigma-Aldrich and used directly unless otherwise stated.

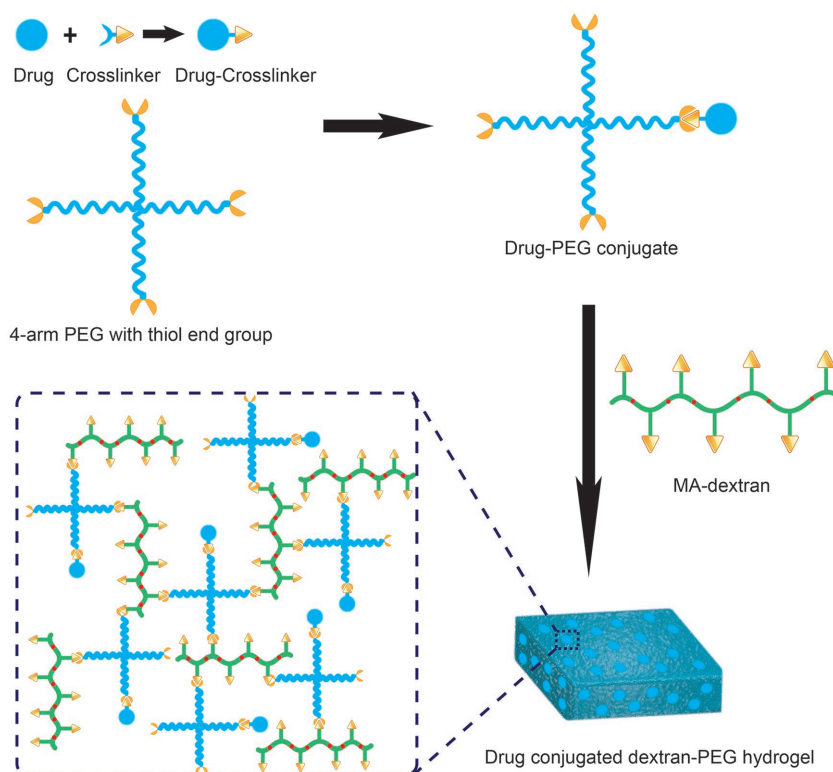
### 2.2. Synthesis and Characterization of Functionalized Dextran

Functionalized dextran (MA-Dextran) was synthesized according to a previous reference,<sup>[48]</sup> as shown in Scheme S1 (Supporting Information). Dextran (1.62 g, 10 mmol repeating unit) and LiCl (1 g, 10 wt % of solvent) were dissolved in anhydride dimethyl formamide (10 mL) at 90 °C under nitrogen atmosphere and stirred till a complete dissolution. After it was cooled down to 70 °C, triethylamine (0.288 mL) was slowly added into the system and stirred for another 15 min. Methacrylic anhydride (1.53 mL, equal eqv. with dextran repeating unit) was then slowly injected into the reaction system and stirred for 14 h at 70 °C protected from light. The functionalized dextran product was precipitated out in cold isopropyl alcohol and washed several times with isopropyl alcohol, then dried at room temperature in vacuum. <sup>1</sup>H NMR and Fourier transform infrared spectroscopy (FTIR) were used to prove the successful introduction of acrylate group. The degree of substitution, which is defined as the number of substituents of the methacrylate group per 100 anhydroglucose (AHG) rings in dextran, was calculated using <sup>1</sup>H NMR (D<sub>2</sub>O as solvent) by comparing the ratio of the areas under the proton peaks of the double bond (at around 6 ppm) and peaks of AHG units (at 3.4–4.2 ppm).

### 2.3. Synthesis and Characterization of Antibiotics Conjugated PEG

The conjugation process of the antibiotic and PEG was shown in Scheme 2. Antibiotic drug (PMB or Vanco, 50 μmol) was dissolved in 3.125 mL phosphate buffer (32 × 10<sup>-3</sup> M, pH 8) and stirred. Crosslinker BMPS (16 mg, 60 μmol) was dissolved in 1.875 mL acetonitrile, added dropwise into antibiotic drug solution, and stirred for 3 h at room temperature to produce the maleimide functionalized drug. Subsequently, 4-arm PEG with thiol groups at each terminal (250 mg, 50 μmol) was added into crosslinker-drug solution and kept for another 3 h to yield the antibiotic conjugated PEG.

The PMB and Vanco conjugation efficiency were characterized by high performance liquid chromatography (HPLC) performed on a Agilent 1200 HPLC system equipped with a C18 column (4.6 × 50 mm, pore size 300 Å, 3 μm particle size) that employed a binary mobile phase formed from 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). The column was eluted with a gradient of 0–60% solvent B over 30 min using a flow rate of 1.0 mL min<sup>-1</sup> at 25 °C. The detection was made at 210 nm. The elution time was 16.3/17.2 min for PMB and 9.9 min for Vanco. Stock solution of PMB or Vanco at a concentration of 1 mg mL<sup>-1</sup> in water was prepared and a series of dilutions were made to get a standard working solution for



**Scheme 1.** Preparation of antibiotics conjugated dextran-PEG hydrogel.

drawing a standard curve. PEG conjugated antibiotics samples were also prepared at  $1 \text{ mg mL}^{-1}$  and the concentrations of free PMB and Vanco were calculated according to the standard curve. The PMB and Vanco conjugation efficiency were defined as (total antibiotic-free antibiotic)/total antibiotic.

#### 2.4. Hydrogel Formation and Characterization

The antibiotics conjugated hydrogel was synthesized by mixing MA-dextran solution and 4-arm PEG solution containing a certain amount of PEG conjugated antibiotics in phosphate buffer saline (PBS) ( $0.1 \times 10^{-3} \text{ M}$ , pH 8), and finally formed as a cylinder with 9 mm of diameter and 2 mm of height. As a typical hydrogel preparation, PEG conjugated antibiotics with 4-arm PEG solution ( $50 \mu\text{L}$ , 10 wt%) was added to MA-dextran solution (50  $\mu\text{L}$ , 10 wt%) to give a certain antibiotics concentration, then gelled at room temperature for 2 h to yield a 10 wt% hydrogel composed of 5 wt% dextran and 5 wt% PEG. The hydrogel samples at final antibiotics concentration of 10, 100, and 1000  $\mu\text{M}$  were abbreviated as HG-10, HG-100, and HG-1000, respectively. Hydrogel without any antibiotics (HG-0) was also prepared as control.

The mechanical property of the hydrogel was evaluated using rheometer (Discovery HR-3 Hybrid Rheometer, TA instruments) with a 500  $\mu\text{m}$  gap. Briefly, MA-dextran and 4-arm PEG solutions were freshly prepared, mixed, and put into the gap quickly and gelled at 25 °C. The gelation time, storage modulus ( $G'$ ) and loss modulus ( $G''$ ) was monitored under a condition of  $1 \text{ rad s}^{-1}$

shear rate and 1% strain. The swelling ratio of the hydrogels was calculated as  $(W_s - W_d)/W_d \times 100\%$ , where  $W_d$  was the weight of the hydrogel after lyophilization and  $W_s$  was the weight of the hydrogel after reaching the swelling equilibrium with PBS at 37 °C overnight.

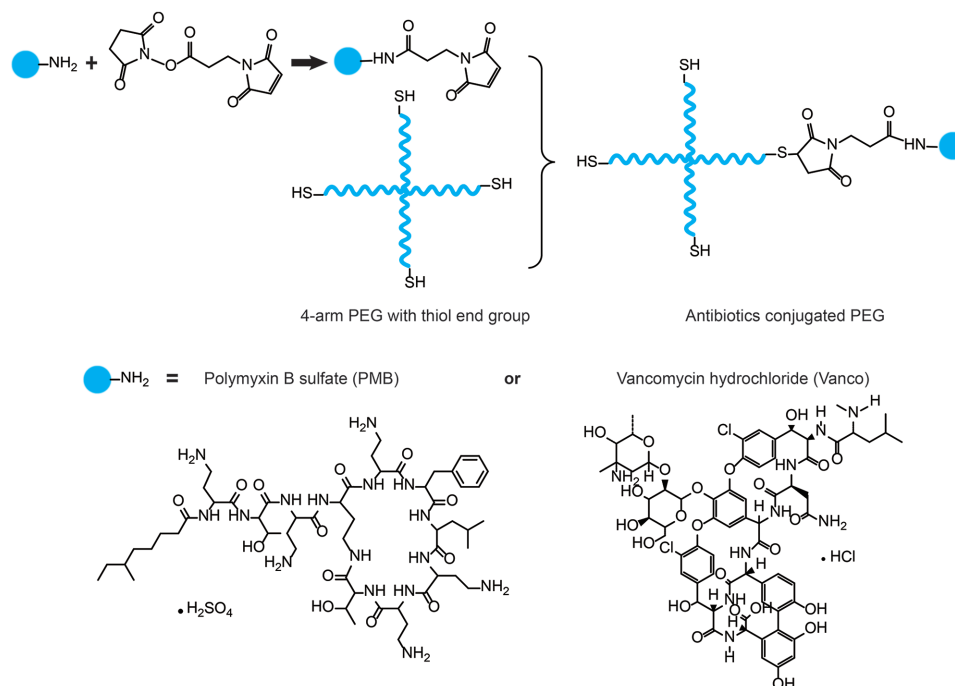
#### 2.5. Antibiotics Release Test

The antibiotics release of drug conjugated hydrogel was investigated by HPLC, using the same conditions as in the characterization of PEG conjugated antibiotics. Hydrogel samples were transferred into glass vials (3 samples per vial) and added with 3 mL PBS ( $10 \times 10^{-3} \text{ M}$ , pH 7.4). The samples were incubated in a shaker at 50 rpm under 37 °C. 100  $\mu\text{L}$  solution was taken periodically, following HPLC characterization and 100  $\mu\text{L}$  of fresh buffer solution was added simultaneously to keep the total volume constant.

#### 2.6. In Vitro Antibacterial Activity Evaluation

The in vitro antibacterial activities of antibiotics conjugated PEG samples were first tested with gram-negative *E. coli* (ATCC 11775) and gram-positive *S. aureus* (ATCC 25923) by agar diffusion assay. Both bacteria were incubated in a liquid Luria–Bertani (LB) broth at 37 °C on a shaker bed at 200 rpm for 12–16 h. The overnight cultured bacteria suspensions were diluted with LB broth for hundred folds and cultured at 37 °C for 2–3 h to achieve logarithmic-phase bacteria suspension, followed by dilution with LB broth to  $10^7 \text{ CFU mL}^{-1}$ . 100  $\mu\text{L}$  of the obtained bacteria suspensions were spread on LB agar plates and incubated for 10 min at room temperature. Cylinder shaped hydrogel samples with 10 mm diameter and 1 mm height were then set onto the bacteria spread petri dishes, followed by 20 h of incubation at 37 °C. The clear zones surrounding the hydrogels were measured to evaluate the antibacterial efficiency of the hydrogel samples.

The antibacterial activities of the antibiotics conjugated dextran-PEG hydrogel were also investigated by time-kill test. All hydrogel samples were washed with 1 mL PBS ( $10 \times 10^{-3} \text{ M}$ , pH 7.4) in a shaker at 50 rpm under 37 °C three times every 20 min to remove nonconjugated antibiotics within hydrogel. *E. coli* and *S. aureus* suspensions with a concentration of  $10^6 \text{ CFU mL}^{-1}$  were obtained by LB broth dilution of logarithmic-phase bacteria culture. 300  $\mu\text{L}$  of the obtained bacteria suspensions was added into 48-well plate with hydrogel, followed by incubation in a shaker at 50 rpm under 37 °C. At a certain time period, 100  $\mu\text{L}$  of the bacterial culture was taken out and added to 96-well plate for  $\text{OD}_{600}$  measurement using Tecan infinite 200 multimode microplate readers. The tested culture was added back for furthering incubation. The antibacterial efficiency percentage was calculated by taking the  $\text{OD}_{600}$  of bacteria without treatment as the control.



**Scheme 2.** Synthesis of antibiotics conjugated PEG.

Live/Dead staining was carried out to characterize the viability of the bacteria seeded on hydrogel. Overnight cultures of *E. coli* and *S. aureus* were diluted to a concentration of  $10^7$  CFU mL<sup>-1</sup>. 20  $\mu$ L of the prepared bacteria suspensions was dropped onto hydrogel surface and incubated at 37 °C for 4 h. 3  $\mu$ L of Live/Dead BacLight (Bacterial Viability Kit; Invitrogen Inc.) was mixed with 1 mL of LB broth and 20  $\mu$ L of the prepared dye solution was added onto the samples. After incubation at 37 °C for 20 min, the samples were rinsed briefly with water in preparation for fluorescence imaging using an IX71 inverted research fluorescence microscope (Olympus) with a 488 nm excitation wavelength at about 488 nm. Fluorescence imaging of bacteria on hydrogel without antibiotics was taken as the control.

The morphology of *E. coli* and *S. aureus* after incubation with the hydrogel was examined by scanning electron microscopy (SEM). Generally, bacteria suspensions with a concentration of  $10^7$  CFU mL<sup>-1</sup> were obtained by LB broth dilution of logarithmic-phase bacteria suspension. 100  $\mu$ L of the prepared bacteria suspensions was added into the hydrogel scaffold dropwise and incubated at 37 °C for 4 h. The samples were then fixed in 4% glutaraldehyde solution for 30 min at room temperature and dehydrated in an ethanol series (30%, 50%, 70%, 90%, 95%, 100% twice) for 10 min each. The dehydrated samples were dried under vacuum overnight and then coated with gold for SEM observation. Bacteria on hydrogel scaffold without antibiotics were also imaged as control.

## 2.7. In Vitro Cytotoxicity Evaluation

Cytotoxicity of the dextran-PEG hydrogel conjugated with different concentrations of antibiotics was evaluated via XTT

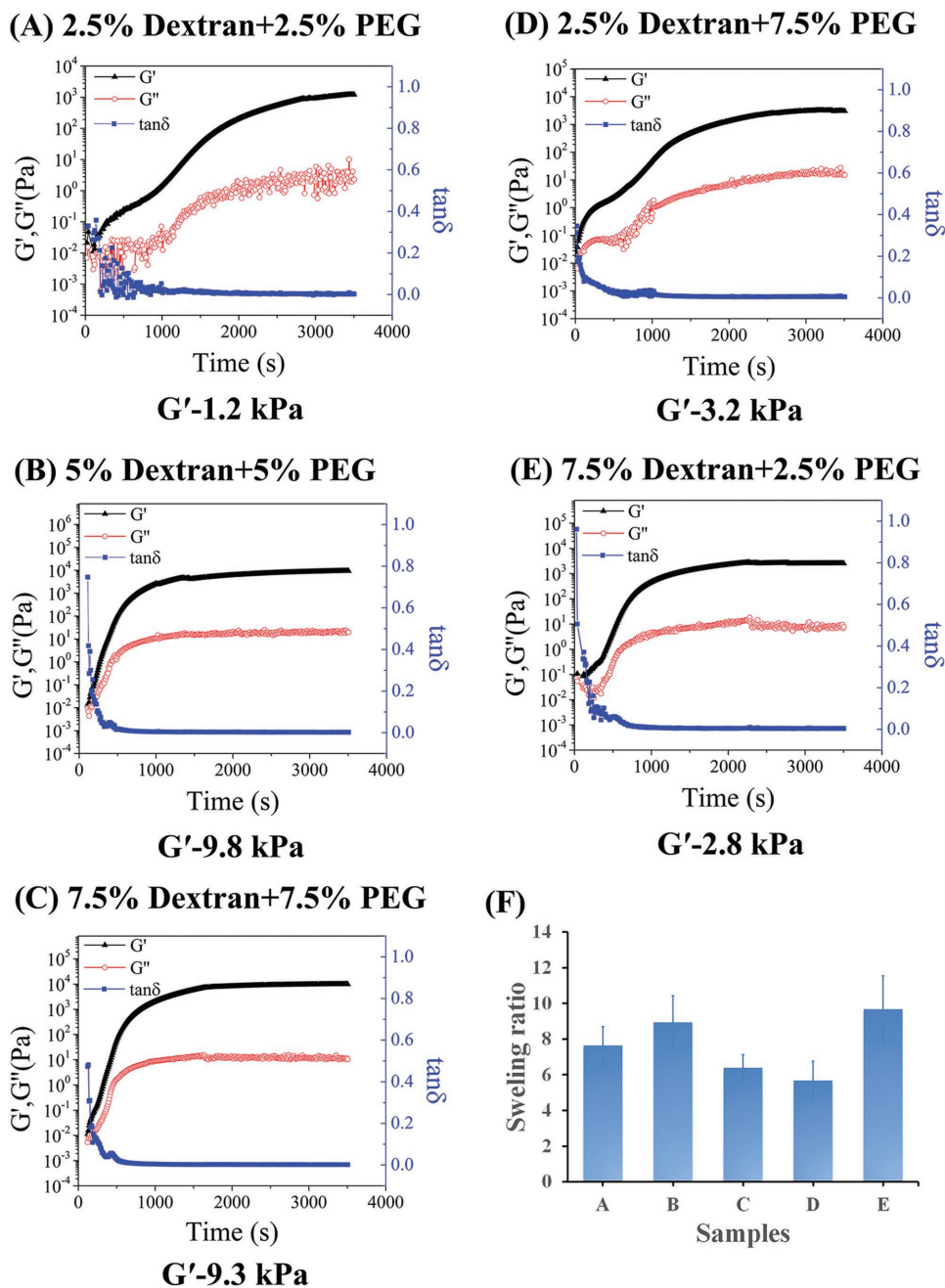
(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell proliferation assay of mouse fibroblast cell line NIH 3T3. Hydrogel without antibiotics conjugation was determined as the control. Specifically, NIH 3T3 cells were seeded in the wells of 48-well cell culture plate and cultured overnight in advance at 37 °C in a 5% (v/v) CO<sub>2</sub> incubator in DMEM supplemented with 10% (v/v) fetal bovine serum and antibiotics (100 U mL<sup>-1</sup> penicillin and 100  $\mu$ g mL<sup>-1</sup> streptomycin). The hydrogel samples were first immersed in 500  $\mu$ L DMEM culture media in another 48-well plate for 1 h then added to well with cultured NIH 3T3 cells layer in 48-well plate for another 1, 2, and 3 days culture (One hydrogel sample in one well). The cell viabilities for each sample were tested by XTT assay (Cell Proliferation Kit II; Roche) according to manufacturer's protocol.

## 3. Results and Discussion

### 3.1. Hydrogel Formation and Characterization

The synthesis of antibiotics conjugated PEG-dextran hydrogel was achieved by crosslinking of methacrylated dextran (MA-dextran) with antibiotics conjugated PEG (Scheme 1). First, functionalizing dextran by reacting it with methacrylic anhydride, provided a double bond in the dextran structure that would facilitate the reaction the thiol group. The acrylation degree can be controlled by changing the molar ratio of dextran unit and methacrylic anhydride. The functionalization of dextran was confirmed by <sup>1</sup>H NMR (Figure S1, Supporting Information) and FTIR (Figure S2, Supporting Information). Antibiotic drug PMB and Vanco were conjugated to crosslinker BMPS respectively by coupling amino and NHS groups, resulting in maleimide group terminated drug (Scheme 2),



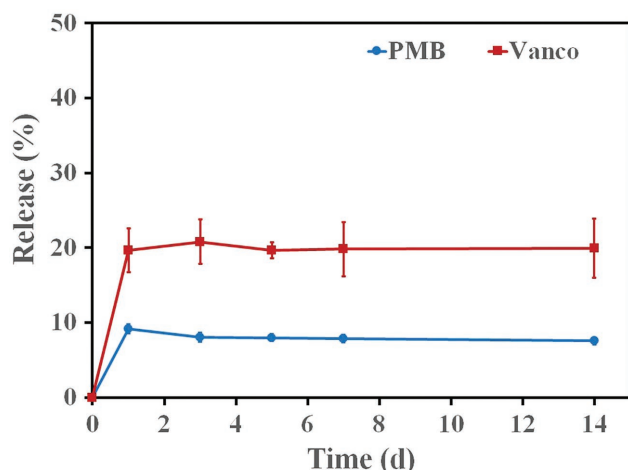


**Figure 1.** A–E) Storage modulus  $G'$  and loss modulus  $G''$  analyses during gelation for hydrogels with different weight ratios (black: storage modulus; red: loss modulus; blue: phase angle) and F) swelling ratio of samples (A)–(E).

which further reacted with the thiol group of 4-arm PEG to generate a drug conjugated PEG (PEG-PMB and PEG-Vanco). Drug conjugation efficiency determined by HPLC showed that 90% PMB and 81% Vanco were conjugated with PEG (10% free PMB and 19% free Vanco). The unreacted thiol groups at the chain end of 4-arm PEG were utilized for crosslinking to the double bond of MA-dextran to form hydrogel.

Rheology analyses were carried out to monitor the gelation process and the mechanical properties of the hydrogel. A series of hydrogels with different wt% MA-dextran and PEG

were prepared. The storage modulus  $G'$  and loss modulus  $G''$  was adjusted by changing the total wt% and weight ratio of two components (Figure 1A–E). The storage modulus  $G'$  value increased from 1.2 to 9.8 kPa when the total wt% of gelators changed from 5% (A) to 10% (B). However, the  $G'$  value exhibited no significant difference as the total wt% increased to 15% (C). When we fixed the total wt% at 10%, the weight ratio of dextran and PEG showed obvious effects on  $G'$  value. Both hydrogels at weight ratio of 1:3 (D) and 3:1 (E) showed lower  $G'$  value than that of 1:1 (B). The swelling ratio of all these samples were



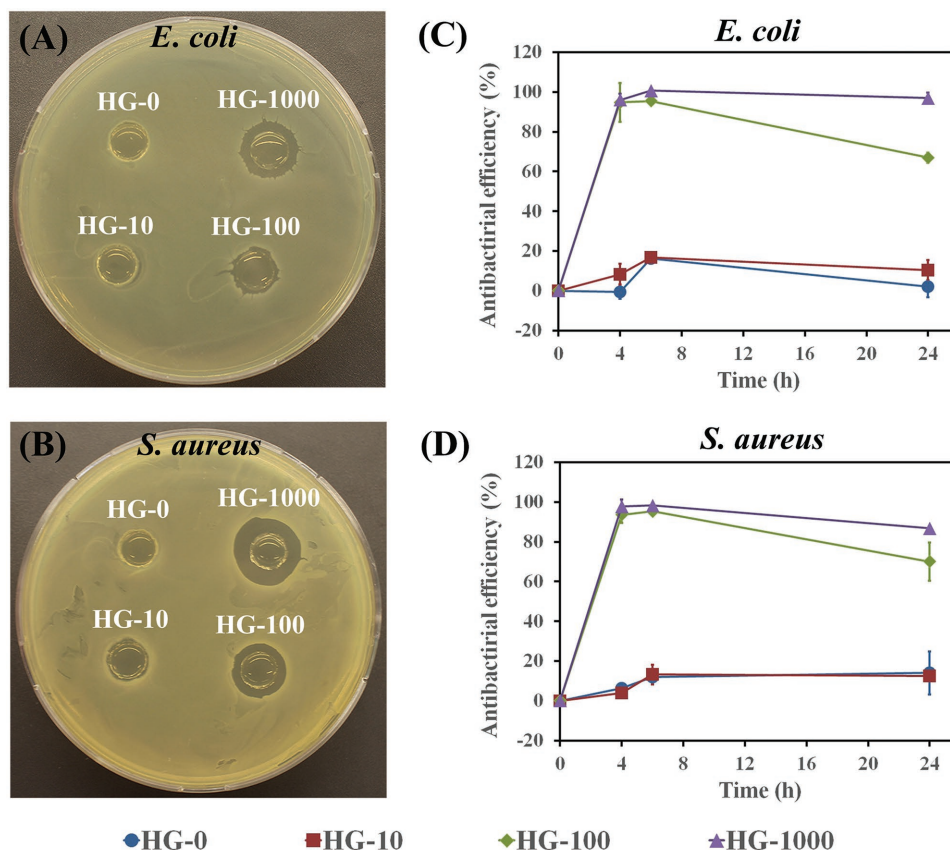
**Figure 2.** Antibiotics release from hydrogel in PBS. Mean  $\pm$  standard deviation and  $n = 3$ .

tested and the results showed that sample B (5% Dextran + 5% PEG) and sample E (7.5% Dextran + 2.5% PEG) gave the higher swelling ratio ( $8.9 \pm 1.5$  for B and  $9.7 \pm 1.9$  for E) than the other samplings (Figure 1F). Therefore, sample B was finally

chosen for the following study for its highest storage modulus  $G'$  and relatively high swelling ratio. Morphology characterization of sample B hydrogel was taken as shown in Figure S3 (Supporting Information).

### 3.2. Antibiotics Release Test

Antibiotics release from HG-1000 was tested in PBS by HPLC. As shown in **Figure 2**, the release of PMB and Vanco from the hydrogel were 9.2% and 19.7% respectively the first day, and the values remained almost unchanged within the first 2 weeks. Together with the conjugation efficiency results showing that 10% PMB and 19% Vanco were nonconjugated within the hydrogel, almost all the nonconjugated antibiotics were released within the first day. We also found that the hydrogels were gradually degraded and totally degraded by the 17th day, indicating the biodegradability of the hydrogels. According to the chemical structure of the hydrogel, the degradation of hydrogel is caused by the hydrolysis of ester bond in the methacrylate group introduced by methacrylate functionalization of dextran. Furthermore, the degradation rate of the hydrogels is much faster in LB broth or cell medium (3 days for total biodegradation) than in PBS.



**Figure 3.** Antibacterial efficiency of the antibiotics conjugated Dextran-PEG hydrogels against A,C) *E. coli* and B,D) *S. aureus* by agar diffusion assay (Left) and time-kill test (Right). Here, a relative efficiency of 100 corresponds to the antibacterial efficiency without hydrogel treatment. Mean  $\pm$  standard deviation,  $n = 3$ .

**Table 1.** Diameter of the clear inhibition zone.

| Samples | <i>E. coli</i>                   | <i>S. aureus</i>                 |
|---------|----------------------------------|----------------------------------|
|         | Diameter of inhibition zone [mm] | Diameter of inhibition zone [mm] |
| HG-100  | 10.68 ± 0.37                     | 13.36 ± 0.88                     |
| HG-1000 | 13.99 ± 0.35                     | 17.46 ± 0.70                     |

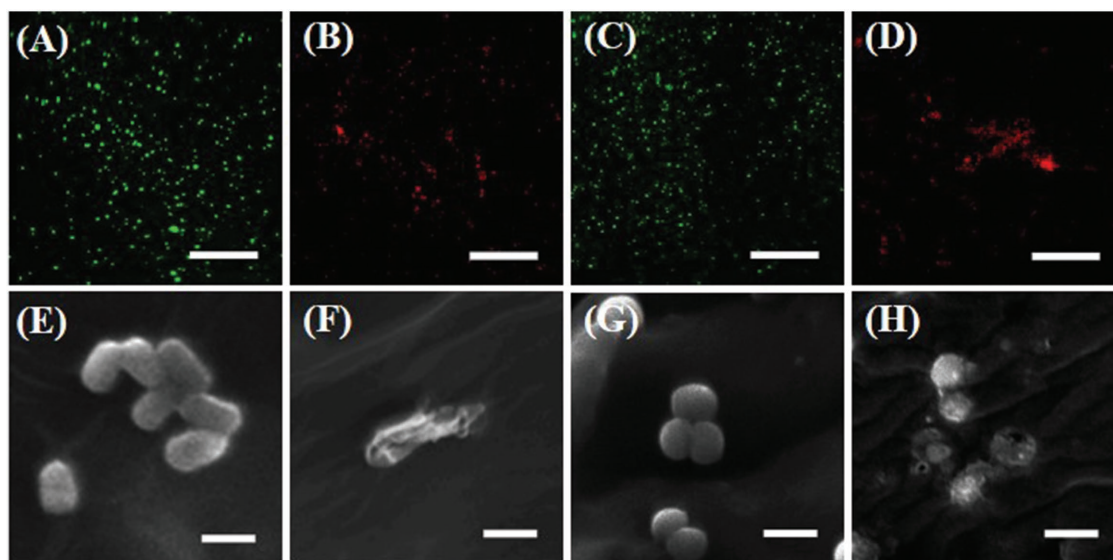
### 3.3. In Vitro Antibacterial Evaluation

The antibacterial activities of the antibiotics conjugated hydrogel against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus* were investigated by agar diffusion assay and time-kill test.

Agar diffusion method was used to evaluate the antibacterial efficiency of the antibiotics conjugated Dextran-PEG hydrogels. As shown in Figure 3A,B, compared with hydrogel without antibiotics (HG-0) and hydrogel with low concentration of antibiotics (HG-10, hydrogel sample at final antibiotics concentration of 10 μm), hydrogel samples HG-100 (hydrogel sample at final antibiotics concentration of 100 μm) and HG-1000 (hydrogel sample at final antibiotics concentration of 1000 μm) showed obvious antibacterial activities against both *E. coli* (A) and *S. aureus* (B). The size of inhibition zone around hydrogel samples HG-100 and HG-1000 was measured as shown in Table 1. The diameter of inhibition zone increased as the concentration of antibiotics increased in the hydrogel.

In order to test the antibacterial efficiency of the antibiotics conjugated hydrogel, we washed the hydrogel with PBS three times every 20 min to remove nonconjugated antibiotics within the hydrogel. A control experiment was designed to ensure all the free antibiotics were released from the hydrogel. After mixing PMB/Vanco with dextran and PEG solution to prepare dextran-PEG hydrogel encapsulated with 1000 μm of PMB and

Vanco noncovalently, the release rate of antibiotics was tested. Almost all antibiotics were released from the hydrogel within the first hour (Figure S4, Supporting Information). Theoretically, we could say that the free PMB and Vanco within the antibiotics conjugated hydrogel could be removed by wash. Hence, the antibacterial activity of the antibiotics conjugated hydrogel after washing was also evaluated by agar diffusion assay (Figure S5, Supporting Information). The results showed that there was no noticeable inhibition zone around the hydrogel after incubation for one day (Figure S5A,B, Supporting Information) or two days (Figure S5C,D, Supporting Information), verifying that no antibiotics were released from the hydrogel to kill bacteria around hydrogel. However, the contact area between hydrogel HG-100/HG-1000 and agar plate was clear after two days incubation. On the contrary, bacterial colonies existed under the HG-0 and HG-10 surface. We further evaluated the antibacterial efficiency of antibiotics conjugated hydrogel after washing by the time-kill test (Figure 3C,D). The results showed that HG-100 and HG-1000 significantly inhibited the growth of *E. coli* and *S. aureus* in the first 6 h. Contrastingly, HG-10 showed little antibacterial activity, indicating that the local concentration of antibiotics conjugated in HG-10 is not sufficiently adequate for efficacious antibacterial performance. Compared with the antibacterial efficiency at 6 h, the antibacterial efficiency at 24 h decreased a little bit (from 100.6 ± 1.2% to 96.9 ± 2.8% for HG-1000 @ *E. coli*; from 98.2 ± 0.1% to 86.8 ± 0.8% for HG-1000 @ *S. aureus*). We think that it is because the conjugated antibiotics were present either within the hydrogel inside or on the surface of hydrogel, however, not in solution, which allowing the bacteria could grow in solution to avoid the contact with hydrogel. We could speculate that the antibacterial efficiency would keep decreasing with time extending. These phenomena were consistent with the previous report.<sup>[27]</sup> Evidenced by these results, conjugated antibiotics still showed antibacterial activity despite removing free antibiotics



**Figure 4.** A–D) Fluorescence imaging and E–H) SEM imaging of *E. coli* and *S. aureus* on hydrogel samples. A,E): *E. coli* on HG-0; B,F): *E. coli* on HG-1000; C,G): *S. aureus* on HG-0; D,H): *S. aureus* on HG-1000. The fluorescence imaging was assessed using the Live/Dead BacLight Bacterial Viability kits (green indicates live cells, red indicates dead cells). Scale bars in fluorescence images, 50 μm; scale bars in SEM images, 500 nm.

after washing, indicating the effectiveness and potency of the antibiotics conjugated hydrogel.

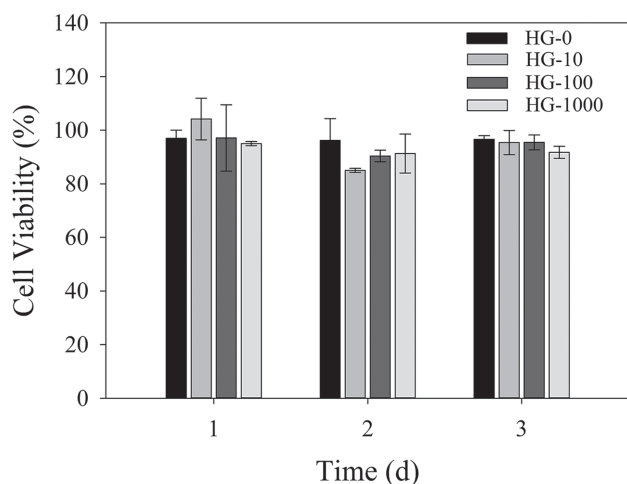
The growth inhibition of bacteria treated with HG-1000 was further observed by fluorescence imaging (Live/Dead staining) and SEM imaging (Figure 4). Almost all the bacteria seeded on HG-1000 were dead (Figure 4A–D). As shown in SEM images (Figure 4E–H), both *E. coli* and *S. aureus* grew well on hydrogel without antibiotics (HG-0) and the shape of cell remained intact. In comparison, bacteria grown on HG-1000 showed damaged cell membrane integrity. It is evident that the treatment of HG-1000 will kill bacteria by damaging cell membrane and protect against potential infections in the field of wound dressing.

### 3.4. In vitro Cytotoxicity Test

As biocompatibility is a critical aspect in the practical application of biomaterials, all materials utilized for the hydrogel synthesis were proven to be biocompatible by XTT test (Figure S6, Supporting Information). Cell viability of antibiotics conjugated dextran-PEG hydrogel in mouse fibroblast cell line NIH 3T3 was also investigated (Figure 5). No obvious cytotoxicity to NIH 3T3 was observed and the antibiotics conjugated hydrogel samples exhibited similar cell viability compared to hydrogels without antibiotics.

## 4. Conclusions

A novel antibacterial dextran-PEG hydrogel was successfully synthesized by the crosslinking of MA-dextran with PMB and Vanco conjugated PEG, exhibiting the proper gel strength and swelling ratio for wound dressing application. The dual-functional hydrogel proved effective against both gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus* and demonstrated exceptional antibacterial activity. More importantly, systemic side effects of these antibiotics can be effectively avoided



**Figure 5.** Cell viability results of antibiotics conjugated Dextran-PEG hydrogel with NIH 3T3 cells by XTT assay. Here, a relative activity of 100 corresponds to the activity of NIH 3T3 cells without hydrogel treatment. Mean  $\pm$  standard deviation and  $n = 3$ .

due to chemical immobilization within the hydrogel, preventing their further release from the gel network. Moreover, by penetrating bacteria cell membrane, the hydrogel samples were able to kill bacteria without presenting cytotoxic effects to mouse fibroblast cell line NIH 3T3. The PMB and Vanco chemical immobilization strategy developed in this work coupled with the biocompatibility of the hydrogel provides an attractive and promising alternative in the field of wound dressing that will successfully accelerate healing, while simultaneously prevent secondary infections.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

antibacterial, biomaterial, hydrogel, wound dressing

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