Nanogel Encapsulated Hydrogels As Advanced Wound Dressings for the Controlled Delivery of Antibiotics

Yanmiao Fan, Mads Lüchow, Yuning Zhang, Jinjian Lin, Lisa Fortuin, Soumitra Mohanty, Annalie Brauner, and Michael Malkoch*

Biocompatible and degradable dual-delivery gel systems based on hyperbranched dendritic–linear–dendritic copolymers (HBDLDs) is herein conceptualized and accomplished via thiol-ene click chemistry. The elasticity of the hydrogels is tunable by varying the lengths of PEG (2, 6, 10 kDa) or the dry weight percentages (20, 30, 40 wt%), and are found to range from 2–14.7 kPa, comparable to human skin. The co-delivery of antibiotics is achieved, where the hydrophilic drug novobiocin sodium salt (NB) is entrapped within the hydrophilic hydrogel, while the hydrophobic antibiotic ciprofloxacin (CIP) is encapsulated within the dendritic nanogels (DNGs) with hydrophobic cores (DNGs-CIP). The DNGs-CIP with drug loading capacity of 2.83 wt% are then physically entrapped within the hybrid hydrogels through UV curing. The hybrid hydrogels enable the quick release of NB and prolonged released of CIP. In vitro cell infection assays showed that the antibiotic-loaded hybrid hydrogels are able to treat bacterial infections with significant bacterial reduction. Hybrid hydrogel band aids are fabricated and exhibited better antibacterial activity compared with commercial antimicrobial band aids. Remarkably, most hydrogels and hybrid hydrogels show enhanced human dermal cell proliferation and could be degraded into non-toxic constituents, showing great promise as wound dressing materials.

1. Introduction

The World Health Organization has listed multidrug resistant pathogens as one of the top three threats to global public health. Since the commercialization of antibiotics in the 1950s, the overuse and misuse by the general population has increased the prevalence of antibiotic resistance. The pace of antibiotic discovery has slowed down while the number of resistant pathogens is on the rise. Nanocarriers and networks, however, are a sophisticated means of overcoming this challenge because older antibiotics can effectively be “repackaged” to have stealth properties, multi-functional features and controllable release profiles. Hydrogels, fabricated from natural or synthetic polymers, are an intriguing class of materials, ideally suited for biomedical applications owing to their unique ability to form self-supporting, 3D viscoelastic networks. The unique physicochemical properties of hydrogels have driven their use in various biomedical applications as engineered scaffolds, in medical diagnostics and therapeutics, as drug delivery systems, hygiene products, and wound dressings. As antimicrobials, hydrogels can either be loaded with antimicrobial agents or exhibit inherent antimicrobial activity. There are, however, several limitations that exist for the clinical use of hydrogels. One such is the low tensile strength, which limits their use in load-bearing applications, another is the quantity and homogeneity of drug loading into hydrogels particularly with regard to hydrophobic drugs due to the functional groups of hydrogels which are largely hydrophilic.

Innovative solutions are needed to overcome these limitations. Hyperbranched polymers with 3D dendritic architectures have attracted much attention during the last decade owing to their unique physicochemical-enhancing properties within nanomaterials, as well as the possibility for mass production through straightforward approaches. An important step in the development of dendritic structures was reported by Aida et al. in the hybridization of linear and dendritic polymers to be used as binders in hydrogels, where cationic dendritic–linear–dendritic hybrids based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) dendrons and linear polyethylene glycol (PEG) chains were constructed through non-covalent interactions. This technique was simplified in an elegant approach by our group, wherein allyl-functional hyperbranched dendritic–linear–dendritic copolymers (HBDLDs) were synthesized and hyperbranched dendritic hydrogels were prepared via thiol-ene chemistry...
(TEC) using organic solvents. Based on allyl-functional HBDDLs, dendritic nanogels (DNGs) with tunable core properties were developed in an aqueous system through TEC, and showed promise as cancer drug carriers. DNGs were also used to encapsulate antimicrobial peptides (AMPs), and the nanocarriers decreased the toxicity of AMPs towards healthy erythrocytes.

Currently, most hydrogel drug delivery systems have been developed for the delivery of a single drug, but the situation is much more complex clinically and multiple drugs or bioactive substances functioning together have been found to elicit optimum treatment responses. Combination therapy has emerged as a tool to reduce the probability of bacterial drug-resistant strains, and served to effectively eradicate the bacteria. The co-delivery of two antibiotics is used to benefit from the different modes of action, clearance times and mitigate the toxicity of certain drugs. In this regard, a dual drug delivery system was developed by Zhong et al. to load and release two drugs with different hydrophobicities. The hydrophobic drug was loaded within poly(ε-caprolactone) (PDLLA) microspheres which were then embedded within calcium alginate hydrogel beads, where the drug release rate was controlled by modifying the ratio of PDLLA and alginate. In another study, two drugs were introduced into different layers of biocompatible double-membrane hydrogels based on cationic cellulose nanocrystals and anionic alginate, achieving the synergistic release of the complexing drugs. Additionally, Alvarez et al. developed silica nanoparticle-collagen composite hydrogels for the co-delivery of two antibiotics. These antibiotics were encapsulated within the silica nanoparticles in a single step and the composite hydrogels were found to prolong the release of the drugs.

Herein, an advanced hybrid hydrogel system with tunable mechanical properties comparable to human skin was developed as a dual antibiotic delivery wound dressing. The incorporation of DNGs within the pristine hydrogels enhanced the mechanical properties of the hybrid hydrogels. The hydrophobic core of DNGs could be used to load the hydrophobic antibiotic ciprofloxacin (CIP), referred to as DNGs-CIP, and DNGs-CIP were embedded within the hybrid hydrogels. This aided in the homogenous distribution of CIP within the hybrid hydrogel network, thus overcoming one of the principal limitations of hydrogels for encapsulating hydrophobic drugs. Following this, the hydrophilic antibiotic NB was introduced via diffusion. The proposed drug delivery system therefore allows for the co-delivery of antibiotics with different hydrophobicities. In vitro cell infection assays were also conducted using human dermal fibroblast (hDF) and human keratinocyte (HaCaT) cell lines in order to verify that the hybrid hydrogel was able to deliver antibiotics to the infected cells. The precursor solutions of the hybrid hydrogels are transparent and are able to be drop cast or injected into any pre-defined shape. Notably, most of the pristine hydrogels and hybrid hydrogels showed enhanced human dermal cell growth ability with cell viability above 100%. The tunable elastic properties and excellent biocompatibility of the hybrid hydrogels led to their application as antibacterial band aids, where it was found to be superior to commercially available antimicrobial band aids.

2. Results and Discussion

2.1. Materials Synthesis and Hybrid Hydrogels Formation

The synthesis of HBDDLs, subsequent formation of DNGs and the formation of the hybrid hydrogels are shown in Scheme 1. Hydroxy-terminated HBDDLs with third generation (G3) dendritic constituents were synthesized through the conventional pseudo polycondensation reaction between bis-MPA and hydroxy-terminated PEG of varying chain lengths, using p-TSA as the catalyst. Following this, hydroxy-terminated HBDDLs were modified with allyl groups through their reaction with anhydride-activated 4-pentenoic acid (PEG2K-G3-allyl, PEG6K-G3-allyl, PEG10K-G3-allyl, PEG20K-G3-allyl, Supporting Information). 1H and 13C NMR spectroscopy as well as SEC (Figures S1 and S2, Supporting Information) were used to confirm that the polymerization and post-functionalization were efficient and of high quality (see details in Supporting Information). As can be seen from Scheme 1b, the amphiphilic property of PEG20K-G3-allyl allows for the spontaneous self-assembly into micelles through solvophobic forces in aqueous solution, and DNGs are formed by crosslinking the micelles via TEC using trimethylolpropane tris(3-mercaptopropionate) as crosslinker, where 5 out of 16 allyl groups per molecule were used for the crosslinking. Post-functionalization was achieved by reacting the remaining 11 allyl groups per molecule with the hydrophobic 1-hexanethiol, introducing a hydrophobic core within the DNGs. The hydrophobic antibiotic CIP was physically entrapped within the DNGs (DNGs-CIP) (Scheme 1b) largely through hydrophobic interactions and hydrogen bonding. The drug loading capacity (DLC) of DNGs was 2.83 wt% as determined by UV-vis spectroscopy, which is higher than poly(lactic-co-glycolic acid) (PLGA) nanoparticles for CIP loading achieving a maximum drug loading capacity of 2.42%. The formation of pristine hydrogels took place in aqueous solution via TEC using allyl-functional HBDDLs (PEG2K-G3-allyl, PEG6K-G3-allyl, PEG10K-G3-allyl), water soluble lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the photoinitiator and thiol-terminated PEG (PEG2K-SH) as the crosslinker with UV curing ($\lambda = 356$ nm) for 10 min. To form the active hybrid hydrogels, DNGs-CIP were dispersed in the aqueous medium containing all of the components before UV curing (Scheme 1c). The concentration of DNGs-CIP in the precursor solution was 1.25 mg mL$^{-1}$. NB is UV sensitive, and was therefore loaded into the hydrogels through diffusion. The final concentrations of CIP and NB were 35.36 and 500 µg mL$^{-1}$, respectively. Those concentrations were chosen because they are below safe dosages and the concentrations were kept constant in the study to accurately assess the release profiles in different hybrid hydrogel systems. Moreover, it is highly advantageous that these processes occur in water, allowing for green chemistry practices and the avoidance of harmful solvents.

2.2. Characterization of DNGs, Pristine Hydrogels, and Hybrid Hydrogels

To achieve optimal performance, wound dressings should have ideal mechanical properties, such as stretchability, high
compression and recovery properties, as well as comparable moduli to soft human tissues\(^{[41]}\). Unlike many hydrogels that are fragile and have low mechanical strength\(^{[23,42]}\), the hydrogels described in this work have tunable mechanical properties. The precursor solutions could be drop cast or injected into different molds, visibly observed as thin film hydrogels (Figure 1a) and cylindrical hydrogels (Figure 1b) that are highly flexible. The hydrogels formed from PEG2K-G3-allyl (H2K) are more opaque than the hydrogels formed from PEG6K-G3-allyl (H6K) and PEG10K-G3-allyl (H10K) due to the limited water solubility of PEG2K-G3-allyl with shorter hydrophilic PEG chains. Noteworthy is that the hydrogels were able to bear a considerable amount of force without breaking, and were able to rebound to their initial shapes (Videos S1 and S2, Supporting Information), demonstrating ideal mechanical properties in a sensory capacity.

The elasticity and swelling ratios of the pristine hydrogels were tested so that the best archetype could be applied as band aid materials. In terms of elasticity, the storage moduli of H2K, H6K, and H10K containing different dry weight contents (20, 30, and 40 wt%) were measured in their swollen state in deionized water (DI water), where it was found that the elasticity of the pristine hydrogels ranged from 2–14.7 kPa (Figure 1c). For all hydrogels, the greater the dry weight content and the shorter...
the length of PEG, the greater the elasticity. This is demonstrated by H2K with a dry weight content of 40 wt%, which showed the highest elasticity of 14.7 kPa. For H10K, there is an increase in elasticity from 2 kPa (H10K, 20 wt%) to 7.5 kPa (H10K, 40 wt%), which were highly comparable to soft tissues such as human skin, between 4.5–8 kPa,[43,44] so H10K was used to prepare DNGs embedded hybrid hydrogels. After the entrapment of DNGs within H10K, referred to as H10K-DNGs, there is an increase in the storage moduli compared with the pristine hydrogels, which is likely due to the interaction between the dendritic nanogel network and the pristine hydrogel networks.[45]

For the swelling ratios (Figure 1d), the hydrogels with 40 wt% dry weight content exhibited higher swelling ratios over the range of PEG lengths compared to the hydrogels with 20 and 30 wt% dry weight percentages, with the lowest swelling ratio of 2.75 obtained from H2K (20 wt%) (Figure 1d). Considering the length of the PEG chains, the hydrogels with longer chains showed much higher swelling ratios than those from shorter PEG chains, with swelling ratios of 6.85, 5.22, and 3.28 obtained from the 40 wt% hydrogels of H10K, H6K, and H2K, respectively. In general, the swelling ratios correlate to the density of the hydrogel network and the length of the PEG chain, as more hydrophilic functional groups and longer hydrophilic PEG chains can bind with more water molecules within the hydrogel system. Hydrogels formed from PEG10K-G3-allyl exhibited the greatest swelling potential, which is an indication of their ability to absorb more secretions from the wound or the infection site, whilst maintaining adequate elasticity comparable to the elastic modulus range of human skin.[44] This further confirmed that PEG10K-G3-allyl was an ideal candidate to form the hybrid hydrogels for wound dressings.

The DNGs were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM). From DLS data, the average hydrodynamic size of the dendritic component of DNGs is 170 ± 1.51 nm (Figure 1g), with a dispersity index of 0.13, and the average size obtained from SEM images is ~113 nm (Figure 1e). The average diameter of DNGs obtained from DLS is greater than that measured from SEM, likely because DLS measures the hydrodynamic diameter of DNGs free to relax their polymer chains in the aqueous state, whilst SEM measures DNGs in a dried, compact state. In order to visualize the DNGs within the hybrid hydrogel, H10K-DNGs with 40 wt% dry weight content was freeze dried for SEM analysis. DNGs were found to be distributed evenly within the hybrid hydrogel network (Figure 1f), which aided in the homogeneous distribution of CIP within the hybrid hydrogel network. SEM images of H10K-DNGs with different dry weight contents exhibited micro-porous structures (Figure S3, Supporting Information) that would be beneficial for wound healing.[46]

The degradation properties of wound dressing materials is important as it is essential to meet the requirements of skin in remodeling and inducing morphogenesis to form new tissues.[47,48] The dendritic component of the allyl-functional HBDLDs contains ester bonds, and readily undergo hydrolytic degradation to non-toxic constituents. The degradation study of H10K with different dry weight contents was conducted in phosphate buffered saline (PBS) pH 7.4 at 37 °C (Figure S4), where it was found that the hydrogels with the lower dry weight content of 20 wt% degraded faster (reaching half its original mass in 17 days) compared to the hydrogels with higher dry weight content of 40 wt% (reaching half its original mass in 21 days). The degradation rate of the hybrid hydrogels can be adjusted by varying the dry weight contents to meet the requirement of
short-term or long-term wounds in terms of severity, thereby providing an optimal environment for wound healing as well as limiting the incidence of secondary infections. The degradability of the hybrid hydrogels is important as it creates space for the skin tissue to regenerate during the wound healing process.

2.3. Biocompatibility of the Pristine Hydrogels, Hybrid Hydrogels and Their Constituents

As wound dressing materials, minimal cytotoxicity should occur. To assess this, the cytotoxicity of the hydrogels and their constituents were evaluated in vitro using human dermal fibroblast (hDF) and mouse monocyte (Raw 264.7) cells. No obvious cytotoxicity was found for any of the constituents towards both Raw 264.7 (Figure 2a, cell viability above 82%) and hDF (Figure 2b, cell viability above 87%) cells, even at the high concentration of 1000 µg mL⁻¹. Notably, the copolymer of PEG10K-G3-allyl improved the cell proliferation at the incubation concentration of 1000 µg mL⁻¹, with cell viability values reaching 105% for Raw 264.7 cells and 107% for hDF cells, thus causing enhanced cell proliferation. DNGs incubated with both cell lines also caused cell viability values to reach >100% at 1000 µg mL⁻¹. As for the pristine hydrogels and hybrid hydrogels, most exhibited good biocompatibility towards both Raw 264.7 (Figure 2c, cell viability above 80%) and hDF (Figure 2d, cell viability above 100%) cells. Notably, H10K, H2K, and H10K-DNGs substantially enhanced hDF growth, causing the highest cell viability of 139% by H2K with the 20 wt% dry weight content (Figure 2d). Antibiotic-loaded H10K-DNGs also showed good biocompatibility towards both hDF (Figure S5, Supporting Information, cell viability above 90%) and Raw 264.7 (Figure S5, Supporting Information, cell viability above 71%), suggesting that the indicated concentration of antibiotics loaded within H10K-DNGs is safe to use. H10K-DNGsA did not impose any cytotoxicity towards Hacat (Figure S9, Supporting Information) with cell viability above 100%. Considering the enhanced proliferation of human skin cells, these materials show great promise as wound dressings for skin wounds.

2.4. Antimicrobial Study of Hybrid Hydrogels As Dual Drug Delivery System

2.4.1. The Disk Diffusion Test

The disk diffusion test was used to assess the hybrid hydrogels as an effective drug delivery system. The antibiotics were loaded within the cylinder-shaped hybrid hydrogels, formed from 50 µL precursor solution of H10K-DNGs containing final concentrations of 35.36 µg mL⁻¹ CIP and 500 µg mL⁻¹ NB (referred to as H10K-DNGsA). Staphylococcus aureus 2569 (S. aureus 2569) and Escherichia coli 208 (E. coli 208), as typical representatives of gram-positive and gram-negative bacteria, were used in the assay. Pristine DNGs and allyl-functional HBDLDs exhibited no obvious antibacterial activity as the minimum inhibitory concentration (MIC) values were above 1.25 mg mL⁻¹ (Table S1, Supporting Information), and H10K-DNGs showed no antibacterial activity as no inhibition zone is seen on the agar plates (Figure S6, Supporting Information) indicating that the building blocks exhibit no antibacterial activity. H10K-DNGs loaded with both CIP and NB showed larger inhibition zones

![Figure 2. AlamarBlue assay for the cytotoxicity evaluation of a) the component polymers on Raw 264.7 and b) hDF for 72 h. c) The hydrogels and hybrid hydrogels on c) Raw 264.7 and d) hDF for 72 h.](attachment:image)
than 10K-DNGs loaded with a single antibiotic towards both S. aureus 2569 and E. coli 208 (Figure 3 and Figure S7, Supporting Information), indicating that the dual drug delivery system is more efficient at killing bacteria than a single loaded drug delivery system due to both antibiotics functioning together to enhance the antibacterial activity. For 10K-DNGs containing NB only, the diameters of the inhibition zones against E. coli 208 (Figure 3b and Figure S7, Supporting Information) and S. aureus 2569 (Figure 3a and Figure S7, Supporting Information) were 0.73 and 2.57 cm, respectively, regardless of the dry weight percentages, suggesting that the hydrophilic drug NB was released freely from the hydrogels. Moreover, the agar plate is a hydrophilic gel system that would have a higher affinity for hydrophilic compounds such as NB, allowing diffusion through the gel network to kill bacteria rapidly. Conversely, 10K-DNGs containing CIP alone had different diameters of inhibition zones which were inversely proportional to the dry weight contents (Figure 3 and Figure S7, Supporting Information). The 40 wt% H10K-DNGsA led to smaller inhibition zones compared with the 20 and 30 wt% H10K-DNGsA against both bacterial strains, regardless of those hybrid hydrogels containing the same amount of CIP. This is likely due to the 40 wt% H10K-DNGsA consisting of more densely crosslinked hydrophilic networks, so the movement of the hydrophobic CIP within the network was prolonged. The encapsulation of CIP within DNGs also prolonged the release of CIP. The different sizes of the inhibition zones indicated that there was CIP entrapped within the hybrid hydrogels. In order to investigate if the degree of the swollen state of H10K-DNGs affected the release of CIP, the 40 wt% H10K-DNGs only containing CIP were allowed to swell in PBS to varying degrees (0, 40, and 80 µL), and the hybrid hydrogels that absorbed 40 and 80 µL of PBS had average inhibition zones of 1.03 cm (Figure S8b, Supporting Information) and 1.27 cm (Figure S8c, Supporting Information) against E. coli, respectively, while the average diameter of the inhibition zone without any treatment is 0.83 cm (Figure S8a, Supporting Information), indicating that the swelling process of the hybrid hydrogels could increase the amount of CIP released from the

Figure 3. Diameters of inhibition zones of the antibiotic-loaded hydrogels and hybrid hydrogels using the disk diffusion test with bacterial concentration of 10⁷ CFU mL⁻¹ towards a) S. aureus 2569 and b) E. coli 208. c) In vitro release of CIP and d) NB from H10K-DNGsA in PBS (pH 7.4) at 37 °C for 72 h.
hybrid hydrogels. The procedure mimicked the medical setting of the wound, where NB is released freely and quickly from the hybrid hydrogels to combat the infection at the early stage of the wound, and after the hybrid hydrogels absorb the secretions of the wound, more CIP would be released in a controlled manner to kill the bacteria at the late stage of the wound. The 40 wt% H10K-DNGsA exhibited the highest swelling ratio, indicating its capability to absorb more wound secretions.

2.4.2. In Vitro Release

The in vitro release profiles of CIP (Figure 3c) and NB (Figure 3d) were also explored in PBS (pH 7.4) at 37 °C. The release of NB was relatively fast, and more than 87% of NB was released within 4 h regardless of the dry weight contents, which is consistent with the disk diffusion test (Figure S7, Supporting Information). NB was freely released due to the high swelling ratio of H10K for the hydrophilic NB to release freely within the loose hydrophilic hydrogel network. The release of CIP was prolonged due to its hydrophobicity and was regulated by varying the cross-linking density of the hybrid hydrogels. 40 wt% H10K-DNGsA showed slowest release rate, and reached its maximum cumulative release after 48 h, while it took 36 h for 20 wt% H10K-DNGsA and 30 wt% H10K-DNGsA. This is mainly because 40 wt% H10K-DNGsA has the most densely crosslinked network that prolonged the release of CIP. The quick release of NB and controlled release of CIP were highly effective in killing bacteria in different timescales to reduce the development of drug-resistant strains.

2.4.3. In Vitro Cell Infection Assay

Hacat cells are primarily located in the surface layer in the epidermis, while hDF are located in the deeper layer in the dermis of the skin. To mimic the infection scenario, Hacat and hDF were chosen to build the in vitro infection models using *S. aureus* 2569. The cells were either infected for 2 h before applying H10K-DNGsA (both antibiotic-loaded) and H10K-DNGs (antibiotic free), or infected at the same time of adding the materials. H10K-DNGsA caused a pronounced reduction in the bacterial number, 99% for Hacat (Figure 4a) and 97% for hDF (Figure 4b), when bacteria and H10K-DNGsA were applied at the same time. The antibacterial activity of H10K-DNGsA caused a similar reduction of 98% (Figure 4a) and 89% (Figure 4b) for Hacat and hDF, respectively, even in the case that the cells were infected with *S. aureus* for 2 h before applying the materials. These biocompatible and degradable hybrid hydrogels are therefore promising as multidrug delivery systems to meet the requirements of a complex clinical milieu.

The advantages of the hybrid hydrogels make them forerunners as wound dressing materials, some including i) that the precursor solution is manageable and transparent, able to be drop cast or injected into any pre-defined shape, ii) the high functionality of the system allowing for tailored outcomes, iii) the ability to form hybrid hydrogels with excellent flexibility and tunable mechanical properties via facile procedures, iv) the easy handling and storage of the materials, v) excellent biocompatibility and enhanced human dermal cell growth, vi) the ability to degrade into non-toxic constituents, vii) the assembly of DNGs and the formation of the hybrid hydrogels using green chemistry to ultimately to deliver multiple drugs with different hydrophobicities.

Taking advantage of the impressive flexibility of the hybrid hydrogels, the precursor solution of the hybrid hydrogels (H10K-DNGs, 30 wt%) was used to fabricate antibacterial band aids (Figure 5a) and antibacterial bandages (Figure 5b). The precursor solution could penetrate through the polyester medical bandage, and a more stable and flexible antimicrobial bandage was achieved upon UV curing (Figure 5b). The final weight of the treated medical bandage increased ×7.2 of the original bandage weight. The facile procedure was applied to fabricate antimicrobial band aids (Figure 5a), and shape molding was easily achieved (Figure 5c) due to the viscosity and easy handling of the precursor solution.

The antibacterial activity of the hybrid hydrogel (H10K-DNGs, 30 wt%) treated band aid (HHBA) was evaluated using the disk diffusion test and was compared with commercially available
“normal” band aids (NBA) and silver-containing antimicrobial band aids (AgBA). All of the band aids were cut into circles ($\varnothing = 0.9$ cm) and were assessed using a bacterial concentration of $10^7$ CFU mL$^{-1}$. The HHBA displayed obvious inhibition zones against both *S. aureus* 2569 ($\varnothing = 2.56$ cm, Figure 6a) and *E. coli* 208 ($\varnothing = 1.13$ cm, Figure 6b). The AgBA, however, showed no obvious inhibition zone against both *S. aureus* 2569 (Figure 6c) and *E. coli* 208 (Figure 6d), except that the bacterial density below the band aid decreased compared with the bacterial density surrounding the band aid, which indicates that the AgBA would have some, but limited effect in the treatment of severe infections caused by high concentrations of bacteria. The NBA showed no antibacterial activity (Figure 6e,f), indicating that the NBA is not able to treat bacterial infections. The HHBA are superior to commercial band aids in that appreciable antibacterial activity was found at high bacterial concentrations. Unlike traditional band aids made from hard materials, the HHBA is soft and pliable, which would provide comfortable contact to the skin, and maintain a moist environment able to absorb secretions from the wound.

Figure 5. Facile procedures for a) fabricating antibacterial band aids and b) fabricating flexible antibacterial bandage using the precursor solution. c) Molding of precursor solution with pre-defined shape.

Figure 6. The disk diffusion test with bacterial concentration of $10^7$ CFU mL$^{-1}$ a) HHBA against *S. aureus* 2569. b) HHBA against *E. coli* 208. c) AgBA against *S. aureus* 2569. d) AgBA against *E. coli* 208. e) NBA against *S. aureus* 2569. f) NBA against *E. coli* 208.
3. Conclusions

In summary, a series of novel biocompatible and degradable dendritic hybrid hydrogels were developed using facile TEC chemistry for the co-delivery of hydrophobic and hydrophilic antibiotics. The hydrophobic antibiotic CIP was incorporated within the hybrid hydrogels using DNGs bearing hydrophobic cores, and were later homogeneously embedded within the hybrid hydrogels, thereby overcoming one of the principal limitations of hydrogels which is the encapsulation of hydrophobic drugs. The hydrophilic antibiotic NB was introduced within the hybrid hydrogel networks via diffusion to achieve the co-loading of antibiotics. The pristine hydrogels and hybrid hydrogels possess flexible and tunable mechanical properties with elasticities in the range of 2–14.7 kPa in their swollen state, that meet the high flexural requirements of soft tissues. Notably, some of the hydrogels and polymers promoted the proliferation of human dermal cells in vitro and could be degraded into nontoxic substituents, indicating their excellent biocompatibility and great potential as wound dressing materials. The in vitro cell infection assay showed that the antibiotic-loaded hybrid hydrogels were able to treat bacterial infections towards both Hacat and hDF cells, causing a significant decrease in bacteria after applying the materials. The transparent, and workable precursor solutions of the hybrid hydrogels make drop casting and injection into any pre-defined shape possible, and able to be absorbed by the medical bandage to make superior antibacterial band aids. The hybrid hydrogels formed band aids that are softer and more pliable compared with commercial band aids, and would be much more effective in providing a moist environment to accelerate wound healing. The results suggest that these biocompatible and degradable hybrid hydrogels are promising drug delivery carriers that can be applied as cutting-edge wound dressings to prevent local bacterial infections.

4. Experimental Section

Materials Synthesis and Characterization: Synthesis and characterization details for the materials can be found in the Supporting Information.

Synthesis Dendritic Nanogels: The synthesis of synthesis dendritic nanogels (DNGs) was conducted using the method reported previously.[29] Briefly, PEG20K-G3-Allyl (200 mg, 8.75 μmol), trimethylpropane tris-(3-mercaptopropionate) (TMP-SH) (5.79 mg, 14.5 μmol) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) (20.6 mg, 168.8 μmol) were dissolved in DCM (4 mL) in a round bottom flask. The solvent was slowly removed under reduced pressure to form a thin film. Milli-Q water (40 mL) was added to the flask and it was vortexed for 1 min before being transferred to an ultrason bath for ultrasonication (15 min, 35 kHz). The resulting colloidal dispersion was exposed to UV light with constant stirring for 60 min (1526 J mm−2) (UVP Blak-Ray bench-top lamp 665 nm, 230 V, 50 Hz). 1-Hexanethiol (56.7 mg, 480 μmol) and DMPA (20.6 mg, 168.8 μmol) were then dissolved in THF (2 mL) and this was added to the colloidal dispersion. The sample was vortexed for 1 min and dispersed with ultrasonication (15 min, 35 kHz). The same UV dosage was applied and the liquid was then filtered through a filter (pore size 4) and poured into a dialysis membrane (MWCO 10 kDa) and dialyzed against water for 3 days. The resulting slightly opaque liquid was freeze dried and a fluffy while solid was obtained.

CIP Loading in DNGs: DNGs were dissolved in DI water (5 mg mL−1, 9 mL) in a glass flask and CIP (9 mg, 27.2 μmol) was added. The mixture was vortexed for 1 min, and transferred to a shaking table for mixing overnight. The mixture was filtered and transferred into a dialysis bag (MWCO 12–14 kDa) immersed in DI water (4 L) for 1 h (water changed every 15 min) to remove free and loosely attached CIP. The CIP concentration was determined via fluorescence with an excitation wavelength of 278 nm and an emission wavelength of 455 nm using the plate reader of infinite M200 PRO. Drug loading capacity was calculated using Equation (1):

\[
\text{Drug loading capacity (DLC)} = \frac{m_{\text{drug}}}{m_{\text{DNGs}}} \times 100\% \tag{1}
\]

Formation of Pristine Hydrogels and Antibiotic-Loaded Hybrid Hydrogels: TEC chemistry was used for the formation of the hydrogels. PEG-G3-allyl and 2K-PEG-SH (molar ratio = 1:8) were dissolved in DI water to form a series of concentrations (20, 30, and 40 wt%). To form the hydrogels containing DNGs-CIP, the diluted DNGs-CIP solution was added to the hydrogel precursor solution and was fully mixed. The initiator LAP was then added and the flask was vortexed for 1 min. The flask was kept at room temperature until the bubbles in the precursor solution disappeared, and the dispersion was UV cured for 10 min. The hydrophilic antibiotic NB is UV sensitive, so it was introduced within the hydrogel network by diffusion. The cylindrical hydrogels were transferred to vials containing 50 μL NB solution (0.5 mg mL−1), and were allowed to absorb the NB solution until reaching equilibrium after 6 h.

Rheology: The viscoelastic properties of the hydrogels were measured using a TA rheometer (DHR-2) equipped with a Peltier plate accessory using a stainless-steel upper geometry (Ω = 8 mm). The hydrogels were prepared and allowed to swell overnight in DI water before testing. The measurement was conducted in DI water at 25 °C. Amplitude sweep was used to evaluate the storage modulus of the hydrogels. The tests were conducted at the strain (ϒ) ranging from 0.01 to 100%, and the frequency (ω) ranging from 0.8 to 1.2 Hz depending on the stiffness of the hydrogels. All measurements were performed in triplicate.

Swelling of the Hydrogels: The swelling ratios of the hydrogels were measured in DI water at room temperature. The weighed hydrogels after UV curing were immersed in DI water. At different time intervals, excess water on the hydrogels was removed by tissue and the samples were weighed until their weight remained constant, which meant that equilibrium had been reached. The experiment was done in triplicate. The equilibrium swelling ratio (ESR) was calculated using the Equation (2):

\[
\text{ESR(\%)} = \left( \frac{W_e - W_d}{W_d} \right) \times 100\% \tag{2}
\]

where \(W_e\) is the weight of swollen hydrogel and \(W_d\) is the starting weight of the hydrogel after UV curing.

Cytotoxicity Assay: A monolayer of human dermal fibroblast (hDF) and mouse monocyte (Raw 264.7) cells were used for the cytotoxicity assays. The cells were maintained in tissue culture flasks at 37 °C, 5% CO₂. Subsequently, Alamar Blue (10 μL) was added and the incubation was continued for 4 h (37 °C, 5% CO₂). The plate was then shaken for 20 s, and the fluorescence intensity was measured at ex/em 560/590 nm.

The cylinder-shaped hydrogels with different dry weight percentages were formed from 50 μL precursor solution. All hydrogels were exposed to UV light for 30 min to sterilize the surface and were allowed to swell in sterilized DI water overnight. Antibiotic-loaded H10K-DNGsA were swollen in 500 μL sterilized DI water without UV sterilization (NB is sensitive to UV). The swollen hydrogels were then transferred to 24-well plates containing cells and were incubated for 72 h at 37 °C, 5% CO₂. After incubation, of Alamar Blue (50 μL) was added and incubation was continued by changing the medium every 15 min to remove free and loosely attached CIP. The CIP concentration was determined via fluorescence with an excitation wavelength of 278 nm and an emission wavelength of 455 nm using the plate reader of infinite M200 PRO.
continued for 4 h (37 °C, 5% CO₂). The plate was then shaken for 20 s, and the fluorescence intensity was measured at ex/em 560/590 nm. Cytotoxicity of H10K-DNGs (40 wt%) and H10K-DNGsA (40 wt%) were tested using XTT. After the incubation with Hacat for 24 h, the hybrid hydrogels were removed, cells were washed with PBS and then incubated with 200 µL of 20% solution of 1 mg mL⁻¹ XTT and 12.5 mM menadione for 4 h. The absorbance was measured at 450 nm in a 96-well plate. All results are shown as mean ± SD.

Degradation of H10K with Different Dry Weight Percentages: The degradation of H10K (20, 30, and 40 wt%) was performed in PBS (pH 7.4) at 37 °C. After UV curing, the hydrogels were freeze dried and weighed. The samples were then immersed in PBS and were transferred to the incubator and incubated at 37 °C for 4 h. The absorbance was measured at 450 nm in a 96-well plate. All results are shown as mean ± SD.

Minimum Inhibitory Concentration: The minimal inhibitory concentration (MIC) of the antibiotics and DNGs were tested using the broth dilution method. Briefly, a single colony of the bacteria from the agar plate was suspended in MHB II broth and incubated with shaking at 37 °C to log phase. The suspended bacteria were then diluted with broth to reach a concentration of 10⁶ CFU mL⁻¹. The compounds were diluted in 96-well plates using the double dilution method, and the bacteria solution was added to the wells to yield a final concentration of 5 x 10² CFU mL⁻¹. Wells containing bacterial cells only and containing antibiotics only were used as positive and negative controls, respectively. The plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured after this time. The test was done in triplicate for each sample.

The Disk Diffusion Test: Antibiotics-loaded and antibiotics-free hybrid hydrogels (negative control) were prepared for the disk diffusion test. The cylinder-shaped hybrid hydrogels with dry weight percentages of 20, 30, and 40 wt% were formed from 50 µL of precursor solution. The final concentrations of CIP and NB in the hydrogels were 35.4 and 500 µg mL⁻¹, respectively. The hydrogels containing antibiotics were then placed on MHB II agar plates containing bacteria (concentration ≈ 10⁸ CFU mL⁻¹). The plates were incubated at 37 °C for 24 h. The inhibition zones were measured after this time. The test was done in triplicate for each sample.

In Vitro Cell Infection Assay: In vitro cell infection assays used the infection protocol with some modifications, and were conducted in a 24-well plate using antibiotic and serum-free medium. Hacat and hDF cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. Cells were infected with 10⁶ CFU mL⁻¹ of S. aureus 2569 in 100 µL PBS. To enhance the bacterial attachment, the culture plates were centrifuged for 1 min at 350 g. Two infection models were built. In the first one, cells were infected with S. aureus for 2 h, washed with PBS to remove non-adherent bacteria and supplemented with fresh medium. Afterwards H10K-DNGs (control, 40 wt%) and H10K-DNGsA (40 wt%) were added and incubated for 6 h. In the second model, cells were infected with S. aureus, H10K-DNGs (control) and H10K-DNGsA were added at the same time and incubated for 8 h. After the incubation in the incubator, bacterial suspensions were serially diluted and plated on blood agar plates. The total bacterial number was evaluated in relation to the number of bacteria added from the same experiment and normalized with respect to untreated infected control. Three independent experiments, each in duplicate were performed. Results are presented as mean ± SD.

In Vitro Drug Release: The release experiments of NB and CIP were performed in PBS (pH 7.4) at 37 °C for 3 days using the release protocol with some modifications. After UV curing and NB encapsulation, H10K-DNGsA was transferred to a bottle containing 15 mL PBS. The bottle was incubated at 37 °C with 100 rpm agitation. Aliquots (100 µL) were withdrawn at different time intervals to determine the drug concentrations, and in all cases, equal volumes of buffer were added to maintain the constant volume. Antibiotic-free H10K-DNGs was used as control. The concentrations of NB and CIP were determined spectrophotometrically at 306 nm (Absorbance) and ex/em 278/455 nm (Fluorescence), respectively. The test was done in triplicate for each sample.

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

Acknowledgements
Y.F. thanks the China Scholarship Council for a special scholarship award. Knut and Alice Wallenberg Foundation KAW (2012.0196 and 2017.0300) are also acknowledged for their financial support.

Conflict of Interest
The authors declare no conflict of interest.

Keywords
antimicrobial, combination therapy, dendritic hybrid hydrogels, dendritic nanogels, drug delivery

Received: July 31, 2020
Revised: September 22, 2020
Published online: November 2, 2020

1. M. Bassetti, F. Ginocchio, M. Mikulska, in Annual Update in Intensive Care and Emergency Medicine 2011, Springer, Berlin 2011, p. 501.
2. R. I. Aminov, Front. Microbiol. 2010, 1, 134.
3. K. Lewis, Nat. Rev. Drug Discovery 2013, 12, 371.
4. S. Salmapro, P. Caliceti, J. Drug Delivery 2013, 2013, 374252.
5. P. V. Baptista, M. P. McCusker, A. Carvalho, D. A. Ferreira, N. M. Mohan, M. Martins, A. R. Fernandes, Front. Microbiol. 2018, 9, 1441.
6. E. M. Ahmed, J Adv Res 2015, 6, 105.
7. K. Y. Lee, D. J. Mooney, Chem. Rev. 2001, 101, 1869.
8. A. S. Hoffmann, J. Controlled Release 1987, 6, 297.
9. J. Li, D. J. Mooney, Nat. Rev. Mater. 2016, 1, 16071.
10. X. Zhang, B. Soontornvorajit, Z. Zhang, N. Chen, Y. Wang, Biomacromolecules 2012, 13, 2202.
11. E. Caló, V. V. Khutoryanskiy, Eur. Polym. J. 2015, 65, 252.
12. B. Balakrishnan, M. Mohanty, P. Umashankar, A. Jayakrishnan, Biomaterials 2005, 26, 6335.
13. M. Kokabi, M. Sirousazar, Z. M. Hassan, Eur. Polym. J. 2007, 43, 773.
14. M. I. Gonzalez-Sanchez, S. Perni, G. Tommasi, N. G. Morris, K. Hawkins, E. Lopez-Cabarcos, P. Prokopovich, Mater. Sci. Eng. C Mater. Biol. Appl. 2015, 50, 332.
15. H. Li, J. Yang, X. Hu, J. Liang, Y. Fan, X. Zhang, J. Biomed. Mater. Res., Part A 2011, 98A, 31.
16. Z. Li, C. He, B. Yuan, X. Dong, X. Chen, Macromol Biosci 2017, 17.
[17] M. V. Risbud, R. R. Bhonde, *Drug Delivery* **2000**, *7*, 69.
[18] A. M. Carmona-Ribeiro, L. D. de Melo Carrasco, *Int. J. Mol. Sci.* **2013**, *14*, 9906.
[19] H. Du, Y. Wang, X. Yao, Q. Luo, W. Zhu, X. Li, Z. Shen, *Polym. Chem.* **2016**, *7*, 5620.
[20] A. Pascual, J. P. Tan, A. Yuen, J. M. Chan, D. Mecerreyes, J. L. Hedrick, Y. Y. Yang, H. Sardon, *Biomacromolecules* **2015**, *16*, 1169.
[21] C. Zhang, Z. Ying, Q. Luo, H. Du, Y. Wang, K. Zhang, S. Yan, X. Li, Z. Shen, W. Zhu, *J. Polym. Sci. A: Polym. Chem.* **2017**, *55*, 2027.
[22] C. Zhao, X. Li, L. Li, G. Cheng, X. Gong, J. Zheng, *Langmuir* **2013**, *29*, 1517.
[23] T. R. Hoare, D. S. Kohane, *Polymer* **2008**, *49*, 1993.
[24] D. C. Coughlan, F. P. Quilty, O. I. Corrigan, *J. Control Release* **2004**, *98*, 97.
[25] C. Gao, D. Yan, *Prog. Polym. Sci.* **2004**, *29*, 183.
[26] M. V. Walter, M. Malkoch, *Chem. Soc. Rev.* **2012**, *41*, 4593.
[27] Q. Wang, J. L. Mynar, M. Yoshida, E. Lee, M. Lee, K. Okuro, K. Kinbara, T. Aida, *Nature* **2010**, *463*, 339.
[28] O. C. J. Andrén, M. V. Walter, T. Yang, A. Hult, M. Malkoch, *Macromolecules* **2013**, *46*, 3726.
[29] Y. Zhang, O. C. Andrén, R. Nordström, Y. Fan, M. Malmsten, S. Mongkhontreerat, M. Malkoch, *Adv. Funct. Mater.* **2019**, *29*, 1806693.
[30] R. Nordström, O. C. Andrén, S. Singh, M. Malkoch, M. Davoudi, A. Schmidtchen, M. Malmsten, *J. Colloid Interface Sci.* **2019**, *554*, 592.
[31] M. Mahmoudian, F. Ganji, *Prog. Biomater.* **2017**, *6*, 49.
[32] Q. Hu, W. Sun, C. Wang, Z. Gu, *Adv. Drug Delivery Rev.* **2016**, *98*, 19.
[33] B. D. Brooks, A. E. Brooks, *Adv. Drug Delivery Rev.* **2014**, *78*, 14.
[34] D. Zhong, Z. Liu, S. Xie, W. Zhang, Y. Zhang, W. Xue, *J. Appl. Polym. Sci.* **2013**, *129*, 767.
[35] N. Lin, A. Geze, D. Wouessidjewe, J. Huang, A. Dufresne, *ACS Appl. Mater. Interfaces* **2016**, *8*, 6830.
[36] G. S. Alvarez, C. Hélary, A. M. Mebert, X. Wang, T. Coradin, M. F. Desimone, *J. Mater. Chem. B* **2014**, *2*, 4660.
[37] C. Choipang, P. Chuyasinuan, O. Suwantong, P. Eakabutr, P. Supaphol, *J. Drug Delivery Sci. Technol.* **2018**, *47*, 106.
[38] P. Schacht, V. Chyský, G. Gruenwald, R. Hullmann, H. Weuta, G. Arcieri, E. Griffith, B. O’Brien, J. Branolte, H. Bruck, *Infection* **1988**, *16*, S29.
[39] V. Rodríguez-Cerrato, G. Del Prado, L. Huelves, P. Naves, V. Ruiz, E. García, C. Ponte, F. Soriano, *Int. J. Antimicrob. Agents* **2010**, *35*, 544.
[40] J. Qu, X. Zhao, Y. Liang, T. Zhang, P. X. Ma, B. Guo, *Biomaterials* **2018**, *183*, 185.
[41] Q. Chai, Y. Jiao, X. Yu, *Gels* **2017**, *3*, 6.
[42] S. Sartori, V. Chiono, C. Tonda-Turo, C. Mattu, C. Gianluca, *J. Mater. Chem. B* **2014**, *2*, 4660.
[43] C. Pailler-Mattei, S. Bec, H. Zahouani, *Med. Eng. Phys.* **2008**, *30*, 599.
[44] Y. Shi, C. Ma, L. Peng, G. Yu, *Adv. Funct. Mater.* **2015**, *25*, 1219.
[45] X. Guo, D. Xu, Y. Zhao, H. Gao, X. Shi, J. Cai, H. Deng, Y. Chen, Y. Du, *ACS Appl. Mater. Interfaces* **2019**, *11*, 34766.
[46] A. D. Metcalfe, M. W. Ferguson, *J. R. Soc. Interface* **2007**, *4*, 413.
[47] Y. Ma, L. Xin, H. Tan, M. Fan, J. Li, Y. Jia, Z. Ling, Y. Chen, X. Hu, *Mater. Sci. Eng. C* **2017**, *71*, 522.
[48] I. Wiegand, K. Hilpert, R. E. Hancock, *Nat. Protoc.* **2008**, *3*, 163.
[49] Y. Kai-Larsen, P. Lüthje, M. Chromek, V. Peters, X. Wang, Å. Holm, L. Kádas, K.-O. Hedlund, J. Johansson, M. R. Chapman, *PLoS Pathog.* **2010**, *6*, e1001010.
[50] T. R. Thatiparti, A. J. Shoffstall, H. A. von Recum, *Biomaterials* **2010**, *31*, 2335.