



Antimicrobial hydrogels: A new weapon in the arsenal against multidrug-resistant infections[☆]



Victor W.L. Ng^{a,1}, Julian M.W. Chan^{b,2}, Haritz Sardon^{b,c,2}, Robert J. Ono^{b,2}, Jeannette M. García^{b,2}, Yi Yan Yang^{a,*,1}, James L. Hedrick^{b,*,2}

^a Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore

^b IBM Almaden Research Center, 650 Harry Road, San Jose, CA 95120, USA

^c POLYMAT, University of the Basque Country UPV/EHU Joxe Mari Korta Center, Avda. Tolosa 72, 20018 Donostia-San Sebastián, Spain

ARTICLE INFO

Available online 1 November 2014

Keywords:

Hydrogels
Antimicrobials
Polymers
Drug delivery
Biofilm dispersion

ABSTRACT

The rapid emergence of antibiotic resistance in pathogenic microbes is becoming an imminent global public health problem. Treatment with conventional antibiotics often leads to resistance development as the majority of these antibiotics act on intracellular targets, leaving the bacterial morphology intact. Thus, they are highly prone to develop resistance through mutation. Much effort has been made to develop macromolecular antimicrobial agents that are less susceptible to resistance as they function by microbial membrane disruption. Antimicrobial hydrogels constitute an important class of macromolecular antimicrobial agents, which have been shown to be effective in preventing and treating multidrug-resistant infections. Advances in synthetic chemistry have made it possible to tailor molecular structure and functionality to impart broad-spectrum antimicrobial activity as well as predictable mechanical and rheological properties. This has significantly broadened the scope of potential applications that range from medical device and implant coating, sterilization, wound dressing, to antimicrobial creams for the prevention and treatment of multidrug-resistant infections. In this review, advances in both chemically and physically cross-linked natural and synthetic hydrogels possessing intrinsic antimicrobial properties or loaded with antibiotics, antimicrobial polymers/peptides and metal nanoparticles are highlighted. Relationships between physicochemical properties and antimicrobial activity/selectivity, and possible antimicrobial mechanisms of the hydrogels are discussed. Approaches to mitigating toxicity of metal nanoparticles that are encapsulated in hydrogels are reviewed. In addition, challenges and future perspectives in the development of safe and effective antimicrobial hydrogel systems especially involving co-delivery of antimicrobial polymers/peptides and conventional antimicrobial agents for eventual clinical applications are presented.

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[☆] This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Emergence of multidrug resistance bacteria: Important role of macromolecules as a new drug targeting microbial membranes".

* Corresponding authors.

E-mail addresses: yyang@ibn.a-star.edu.sg (Y.Y. Yang), hedrick@us.ibm.com (J.L. Hedrick).

¹ Tel.: +65 6824 7106.

² Tel.: +1 408 927 1632.

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1. Introduction

Despite major advancements in the standards of health care and medical technology, infectious diseases caused by pathogenic microorganisms such as bacteria, viruses, parasites or fungi, remain major public health threats that can translate into extensive socio-economic problems. In fact, according to a World Health Organization (WHO) report, infectious diseases are the second leading cause of global mortality [1]. Furthermore, over-prescription and misuse of conventional antibiotics have also led to escalating drug-resistance associated with various pathogens [2–4]. Coupled with this problem is the dwindling pipeline in development and production of new antibiotics and antimicrobials against resistant microbes [5–8]. In view of this, there is an imperative need to develop new antimicrobials and their relevant formulations to address this urgency.

Several classes of unique materials, such as antimicrobial peptides (AMPs) [9–16], synthetic cationic polymers [17–19] and antimicrobial nanoparticles [20–22], have emerged as potential substitutes for conventional antibiotics. While conventional antibiotics function by acting on specific molecular targets such as inhibiting protein synthesis or cell wall biosynthesis within/on the organisms, AMPs and their natural/synthetic cationic polymers counterparts are postulated to act upon the entire cellular membrane through electrostatic interactions, and subsequent insertion of their hydrophobic components into the lipid domains of the microbial membrane to generate pores [23–25]. This eventually leads to cell membrane rupture, leakage of cytoplasmic content, and cell death. On the other hand, the antimicrobial mechanisms of AMPs are more diverse and they have been proposed to include: (1) photocatalytic production of reactive oxygen species (ROS) that target cellular and viral structures, (2) increased permeability and disruption of cell membrane, (3) interruption of energy transduction pathway and (4) inhibition of enzymatic activity and DNA synthesis [20]. All these mechanisms are generally believed to circumvent the problem of microbial resistance.

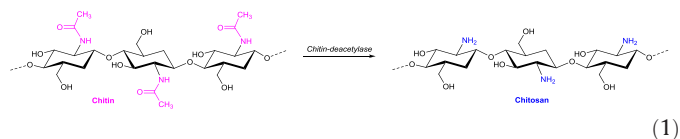
Hydrogels are a class of materials formed from natural or synthetic polymers that exhibit three-dimensional (3D) networks with high to ultra-high degree of water content. While the term has been used as early as 1894 [26,27], the first biological use of such gels was only reported by Wichterle and Lim in 1960 [28]. Since then, there has been an explosion of investigations documenting the use of hydrogels in many biomedical applications [29–31], including drug and protein delivery [32–34], tissue engineering [33–35], cell culture [36,37], coatings and wound dressing [38–40]. The methodologies involving the production of such materials have also seen substantial increase in recent years especially with regards to the type of physical and chemical crosslinking processes [41].

This succinct review article pays special attention to the integration of antimicrobials and hydrogels. Specifically, we focus on (1) natural and synthetic hydrogels having intrinsic antimicrobial properties and (2) hydrogels that are loaded with antimicrobial substances, e.g., antibiotics, AMPs, synthetic cationic polymers and antimicrobial nanoparticles. There are a number of excellent reviews in the literature pertaining to this topic [42,43]. In this review, we aim to illustrate a clear relationship between the synthetic chemistry and biological implications of antimicrobial hydrogels based on current literature.

2. Hydrogels possessing antimicrobial properties

2.1. Natural polymeric hydrogels

Natural biopolymers are polymers that are produced by living organisms. They can be loosely classified into three main categories, including polypeptides (e.g. proteins), polynucleotides (e.g. DNA and RNA), and sugars (e.g. cellulose, chitin). Chitosan is a linear polysaccharide commercially manufactured by the de-acetylation of chitin (Eq. (1)), one of the most abundant sugar-based biopolymers. There has been tremendous research effort directed at the investigation of chitosan and its derivatives for biomedical applications due to their biocompatibility, low toxicity, biodegradability, hydrophilicity and low cost [44–46]. Chitosan is also weakly basic having an intrinsic pK_a value of 6.5. The primary amino groups are readily protonated in acidic medium, enhancing water solubility at varying pH levels, and imparting unto the polymer an intrinsic antimicrobial property. As such, these materials have been reported as potentially useful wound dressing materials on numerous occasions [38]. In fact, chitosan products have been approved and used by USA and UK as field bandages in the Iraq and Afghanistan wars owing to their hemostatic, hypoallergenic and natural antibacterial properties [47]. In addition, chitosan hydrogelation can also easily be achieved by simple physical and chemical methods through blending or crosslinking with suitable reagents. In short, these biopolymers are highly versatile materials that can be modified to meet the needs of the desired biomedical application.



In 2010, a chitosan- γ -poly(glutamic acid) polyelectrolyte complex hydrogel system was reported by Hsieh and co-workers [48]. These hydrogels showed positive antimicrobial activities against *S. aureus* and *E. coli*, and at the same time demonstrated effectiveness in promoting 3T3 fibroblasts cell proliferation. The hydrogels were also evaluated and proved useful for wound-healing capabilities [49]. Almost concurrently, Chan-Park and co-workers independently developed a novel antimicrobial hydrogel based on dimethylalkylammonium chitosan (with various degrees of quaternization)-graft-poly(ethylene glycol) methacrylate (qC-g-EM) and poly(ethylene glycol) diacrylate (Fig. 1a) [50]. In this investigation, the authors found that increasing the alkyl chain length of the quaternizing agent from trimethylammonium (TM) to dimethyldecylammonium (DMD) led to greater efficacy against Gram-positive *S. aureus* but not the Gram-negative bacteria *E. coli* and *P. aeruginosa*. Furthermore, DMDC-Q-g-EM (Q denoting higher degree of quaternization) demonstrated exceptional antimicrobial efficacy against major classes of bacteria and fungus including *P. aeruginosa* (Gram-negative), *E. coli* (Gram-negative), *S. aureus* (Gram-positive) and *Fusarium solanis* (fungi). The choice of chitosan was logical as it is biocompatible and possesses intrinsic antimicrobial activity. Furthermore, modification and functionalization can easily be carried out to enhance the polymer's prowess against pathogenic microbes. These cationic nanoporous hydrogels are hypothesized to act via bacterial

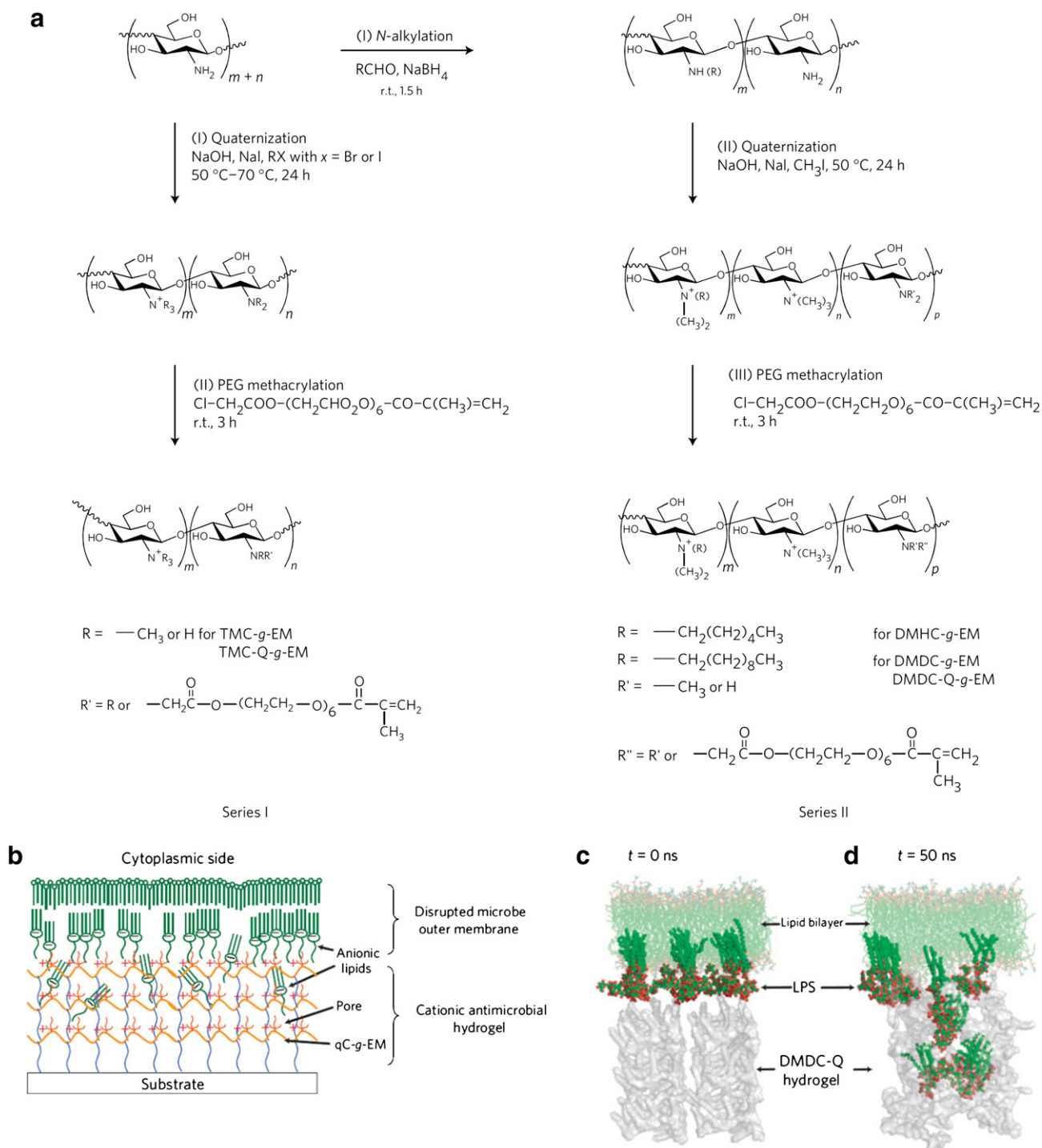


Fig. 1. (a) Synthesis of modified chitosan antimicrobial polymers. (b) Schematic diagram of the 'anion sponge' model showing parts of the negatively-charged bacterial membrane being 'suctioned' into the pores of the hydrogel. (c,d) Computer simulation of the killing mechanism showing the 'suctioning' of *P. aeruginosa* bacterial membrane (lipid bilayer) LPS molecules into the hydrogel after 50 ns. Reprinted from Ref. [50] by permission from Macmillan Publishers Ltd, Copyright (2010).

membrane disruption through ionic interactions with the anionic microbial surfaces (Fig. 1b, c). The contact-active hydrogels showed good *in vitro* and *in vivo* biocompatibility and are non-hemolytic. Hydrogel coated onto a fluoropolymer substrate also gave antimicrobial efficiency comparable to the free hydrogel films.

Subsequently, Aziz et al. described the development of an antimicrobial chitosan-dextran (CD) hydrogel for use in endoscopic sinus surgery [51]. Here, the CD hydrogel was synthesized by reacting *N*-succinyl chitosan (5% in water) with an oxidized dextran aldehyde (5% in water) in a Michael-type addition at a 1:1 mixture using a previously

reported protocol [52]. Gelation was achieved within a minute of addition. The resultant CD gel was found to be active against *S. aureus*, *Streptococcus pyogenes* (Gram-positive), *Clostridium perfringens* (Gram-positive) and *E. coli* at its surgical concentration of $50,000 \text{ mg L}^{-1}$. Interestingly, the authors found that dextran aldehyde was the antimicrobial constituent of the CD hydrogel rather than the derivatized chitosan. Furthermore, they postulated that such activity could be due to addition reactions occurring between the aldehyde moiety and the amino groups on the bacterial membrane, or it could be a result of rupturing peptido-bonds on the cellular envelope.

More recently, Mohamed and coworkers detailed the synthesis and antimicrobial activity of some chitosan hydrogels which were crosslinked with various concentrations of oxaly bis-4-(2,5-dioxo-2H-pyrrol-1(5H)-yl)benzamide [53]. They treated the hydrogels with five types of bacteria, namely, *Bacillus subtilis* (Gram-positive), *S. aureus*, *Streptococcus pneumonia* (Gram-positive), *Salmonella typhimurium* (Gram-negative), *E. coli*, as well as two crop-threatening pathogenic fungi, *Aspergillus fumigatus* and *Aspergillus niger*. The crosslinked hydrogels proved to be more effective antimicrobials compared to the parent chitosan, and they also exert greater activity against the Gram-positive over the Gram-negative bacteria. Significantly, the authors noted that an increase in the degree of crosslinking in the hydrogels corresponded to an enhancement in antimicrobial activity. The incorporation of terephthaloyl thiourea fragments as crosslinkers was postulated to prevent intermolecular hydrogen-bonding between chitosan polymeric chains, thereby increasing their swelling capacity, allowing easy penetration of the hydrogel into the microbes and resulting in enhanced antimicrobial activities. Following this investigation, they reported a series of novel terephthaloyl thiourea crosslinked chitosan hydrogels exhibiting antibacterial and antifungal properties [54]. The hydrogels were challenged with *B. subtilis*, *S. aureus* and *E. coli*, and again showed higher activity against Gram-positive over Gram-negative bacteria. Furthermore, the modified hydrogels were found to be more effective against *A. fumigatus*, *G. candidum* and *C. albicans* when compared to the unmodified chitosan. Here, an increase in the degree of crosslinking in the hydrogels also led to an increase in antimicrobial activity.

Using a similar grafting strategy to Chan-Park's chitosan hydrogel, Jiang et al. synthesized the quaternary ammonium salt of gelatin using 2,3-epoxypropyl trimethylammonium chloride (EPTAC) [55]. The amino functional groups were found to be fully consumed when the molar ratio of the ammonium salt to $-NH_2$ group was at 2.5:1. Excess EPTAC was found to react with hydroxyl groups, thus giving rise to higher degree of cationic charges on the grafted polymers. By increasing the degree of cationic grafting, significant improvement in the polymers' antimicrobial activity was observed. As evaluated from the above examples, factors that influence antimicrobial activity and biocompatibility of a given material include amphiphilicity (i.e. hydrophilicity vs. hydrophobicity), degree of crosslinking, chemical reactivity/compatibility, and physical interactions, e.g. hydrogen-bonding. Although natural polymeric hydrogels have seen significant success in antimicrobial activity, batch-to-batch variation in molecular weights of natural polymers may compromise reproducibility and affect physical properties. Therefore, further studies on the effect of molecular weight of natural polymers should be performed. Immunogenicity is often reported with the use of natural polymers, which is caused by impurities in natural polymers. This should also be taken into consideration when designing and preparing natural antimicrobial hydrogels.

2.2. Synthetic polymeric hydrogels

As mentioned earlier, synthetic cationic polymers have emerged as important antimicrobial alternatives to antibiotics. These materials can be synthesized in a myriad of ways and they can be composed of either biodegradable or non-biodegradable polymer backbones. The development of controlled polymerization methodologies, including atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain-transfer polymerization (RAFT), ring-opening metathesis polymerization (ROMP), ring-opening polymerization (ROP), have also spurred exhaustive research efforts in the development of well-defined synthetic cationic polymers. In fact, with the ever-increasing threat of antibiotic resistance, these materials have attracted tremendous research interests. A plethora of antimicrobial synthetic cationic polymers have since been reported in the literature, including poly(acrylate) [56–62] and poly(norbornene) [63–67] systems, poly(ethyleneimine)s (PEIs) [68–70], poly(arylamide)s [71–73],

poly- β -lactams [74–78], poly- α -amino acids [79,80] and polycarbonates [81–86].

In spite of this, there have only been a few attempts to amalgamate synthetic cationic polymers into a hydrogel form in which the gel would be intrinsically antimicrobial. Liu et al. recently reported a series of antimicrobial and antifouling hydrogels generated in situ from cationic polycarbonate and four-arm poly(ethylene glycol) (PEG) via Michael addition [87]. The strategy involves a two-step thiol-based Michael-type addition, first by partially coupling a thiol-terminated diblock copolymer of PEG and cationic polycarbonate to four-arm PEG tetraacrylate followed by crosslinking using four-arm tetrasulfuryl poly(ethylene glycol) at 37 °C in the presence of triethanolamine (Fig. 2). The diblock copolymers of PEG and cationic polycarbonate were synthesized by metal-free organocatalytic living ring-opening polymerization of functional cyclic carbonate monomers using thiol-functionalized PEG as a macroinitiator. This synthetic approach provides precise control over molecular weight, molecular composition and functionality. It was found that the cationic hydrogels eliminated Gram-positive bacteria more easily than Gram-negative bacteria, and the presence of hydrophobic components in the cationic polycarbonate block was essential for the hydrogels to eliminate *E. coli* and *C. albicans* effectively. An increased hydrophobic content resulted in greater antimicrobial efficacy especially against *E. coli* and *C. albicans*. The envelope of Gram-negative bacterial cell has an additional membrane as the outermost layer when compared to that of Gram-positive bacterial cell, and the cell wall of Gram-positive bacteria has more negative charges than the cell membrane of Gram-negative bacteria [88,89]. Therefore, hydrophobic components are needed to interact with and disrupt the cell membrane [23]. The diblock copolymers of PEG and cationic polycarbonate had low antimicrobial activity in solution. However, when it was incorporated into hydrogel networks, the hydrogels demonstrated strong antimicrobial properties. Particularly, the best hydrogel showed broad-spectrum antimicrobial activity with >99.99% killing efficiency for all microbes tested, e.g., *S. aureus*, *E. coli* and *C. albicans*. The authors hypothesized that the anionic bacterial cell wall/membrane would first interact with the cationic hydrogel surface at several fixed points via electrostatic attraction, followed by insertion of the hydrophobic segments of the hydrogel into the hydrophobic regions of the lipid membrane, thereby inducing leakage of cell contents and subsequent lysis. The contact-active antimicrobial effect of the hydrogel was further verified by agar plate assay. *S. aureus* was incubated on the pre-formed hydrogel on agar plates at 37 °C for one day, and the hydrogel coating demonstrated complete bacterial colony eradication/prevention throughout a 2-week examination period (Fig. 2b) by virtue of a clear inhibition zone. In addition, the surrounding agar region that was not subjected to the hydrogel showed considerable bacterial colonies, suggestive of a contact-active antimicrobial system. Significantly, control PEG gel exposed to *S. aureus* under the same conditions gave rise to microbial over-growth on the agar plate. When the cationic hydrogel was coated onto silicone rubber surfaces, it manifested both antimicrobial and antifouling properties that are highly desirable for catheter coating materials. More notably, these hydrogels did not exhibit significant hemolysis and skin toxicity.

Thereafter, Li et al. described the synthesis of physically-crosslinked broad-spectrum antimicrobial hydrogels derived from the stereocomplexation of biodegradable polymers P_L LA-PEG- P_L LA (P_L LA = poly(L-lactide)) and polycationic P_D LA-CPC- P_D LA (P_D LA = poly(D-lactide), CPC = cationic polycarbonate) (Fig. 3) [90]. Both P_L LA-PEG- P_L LA and P_D LA-CPC- P_D LA precursor triblock copolymers were synthesized via organo-catalyzed ROP using PEG and polycarbonate as the macroinitiators, respectively. This synthetic approach gave rise to polymers with well-controlled chain lengths having the desired degree of polymerizations (DP). The cationic polycarbonates were subsequently obtained by quaternization of their respective precursors with trimethylamine. The systematic combination of these triblock copolymers resulted in the creation of a series of supramolecular

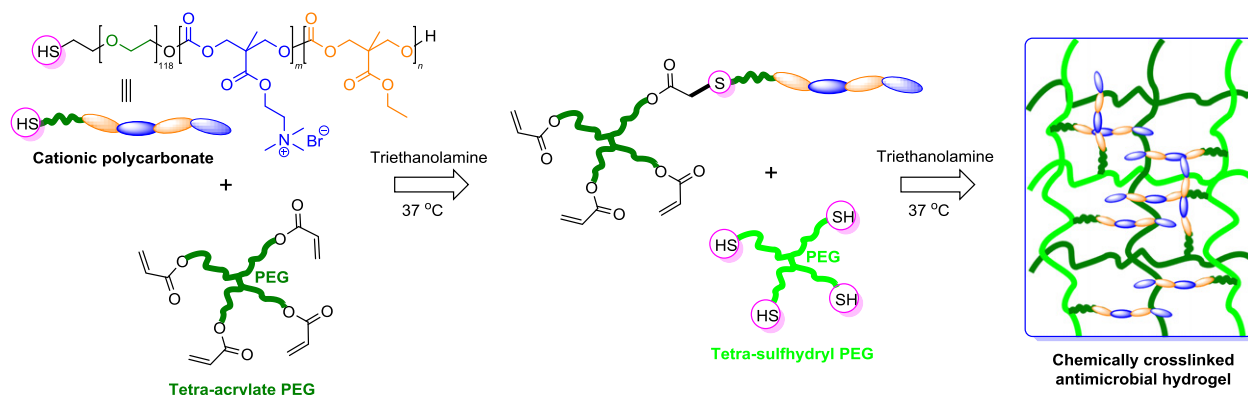


Fig. 2. Synthesis of chemically-crosslinked antimicrobial polycarbonate hydrogels.

hydrogel assemblies that exhibited lower critical solution temperature (LCST) behavior, shear-thinning behavior, and biofilm disrupting capabilities at 37 °C. The antimicrobial activity was evaluated against three pathogenic microbes including *S. aureus*, *E. coli* and *C. albicans*, as well as clinically isolated microbes such as methicillin-resistant *S. aureus* (MRSA, Gram-positive), vancomycin-resistant *enterococci* (VRE, Gram-positive), *P. aeruginosa* (Gram-negative), *A. baumannii* (Gram-negative, resistant to most antibiotics), *K. pneumoniae* (Gram-negative, resistant to carbapenem), and *C. neoformans* (fungi). The hydrogels were able to completely eliminate growth and demonstrate near perfect killing efficiency on all the microbes tested although cationic P_DLA-CPC-P_DLA polymers in solution showed no/low antimicrobial activity. Cell wall/membrane lysis was determined to be the antimicrobial mechanism which was supported by microbial morphological changes through scanning electron microscopy (SEM) investigation. The hydrogel with an optimal composition demonstrated negligible toxicity *in vitro* and *in vivo*.

Zwitterionic polymers such as poly(carboxybetaine) (pCB) and poly(sulfobetaine) (pSB) are another class of materials that have been utilized in the construction of antimicrobial hydrogel systems. Jiang and co-workers are the frontrunners in this area of research and have published several elegant works and reviews on this subject [91,92].

In 2012, Cao et al. reported the synthesis of a smart polymer capable of attacking and defending bacteria under different pH conditions repeatedly [93]. Here, they ingeniously made use of the switching ability between two equilibrium states of a cationic six-membered *N,N*-dimethyl-2-morpholinone (CB-ring; antimicrobial) and a zwitterionic carboxy betaine (CB-OH; antifouling) to achieve such a function. An impressive killing efficiency of greater than 99% of *E. coli* (K12) under the CB-ring state was demonstrated. The Cheng group followed up with two reports on the improvements of such ring-opening–ring-closing strategy using modified methacrylate monomers (Fig. 4) [94, 95]. In the latter report, amide derivatives (CBMAA-1, six-membered; CBMAA-2, seven-membered) were synthesized using the respective methacrylamides [95]. The hydrogels were prepared by UV photopolymerization in the presence of carboxybetaine dimethacrylate (crosslinker), 2-hydroxy-2-methylpropiophenone (initiator) and their respective monomers. They found an increase in the elasticity and stability while retaining switchability and killing efficacy against *E. coli* (K12) for the CBMAA-2 hydrogel.

Earlier in 2000, Perrault and Rouns patented a series of inherently antimicrobial quaternary ammonium hydrogels based on crosslinked acrylate polymers for wound dressing applications [96]. One of the hydrogels was screened for antimicrobial activity using an adaptation

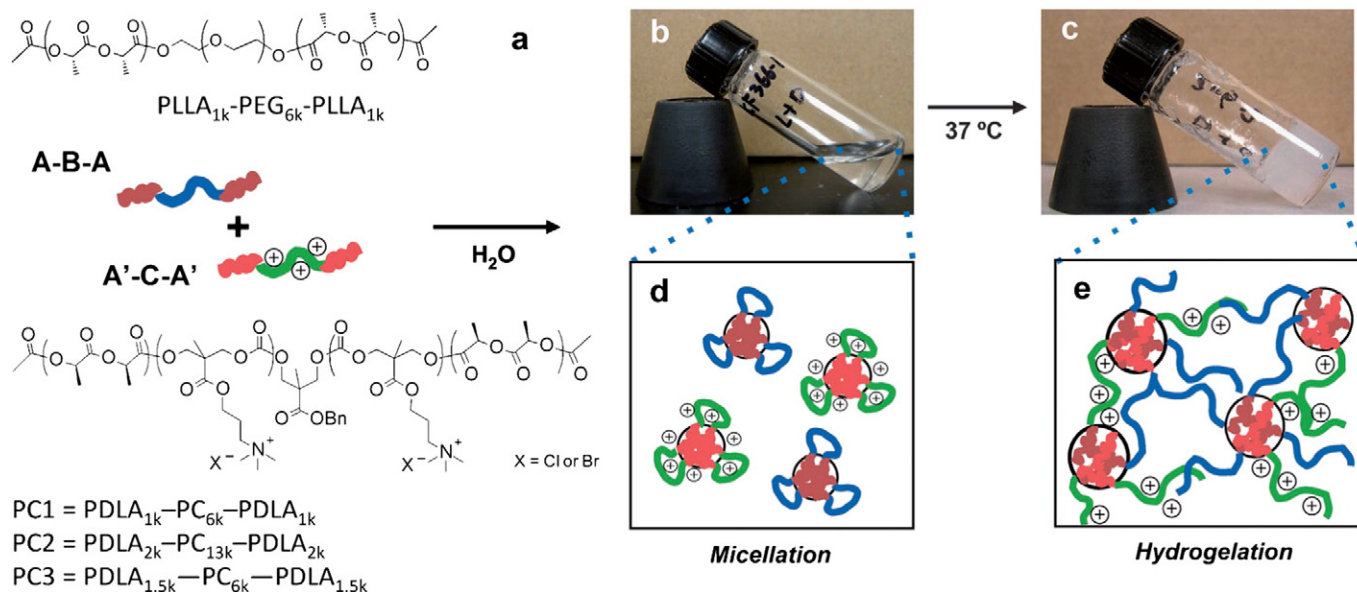


Fig. 3. Formulation of hydrogels derived from PLLA-PEG-PDLA (PLLA = poly(L-lactide)) and polycationic PDLA-CPC-PDLA. Reprinted from Ref. [90] with permission from John Wiley and Sons, Copyright (2013).

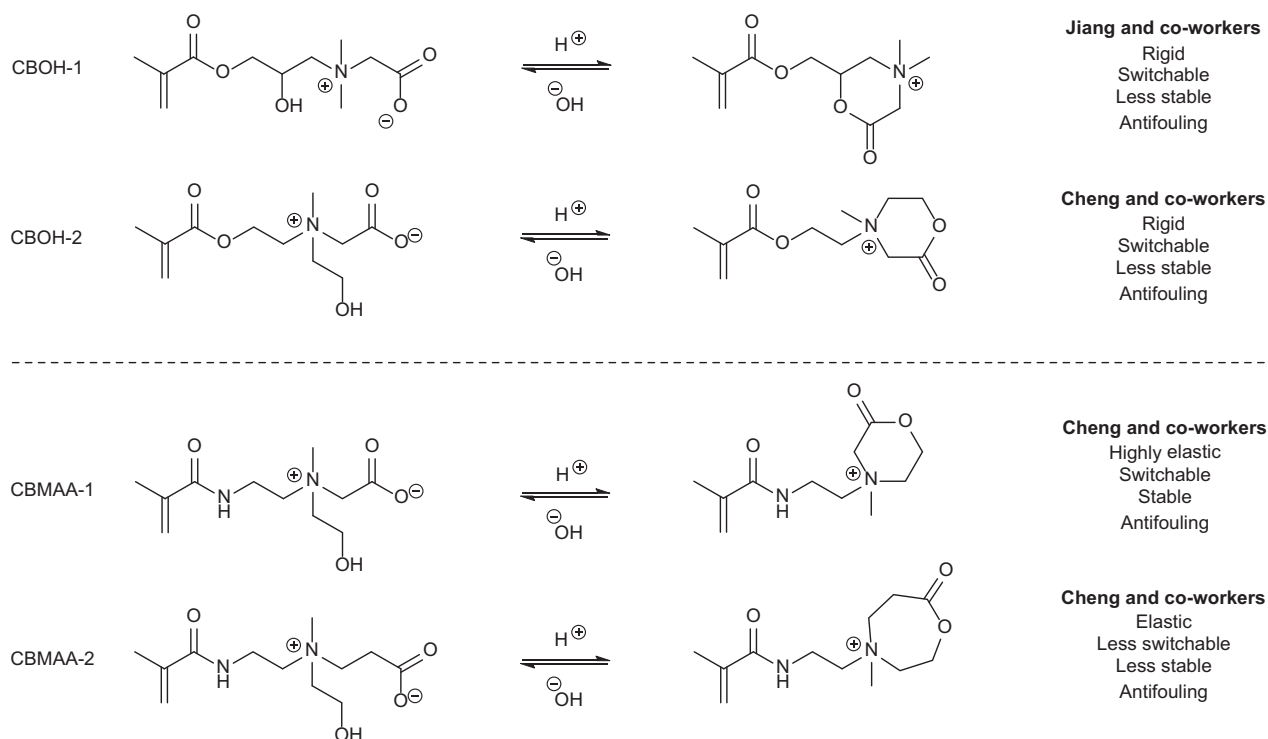


Fig. 4. Chemical structures and characteristics of carboxybetaine derivatives. Adapted from Ref. [95] with permission from Elsevier, Copyright (2013).

of the Kirby–Bauer method, where the potency is determined by the average zone of inhibition. The microbes used were *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecalis* and *C. albicans*. The hydrogel exhibited significant activity to all bacteria except for the fungi, *C. albicans*. Separately, Armes and coworkers published two articles detailing the synthesis and antimicrobial activity of a biocompatible thermo-responsive PHPMA-(PMPC-S-S-PMPC)-PHPMA triblock copolymer gelator (PHPMA = poly(2-hydroxypropyl methacrylate) and PMPC = poly(2-(methacryloyloxy)ethyl phosphorylcholine)) incorporating a cleavable disulfide bond [97,98]. This particular hydrogel produced an inhibitory zone that was six times greater than that of the control (broth without the copolymer) against *S. aureus*. The authors also demonstrated the biocompatibility and non-toxicity on a tissue-engineered skin model, arguing that these gels are potentially useful antimicrobial wound dressing materials. La and coworkers synthesized hydrogel coatings for water purification membranes having bifunctional antifouling and antimicrobial activities [99]. These materials were prepared by the photopolymerization of poly(ethylene glycol) diacrylate and an ammonium-functionalized methacrylate monomer in the presence of a photoinitiator. Poly(sulfone)s ultrafiltration membrane coated with these super-absorbent hydrogels exhibited excellent antifouling behavior. The hydrogel materials were challenged with *E. coli* and the best hydrogel coating (containing 33% ammonium moiety) inhibited *E. coli* growth effectively.

In addition to acrylate/methacrylate systems, poly(allylamine) can also be easily modified into antimicrobial hydrogels by simple crosslinking. Indeed, Andrews et al. illustrated this concept beautifully by using aldaric acid derivatives, such as diethyl L-tartrate, D-glucaro-1,4:6,3-dilactone and N,N'-bis(methoxycarbonylmethyl)-D-glucaramide (D-MCMGA), as crosslinkers [100]. All hydrogels had antimicrobial activity. In particular, 0.1 wt.% hydrogels crosslinked with diethyl L-tartrate and D-MCMGA managed a complete eradication of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* with 4 h of treatment. In addition, a 0.8% aqueous dispersion of the hydrogel crosslinked with D-MCMGA gave a killing efficiency of 99.7% of *Serratia marcescens* (Gram-negative) on human hands within 5 min. This hydrogel also showed no dermal

irritation or allergic contact sensitization using a patch test. The antimicrobial activity of the hydrogels that were fabricated using different crosslinkers could not be compared as the preparation conditions such as crosslinking degree and hydrogel concentration were not fixed.

2.3. Peptide-based hydrogels

In addition to the polymer-based hydrogel systems described above, several notable peptide-based antimicrobial hydrogels have also been reported in recent years. In particular, research efforts by Schneider and Pochan have resulted in peptide hydrogels with excellent inherent antibacterial activity. For example, Salick et al. designed a β -hairpin hydrogel scaffold based on the self-assembling 20-residue peptide MAX1 (2 wt.%) for tissue regeneration purposes (Fig. 5), whereby the hydrogel itself possessed intrinsic broad-spectrum antibacterial activity [101]. This is likely a result of the polycationic, lysine-rich hydrogel surface having the ability to disrupt negatively-charged bacterial cell membranes via electrostatic interactions. Potent activity was observed against both Gram-positive (*S. aureus*, *S. epidermidis*, *S. pyogenes*) and Gram-negative (*E. coli*, *K. pneumoniae*) bacteria. Hemolysis experiments showed the hydrogel surfaces to be non-toxic toward human red blood cells. Additionally, co-culture experiments also showed that the hydrogel surface permitted mammalian cell adhesion and proliferation while simultaneously inhibiting bacterial growth. The inherent antibacterial properties of the hydrogel, combined with its high selectivity, make the MAX1 hydrogel an excellent candidate for tissue regeneration applications.

Following the development of the MAX1 hydrogel, the same group reported an injectable β -hairpin hydrogel based on the 20-residue peptide MARG1 (Fig. 6), which is capable of killing methicillin-resistant *S. aureus* (MRSA) on contact [102]. The gel was found to be more effective than MAX1 in inhibiting the proliferation of this drug-resistant strain of *S. aureus* on surfaces. The higher activity towards MRSA likely stems from the inclusion of arginine residues in addition to lysine, which may bring about specific interactions with the bacteria. Like MAX1, the MARG1 hydrogel was also found to be selectively toxic to

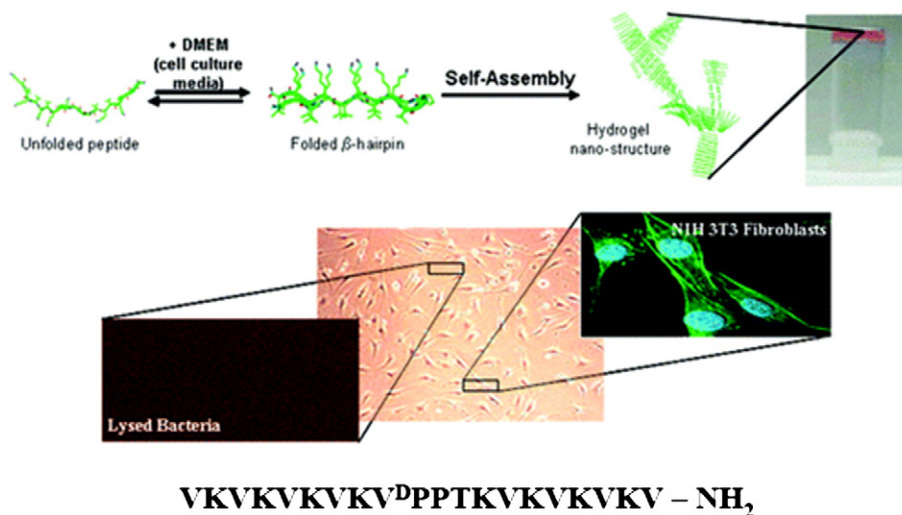


Fig. 5. Folding and self-assembly of the antibacterial MAX1 hydrogel. Reprinted from Ref. [101] with permission from the American Chemical Society, Copyright (2007).

bacterial cells over mammalian cells. MARG1 folds into an amphiphilic β -hairpin that in turn self-assembles into a mechanically rigid β -sheet network. More importantly, the rigid hydrogel exhibits shear-thinning/recovery behavior. Upon application of a shear stress, the hydrogel transforms into a low-viscosity gel that flows, allowing for convenient delivery. Mechanical rigidity of the gel is restored once the shear stress is removed. This property makes the MARG1 hydrogel ideal for delivery to target sites via syringe.

The role of arginine in antibacterial peptide hydrogels was further investigated more recently by Schneider. In that work, systematic structure–activity relationship studies on a series of peptides showed how the antibacterial and rheological properties of the bulk materials were influenced by their arginine content [103]. Specifically, four 20-residue peptides (PEP2R, PEP4R, PEP6R, and PEP8R) containing between two to eight arginine residues respectively were synthesized and tested for their antibacterial, hemolytic, and material rheological properties.

When four or more arginines were present, the peptides were equally active against *S. aureus* and *E. coli* at all weight percentages. With only two arginine residues however, PEP2R showed lower activity against *E. coli* (Fig. 7A, B). Regarding toxicity, cell assays indicated a marked decrease in hemolytic activity with decreasing arginine content, while antibacterial activity diminished slightly as arginine residues were replaced by lysines. In terms of rheological properties, arginine

was found to be important in influencing hydrogel rigidity. An increase in storage modulus (G') was observed when going from eight to six arginine residues, but further lowering of the arginine content resulted in compromised gel mechanical properties. The results of these studies revealed PEP6R to be the optimal hydrogel, with excellent activity against *E. coli*, *S. aureus*, and multi-drug resistant *P. aeruginosa*. The hydrogel is also cytocompatible with mesenchymal stem cells, and only marginally hemolytic. The shear-thinning/recovery behavior of the gel also makes it amenable for delivery via syringe (Fig. 7C, D).

Recently, Liu et al. also designed a Gram-positive antibacterial peptide-containing hydrogel material that self-assembled in response to external stimuli such as pH, ionic strength, and heat [104]. The basic building block used in the work consisted of two antibacterial peptide sequences that were connected by a central tetrapeptide linker. Exposure of the peptide to the aforementioned stimuli (i.e. increasing pH, ionic strength, or temperature) triggered self-assembly into β -sheets, ultimately leading to hydrogel formation. The authors proposed that external stimuli could be used to shift the balance between the effects of electrostatic repulsion, hydrophobic interactions, and hydrogen bonding to favor self-assembly of the peptide (Fig. 8). The antibacterial activity of the hydrogels was evaluated against *E. coli*, and exhibited significant inhibition of bacterial growth when compared to polystyrene controls, although no quantitative measurements were given.

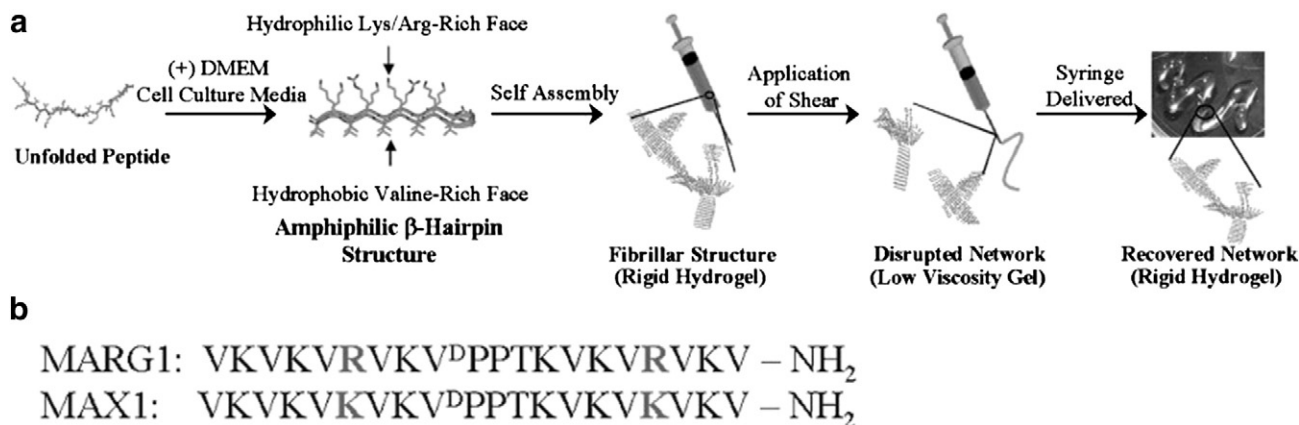


Fig. 6. (A) Folding and self-assembly of the antibacterial MARG1 hydrogel triggered by DMEM cell culture media, followed by shear-thin delivery via syringe. (B) Amino acid sequences of MARG1 and MAX1. Reprinted from Ref. [102] with permission from John Wiley and Sons, Copyright (2009).

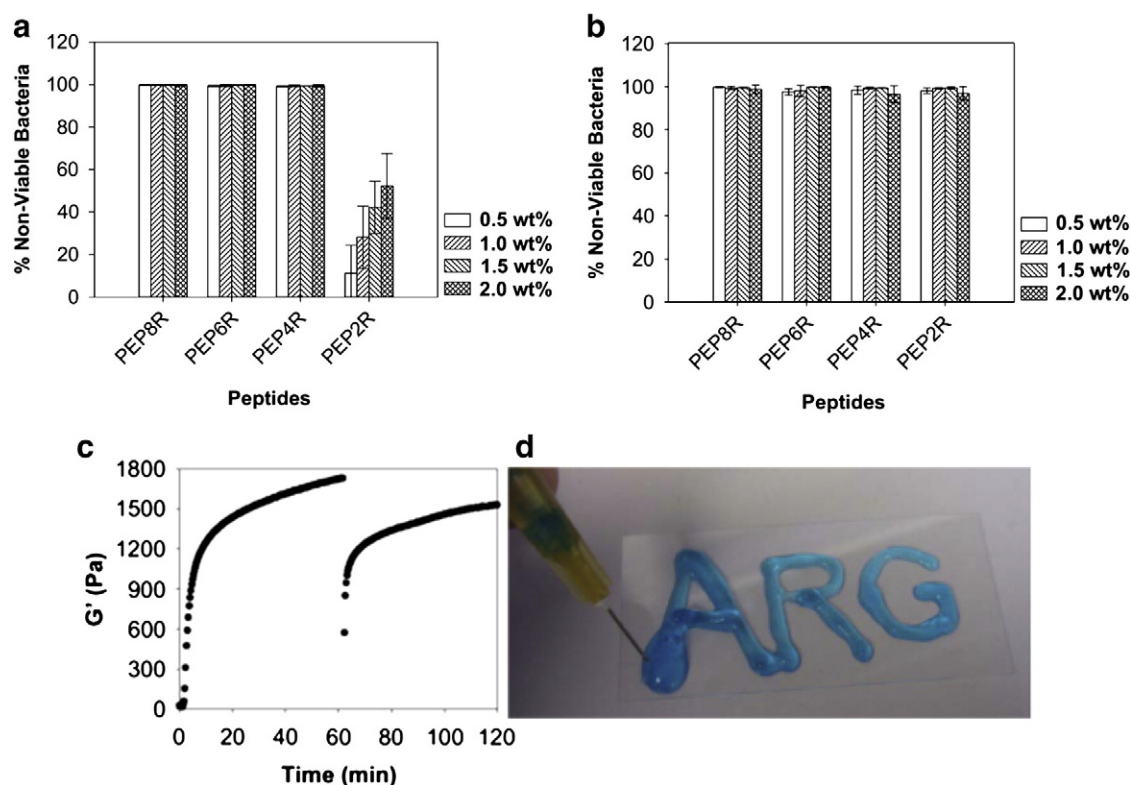


Fig. 7. Antibacterial activity of the hydrogel surfaces against *E. coli* (A) and *S. aureus* (B) after 24 h incubation at 37 °C. Percent non-viable bacteria is reported for 0.5, 1, 1.5, and 2 wt.% hydrogels ($n = 3$). (C) Shear-thinning/recovery behavior of 1 wt.% PEP6R hydrogel at 37 °C. (D) Photograph of shear-thin deposited (by syringe) 1 wt.% PEP6R hydrogel. Reprinted from Ref. [103] with permission from Elsevier, Copyright (2012).

Debnath et al. reported a class of Fmoc-protected peptide hydrogelators that contained terminal pyridinium moieties, which are known to possess antibacterial properties due to their propensity for penetrating cell membranes. All of the peptide amphiphiles studied, which ranged in composition and structure from those containing only aliphatic amino acids (e.g. Ile) to those with only aromatic residues (e.g. Phe) and combinations thereof, were efficient hydrogelators that exhibited minimum gelation concentrations of ~0.6–2.2 wt.%. The peptide hydrogels were also studied using a suite of spectroscopic and microscopic techniques, which revealed that a combination of

intermolecular hydrogen bonding and π - π stacking interactions were implicated in the formation of a complex, three-dimensional gel network. All of the peptide hydrogels tested were effective at killing both Gram-positive and Gram-negative bacteria [105].

A related yet orthogonal approach toward antimicrobial peptide hydrogels was demonstrated by Xu et al. [106] and, more recently, by Hughes et al. [107], who exploited enzymatic hydrolysis mechanisms inside *E. coli* cells to trigger an intracellular molecular self-assembly of amphiphilic peptide hydrogelators. The formation of a hydrogel within the cell was hypothesized to change the viscosity of the cytoplasm,

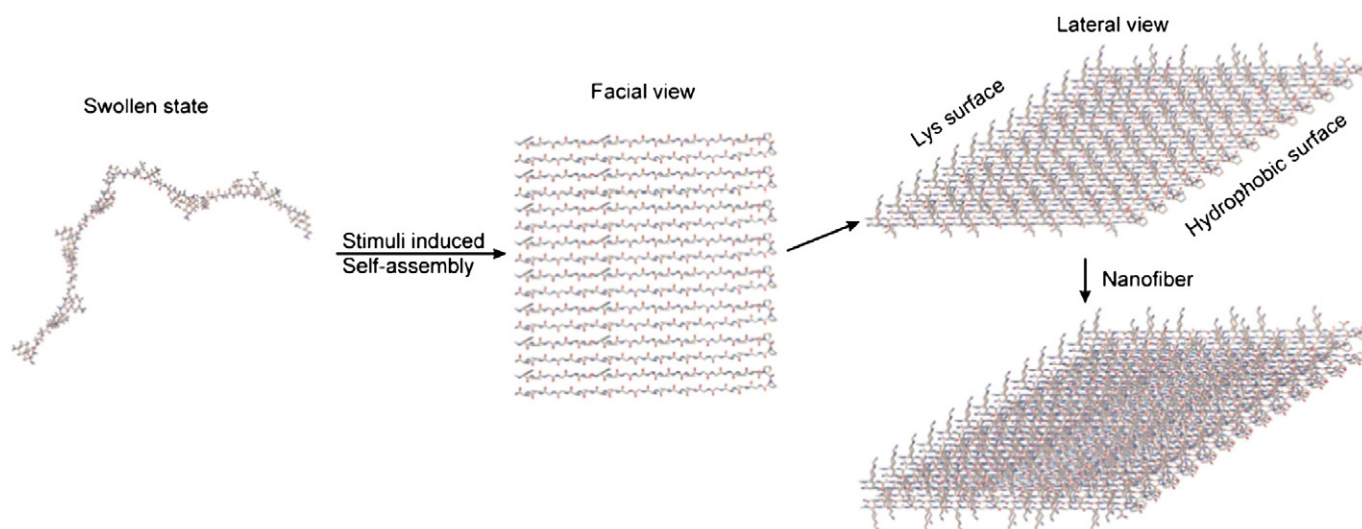


Fig. 8. Proposed molecular model of stimuli-induced self-assembly of a peptide (i.e. tetrapeptide linked (KIGAKI)₃-NH₂) to form a hydrogel with antimicrobial properties. Reprinted from Ref. [104] with permission from the Royal Society of Chemistry, Copyright (2013).

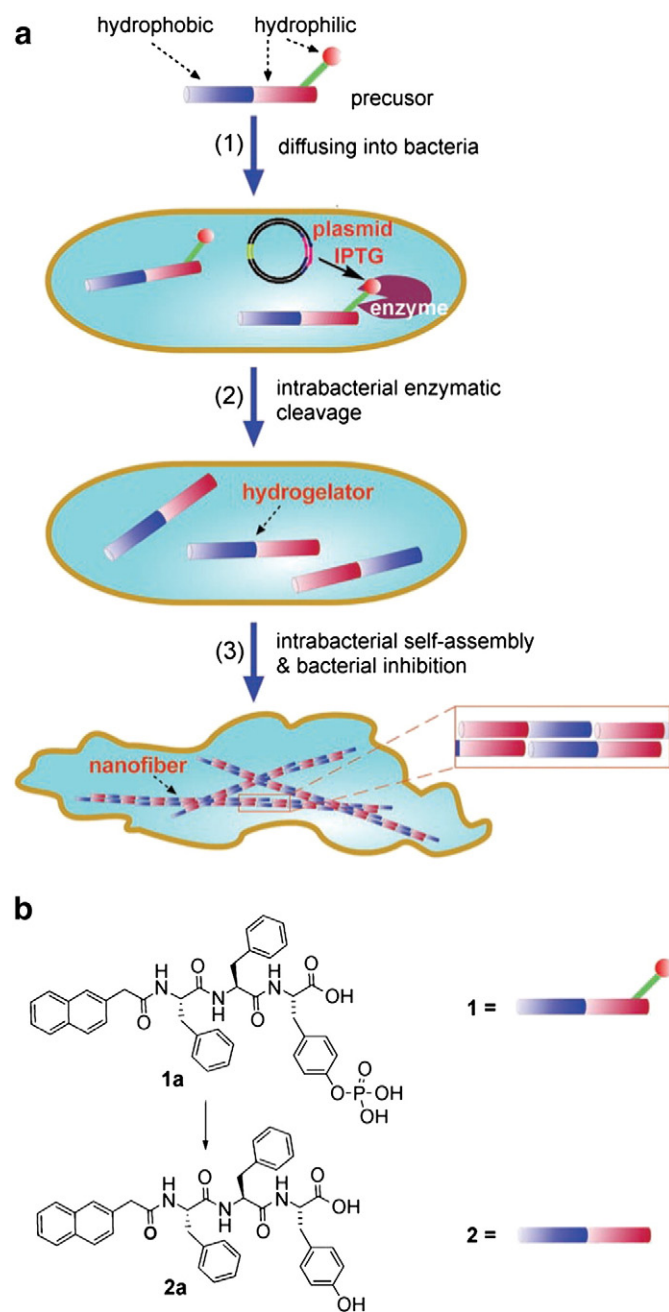


Fig. 9. (a) Scheme outlining the enzymatically-triggered intracellular hydrogelation of an amphiphilic small molecule and subsequent inhibition of cell growth. (b) Chemical structures and cartoon representations of a phosphorylated precursor to a hydrogelator (1a) and the corresponding hydrogelator (2a). Reprinted from Ref. [106] with permission from John Wiley and Sons, Copyright (2007).

leading to cell stress and subsequent inhibition of bacterial growth. Schematically, as shown in Fig. 9, a phosphorylated amphiphilic peptide serving as the precursor to the hydrogelator enters the cell via diffusion, whereby it is enzymatically dephosphorylated (e.g. by a tyrosine phosphatase) to generate a more hydrophobic molecule that self-assembles into a hydrogel, leading to cell growth inhibition. Notably, Xu and co-workers confirmed via HPLC analyses that the hydrogelator successfully accumulated inside the bacteria, as exemplified by a nearly ten-fold increase in intracellular concentration of the hydrogelator in comparison to that found in the culture medium, as well as successful enzymatic cleavage of the hydrogelator. Antibacterial activity (IC_{50}) of the intracellular hydrogels was determined to be $20 \mu\text{g mL}^{-1}$ against bacteria that overexpressed phosphatase. However, this value was more than

100-fold higher against bacteria without phosphatase overexpression. Nevertheless, the reported work demonstrates the potential for targeted delivery of antimicrobial agents and opens up avenues for tailored hydrogel formation, e.g. through the use of multiple enzymatic triggers.

Zhou et al. modified epsilon-poly-L-lysine (EPL), an antimicrobial peptide produced by *Streptomyces albulus*, with methacrylamide moieties, which were then crosslinked with PEG diacrylate to form antibacterial hydrogels (Fig. 10) [108]. EPL was selected for its edibility, non-toxicity, biodegradability, and low production costs. The polymer synthesis is straightforward and utilizes cheap commercially-available starting materials. The resulting materials were found to exhibit excellent activity against a number of clinically relevant bacteria and fungi, including *E. coli*, *P. aeruginosa*, and *S. aureus*, among others. Furthermore, the hydrogels were found to be biocompatible with primary human epidermal keratinocytes (*in vitro*), exhibited low hemolytic activity, and could be immobilized onto plastic surfaces via UV-generated surface radicals crosslinked to free acrylates/methacrylates, making them excellent candidates for use as antimicrobial coatings for medical devices and implants.

Song et al. developed all-synthetic polypeptide hydrogels with antibacterial activity by crosslinking poly(Lys)_x(Ala)_y copolymers with six-armed *N*-hydroxysuccinimide (NHS) terminated PEG [109]. Poly(Lys)_x(Ala)_y of varying compositions were synthesized via ring-opening polymerization (ROP) of the respective α -amino acid *N*-carboxyanhydride (NCA) monomers. In addition to the presence of Lys, which is known to inhibit bacterial growth, the authors reasoned that copolymerization of the former with Ala would allow for tuning of the cationic/hydrophobic balance of the resulting copolymers, which can facilitate binding and insertion into the targeted bacterial membranes. Out of all the formulations that were tested, the authors found that hydrogels comprising poly(Lys)₆₀(Ala)₄₀ exhibited superior cell adhesion and cell proliferation activities whilst maintaining antibacterial activity against *E. coli* and *S. aureus*. The authors also found the gelation and antimicrobial properties to be highly dependent on the concentration of the PEG crosslinker: lower PEG concentrations resulted in no gelation, while higher crosslinker concentrations resulted in loss of antibacterial activity. Further, linear two-armed NHS-terminated PEG crosslinkers used in place of the aforementioned six-armed variant were unable to induce gelation, design features which should not be overlooked in the preparation of related hydrogel materials.

2.4. Functional mechanism of hydrogels having antimicrobial activity

Unlike antimicrobial peptides/cationic polymers in solution, the antimicrobial mechanism of hydrogels with intrinsic antimicrobial activity is not fully understood. From reported SEM analyses, [50,87,108,110] it is likely based on bacterial cell wall/membrane lysis. The anionic bacterial cells are attracted to the cationic nanoporous hydrogels through electrostatic interaction, leading to subsequent cell wall disruption, membrane lysis and ultimately cell death. In addition to the SEM images illustrating highly distorted cellular envelopes for various microbes, Chan and coworkers also carried out computer molecular dynamic simulations to further substantiate this hypothesis [50]. It is thus not surprising that the antimicrobial activity of these hydrogels depends greatly on (1) cationic charges on the polymers, (2) amphiphilicity of the polymers, (3) porosity of the hydrogels, and (4) permeability of the microbial membrane.

3. Hydrogel containing antimicrobial polymers and peptides

The use of a hydrogel to deliver macromolecular antimicrobials has been largely understudied and is an area of research that has yet to reach its full potential. The use of hydrogels to deliver antibiotics has been demonstrated with limited success; however, a few reports have

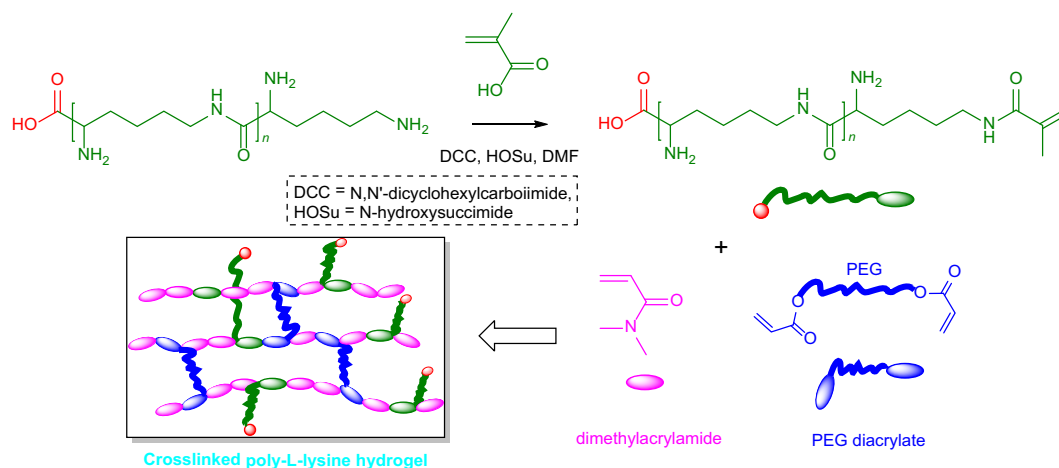


Fig. 10. Synthesis of chemically crosslinked ϵ -poly-L-lysine hydrogel.

focused on the delivery of polymer-based antimicrobial hydrogels. The applications for such molecular composites are diverse and range from the treatment of skin infections and acne to their use as medical device coatings. Interestingly, many medical devices such as contact lenses, intravascular catheters and prosthetic implants utilize hydrogel materials that succumb to microbial attachment or biofilm formation. Therefore, the encapsulation of antimicrobial macromolecules in the functional hydrogel may serve to mitigate biofilm formation. The generation of an effective antimicrobial hydrogel necessitates a thorough understanding of how planktonic cells form biofilms. The deposition of a biofilm begins with the adherence of planktonic cells on an inanimate surface such as a medical device, followed by the anchoring of these cells

to the surface via the production of exopolymers. Microcolonies appear as proliferation occurs along with this polymer matrix, ultimately leading to biofilm formation. The most common pathogens associated with biofilm-induced chronic infections include *S. aureus*, *S. epidermidis*, *S. enteropathogenic*, *E. coli* and *C. albicans*.

Lavery and coworkers reported the use of antimicrobial peptides incorporated into poly(2-hydroxyethyl methacrylate) hydrogel with the objective of preventing *S. epidermidis*-associated biomaterial infections [111]. *S. epidermidis* is a major organism in the infection of peritoneal dialysis and intravascular catheters, which are used in environments conducive to the proliferation of such opportunistic pathogens. The incorporation of several antimicrobial peptides into

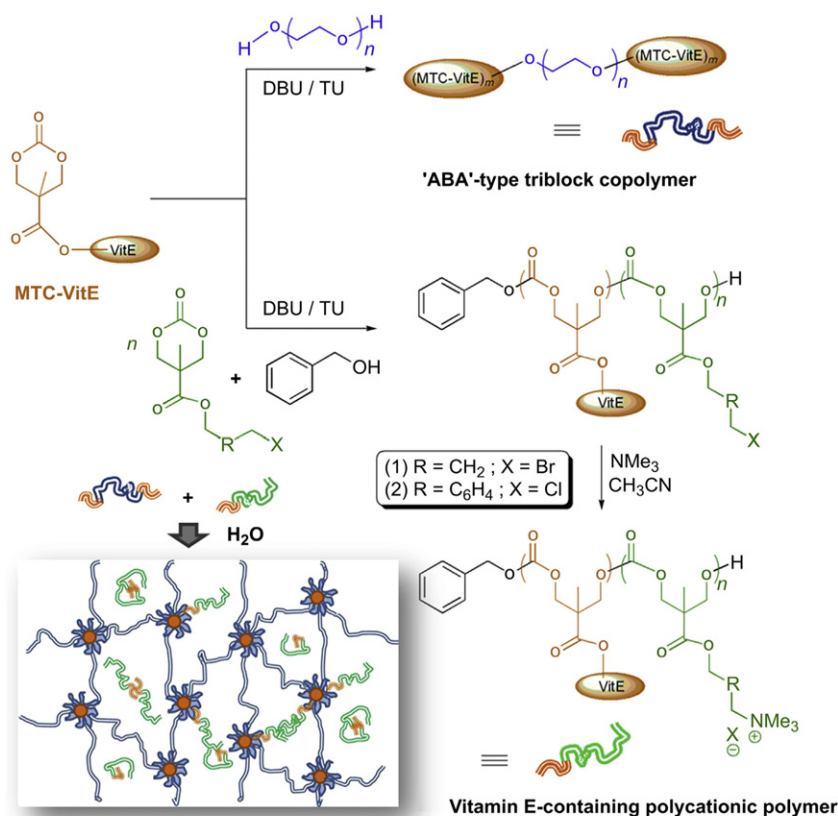


Fig. 11. Syntheses of '(MTC-VE)_n-PEG-(MTC-VE)_n' and vitamin E-containing cationic polymers, and schematic illustration of incorporating cationic polymers into hydrogel system (inset). Reprinted from Ref. [111] with permission from Elsevier, Copyright (2013).

poly(2-hydroxyethyl methacrylate) hydrogels was compared with vancomycin-loaded hydrogel. The cationic antimicrobial peptides in the anionic hydrogels showed controlled and sustained release of the antimicrobial agent, whereas vancomycin was released much faster from the hydrogel. The electrostatic interactions associated with the peptide and the hydrogel are believed to be responsible for the controlled release of the drug cargo. The ability of the microbes to adhere to the biofilms was found to be highly dependent on the drug release rate as well as the inherent MICs of the antimicrobial agent in question. The antimicrobial peptide-loaded hydrogels were found to be extremely effective against biofilm adhesion relative to vancomycin, likely due to the physical mode of action involving membrane disruption. Clearly, this report shows that antimicrobial peptides can function as potent weapons against biomaterial/device-associated infections.

Recently, Lee et al. reported the modification of a hydrogel with a synthetic antimicrobial as a means to produce broad-spectrum antimicrobial gels with a unique ability to disperse mature biofilms [112]. These hydrogels are comprised of a mixture of an 'ABA'-type triblock copolymer, consisting of a hydrophilic PEG middle block flanked on both ends by hydrophobic vitamin E-functionalized polycarbonate blocks and the second component is a biocidal cationic polycarbonate that possesses vitamin E moieties (Fig. 11). Both materials were prepared via the ring-opening polymerization of functionalized cyclic carbonate monomers. The supramolecular assembly and formation of these hydrogels are based on hydrophobic interactions between the vitamin E-functionalized polycarbonate blocks. They were shown to have unique shear-thinning behavior or thixotropic behavior, allowing for deposition through a syringe or topical applications. Antimicrobial efficacy of these physically crosslinked hydrogels were studied against *S. aureus* and *E. coli* bacteria as well as *C. albicans*. They achieved more than 99.9% killing efficiency of these microbes upon contact. In addition, the co-delivery of antimicrobial polycations with a conventional antifungal agent, fluconazole, shows a high degree of synergistic antimicrobial effects on *C. albicans*. From metabolic assessments, biomass removal and scanning electron microscopy studies (Fig. 12), the hydrogels were found to be capable of eradicating biofilms. The cytotoxicity of the hydrogels loaded with cationic polymers and/or fluconazole at minimum biocidal concentrations (MBC) was evaluated against human dermal fibroblasts, and no significant toxicity was observed.

4. Hydrogel containing antibiotics

Although antimicrobial hydrogels-based systems have shown incredible efficacy in breaking down biofilms and destroying multidrug-resistant microbes upon contact, the interactions between the antimicrobial polymers and microbial cell membranes are non-specific, and in most cases the antimicrobial hydrogels also result in mammalian cell death above certain concentrations. Therefore, many applications still require the incorporation of antibiotics in combination with hydrogels to fight bacterial infections without compromising human cell viability. Antibiotics selectively kill bacteria and are considered one of the most powerful medicines that fight bacterial infections. Penicillins and cephalosporins are the most widely used antibiotics followed by fluoroquinolones [113]. However, their overuse and misuse have led to an increasing number of resistant pathogens.

Recent studies have shown that a combination of synthetic antimicrobial polymers and antibiotics could potentially evade problems of drug resistance by virtue of the polymer's membrane-lytic mechanism while mitigating polymer toxicity since the co-usage of antibiotics allows for a smaller amount of polymer to be used. Thus, Ng et al. have shown that combining cationic antimicrobial polycarbonates with conventional antibiotics such as penicillin G, doxycycline or streptomycin was able to effectively kill multidrug-resistant *P. aeruginosa* [84]. This opportunistic pathogen is associated with nosocomial infections and its treatment with conventional antibiotics is difficult due to the limited permeability of the bacterial cell membrane. The authors showed that the polymer could not only increase the membrane permeability and facilitate the penetration of small molecule antibiotic, but also lower the minimum bactericidal concentration of both polymer and antibiotics to kill *P. aeruginosa* without causing significant hemolysis. In a similar fashion, Sung et al. combined PVA/chitosan hydrogels together with minocycline, a broad-spectrum antibiotic [114], and demonstrated improved wound healing effects due to both minocycline and the antifungal effect of chitosan. Specifically, they found improved wound healing behavior when the hydrogel was composed of 5% PVA, 0.75% chitosan and 0.25% of minocycline.

In spite of the advantages of utilizing antimicrobial hydrogels for the delivery of antibiotics, in most of the studies, hydrogels themselves do not possess any antimicrobial activity and they are designed to offer other advantages such as low toxicity, site-specific drug delivery, and

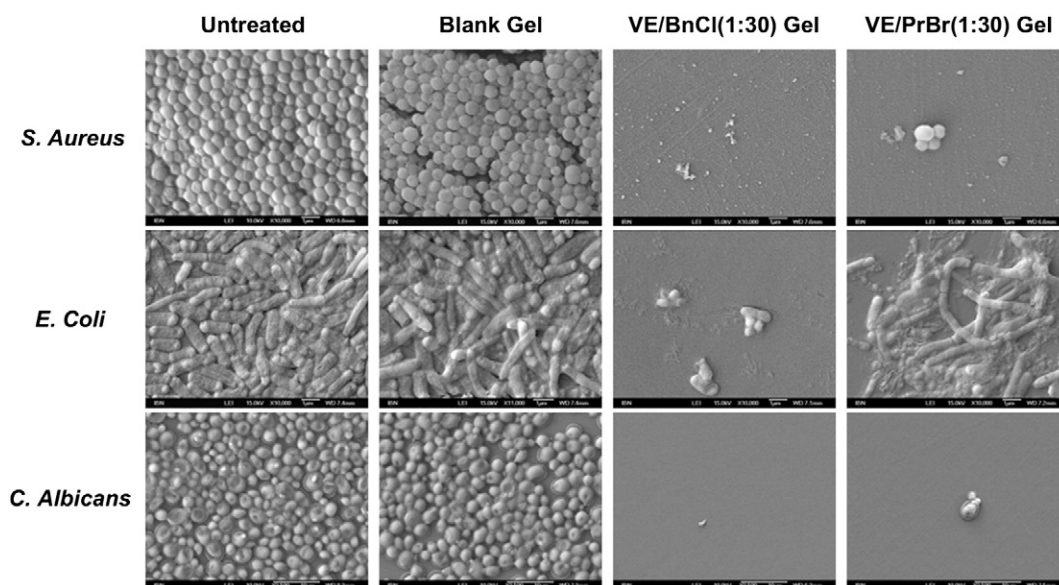


Fig. 12. SEM images of biofilms treated with polycation-loaded (MTC-VE)_{1.25}-PEG(20 k)-(MTC-VE)_{1.25} (4 wt.%) hydrogels. Reprinted from Ref. [111] with permission from Elsevier, Copyright (2013).

controlled degradation. Several polysaccharides have been broadly used for the delivery of therapeutics due to their biocompatibility and biodegradability [115]. For example, Zumbuehl et al. prepared dextran-based hydrogels containing the broad-spectrum antifungal drug amphotericin B. Amphotericin B-loaded hydrogels killed *C. albicans* efficiently within two hours without causing hemolysis [116]. Along the same lines, Glisoni et al. prepared β -chitosan dextran-based hydrogels for the ocular delivery of thiosemicarbazones [117]. Thiosemicarbazone-loaded hydrogels successfully inhibited the growth of *P. aeruginosa* and *S. aureus*, which are two types of bacteria commonly associated with ocular infections.

Beside the polysaccharides, the poly(acrylate)s have been also employed as carriers for the site-specific delivery of antibiotics, despite their lack of biodegradability. For instance, Jones et al. investigated a series of hydrogels prepared via the copolymerization of *N*-isopropylacrylamide and methacrylic acid with different hydroxymethacrylates, with the goal of delivering chlorhexidine diacetate [118]. They found that when the *N*-isopropylacrylamide was copolymerized with 2-hydroxyethyl methacrylate in a 1:1 ratio, an accelerated release of the antibiotic was achieved at 37 °C, resulting in pronounced antimicrobial activity against *S. epidermidis* (Gram-positive). In 2010, Jiang and coworkers reported on a series of integrated antimicrobial and antifouling hydrogels that prevented the growth of planktonic bacterial cells while keeping the surfaces clean [119]. In this work, a mild antimicrobial agent (salicylate) was incorporated into a poly(carboxybetaine) (pCB)-containing methacrylate-based chemically-crosslinked hydrogel as its anion. The hydrogel system was found to prevent the proliferation of both *S. epidermidis* and *E. coli* by 99.9%, making it useful as wound dressings and surface coatings for biomedical equipment. Notably, pCB-based materials without salicylate could only reduce/delay microbial attachment but they inhibited or killed the pathogenic bacteria in the presence of salicylate. In a follow-up investigation, they made use of this concept and modified the methacrylate-based chemically-crosslinked hydrogel into a thermo-responsive antimicrobial wound dressing material by incorporating the popular temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PNIPAM) (Fig. 13) [120]. The incorporation of NIPAM allows thermo-responsiveness towards in situ gelation, a convenient and desirable feature for wound dressing application. Again, one of the salicylate-containing hydrogel completely inhibited *E. coli* growth and also showed negligible cytotoxicity on mammalian fibroblast cell line COS-

7. In both instances, the cationic betaine esters can be hydrolyzed to its zwitterionic configuration, which is known to be anti-fouling thus prevents the formation of pathogenic biofilms.

Similarly, Zhao et al. developed poly(*N*-hydroxyethylacrylamide)/salicylate hydrogels with antimicrobial and antifouling activity [121]. Salicylate is a natural antibacterial compound produced by many plants. They evaluated the antimicrobial properties of the hydrogels against *E. coli* RP437 and *S. epidermidis*, and they found that when the salicylate loading was around 80% (w/w), poly(*N*-hydroxyethylacrylamide) hydrogels inhibited the growth of *S. epidermidis* and *E. coli* RP437 by 97–98%. Recently, Tan et al. also developed seaweed-containing PVA/PVP hydrogel. The seaweed extract has shown activity against clinically relevant wound pathogens such as *S. aureus* (both methicillin-susceptible and resistant strains), *E. cloacae*, and *C. perfringens* [122]. They found that seaweed was effective in inhibiting 70–90% of the bacterial population in the first 30 min of application.

In addition to the non-responsive hydrogels, in some cases stimuli-sensitive hydrogels are employed for the controlled delivery of antibiotics. Thus, Lai et al. synthesized thermosensitive hydrogels based on methoxypoly(ethylene glycol) and aromatic anhydride for cefazolin delivery [123]. They found that these gels exhibited extraordinary aqueous transition temperatures having three different physical states in the range of 5 to 60 °C, i.e. solution, gel, and precipitate. Hence, at low temperature, cefazolin could be completely encapsulated by the hydrogels and it could be released via a diffusion-controlled mechanism over four weeks. These synthesized hydrogels showed effective antibacterial action against *E. coli*.

5. Hydrogel containing antimicrobial metal nanoparticles

Polymer-supported silver ions for use as antimicrobials in consumer products are of current interest for the control of bacterial growth on surfaces. The use of silver as an antimicrobial dates back centuries, when solutions of silver salts were commonly consumed for the treatment of illness. Although silver ions exhibit bactericidal properties against microorganisms, silver treatments also cause apoptosis and necrosis of mammalian cells. In addition, when ingested, silver causes argyria (a cosmetic condition that causes irreversible grey-blue discoloration of the dermis) in humans. Recently, there have been substantial advances towards the use of silver ions and silver nanoparticles (AgNPs) in hydrogels for wound treatment, and thus efforts have been made to

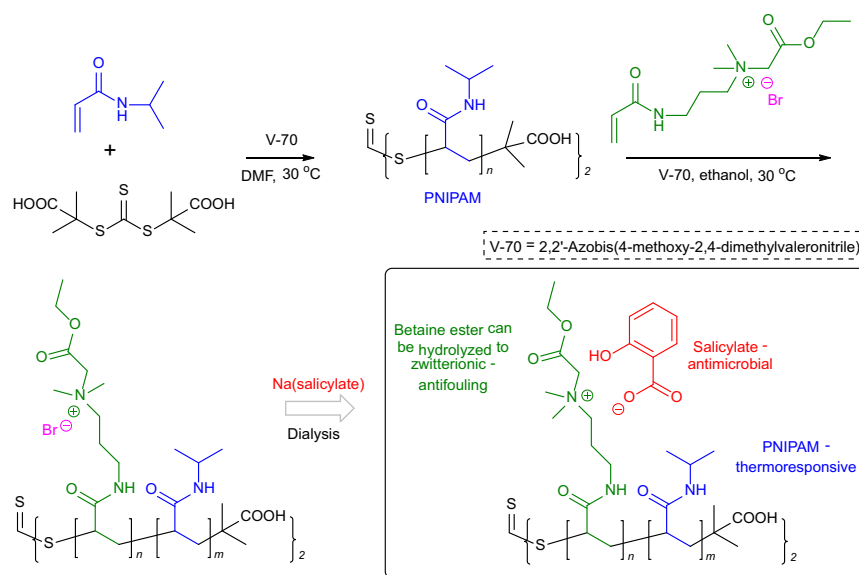


Fig. 13. Synthesis of thermo-responsive wound dressing copolymers.

modulate toxicity. The antimicrobial activity of silver and other metal salts has been attributed to the metal cation causing cell membrane disruption and subsequent lysis. Previously, treatments involving silver ions had been known to achieve bacteriostatic activity at concentrations as low as 30 mg/mL [124], and potential applications for metal-containing hydrogels include the use of silver-impregnated gels for medical devices, coatings, water purification, and contact lenses [125]. Spectroscopic methods for the characterization of metal-containing hydrogels typically involve drying the gels and then performing X-ray diffraction (XRD), Fourier-transform infrared (FT-IR) spectroscopy, dynamic light scattering (DLS), UV-vis measurements, transmission electron microscopy (TEM), selected area electron diffraction (SAED), and energy dispersive X-ray spectroscopy (EDS) techniques. Approaches to determine behavior of Ag-hydrogels on microorganisms and toxicity to mammalian cells vary but scanning electron microscopy (SEM), fluorescence spectroscopy and growth inhibition of cultures are commonly employed. Nanoparticle formation and the development of in situ and ex situ reducing methods towards achieving uniform particle size and shape distributions remains an active area of research. Currently, in situ formation can involve either chemical (i.e. NaBH_4 treatment) or physical methods (gamma radiation [126] and electrochemical synthesis [127]).

5.1. Improving nanoparticle dispersion sustainably

Strategies for achieving even nanoparticle distribution and stabilization in water-insoluble, crosslinked hydrogel matrices involve modifications of the functionality so that it has a strong association with the silver particles (i.e. ionic association through an anionic moiety). Mukherji and Agnihotri developed a hydrogel that contained chitosan as a crosslinker in a poly(vinyl acetate) gel matrix [128]. They found that high percentages of crosslinker in the gel led to increased AgNP concentrations and porosity of the resulting hydrogel. AgNPs were prepared in situ by reducing silver ions incorporated into the gel matrix with sodium borohydride, after which the gels were tested for antimicrobial efficacy against *E. coli*. After 12 h, there was an 83.5% reduction in bacterial growth for the Ag-loaded hydrogel (compared to 24.4% for gel without silver). The authors reasoned that the diminished *E. coli*

growth observed in their control was likely due to charged chitosan interacting with the bacterial cell membrane.

Naturally occurring functionalized polysaccharides have likewise been studied: Raju and co-workers discovered that the sulfonate-decorated carrageenan bound AgNPs effectively and was easily integrated into a biodegradable system with acrylamide [129]. The nanoparticles were synthesized in the hydrogel through a “green” reduction process involving *Azadirachta indica* (neem leaf) extract. Additionally, the ready availability of the starting material and the ease in degradability of the hydrogel minimizes the environmental impact of the synthetic process. Hydrogels were characterized using thermal analysis and spectroscopic techniques, and subsequently tested against *Bacillus* and *E. coli* through a diffusion method (1.2 cm and 0.9 cm respectively). Biodegradability was determined by weight loss measurements of the dried samples. Similarly, styrenesulfonic acid salts have also been used as templates for AgNPs in hydrogels [130] as well as curcumin composites [131] and amino acids to improve uniform distribution [132].

A microorganism initiated “green” synthesis of supported AgNPs was presented in a study by Pawar et al. [133]. In their synthesis, *Rhodococcus* NCIM 2891 was added to a sodium alginate solution and precipitated into CaCl_2 in order to form hardened 2-mm beads. The Ag-alginate biohydrogel was prepared through the addition of a silver nitrate solution and the resulting gel was analyzed by XRD, DLS, and FT-IR. A diffusion assay was then utilized to study the effects on Gram-positive erythromycin-resistant *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*. Gram-negative strains evaluated were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and the authors also evaluated their gels for antifungal activity against *Penicillium chrysogenum*, *Aspergillus oryzae*, *Candida albicans*, *Candida tropicalis*, *Candida krusei* and *Candida lusitanae*. The zone of inhibition for each strain of microbes was measured and SEM images were taken of the changes in the organisms before and after treatment. The gel was effective against all strains tested, albeit the fungi generally had smaller zones of inhibition compared to the bacterial strains (2–8 mm for fungal stains compared to 8–13 mm for bacterial strains). The Gram-negative bacteria were more sensitive to the AgNPs than the Gram-positive bacteria. Notably, the fungus *C. albicans* showed a zone of inhibition of 20 mm, higher than all other microorganisms tested. SEM images were captured of

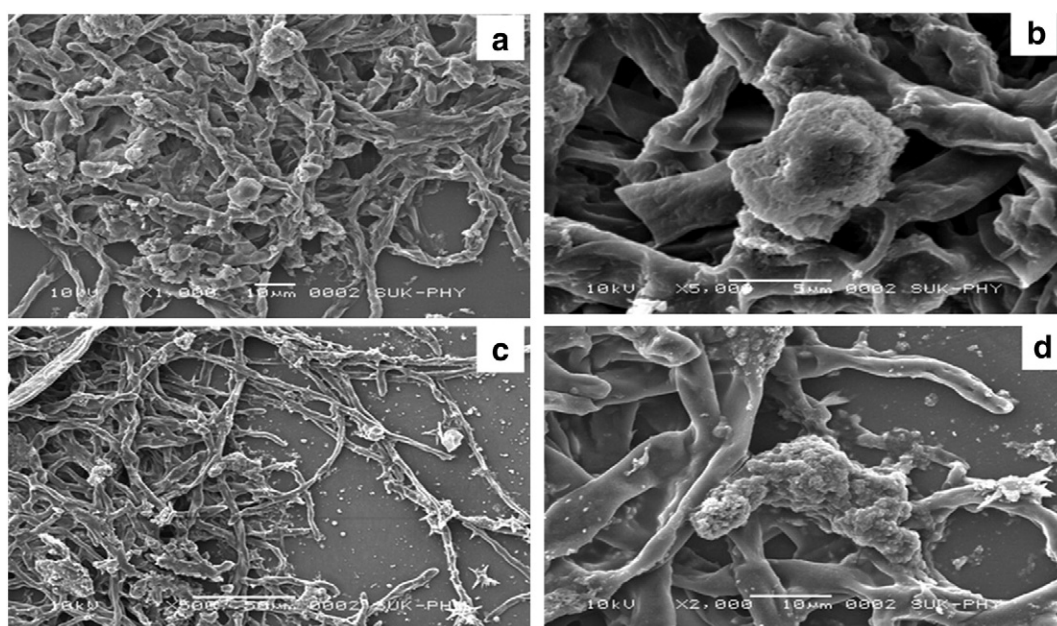


Fig. 14. SEM images of *Penicillium chrysogenum* in the absence (a, b) and in the presence of Ag–Alginate biohydrogel (c, d). Reprinted from Ref. [132] with permission from the Royal Society of Chemistry, Copyright (2013).

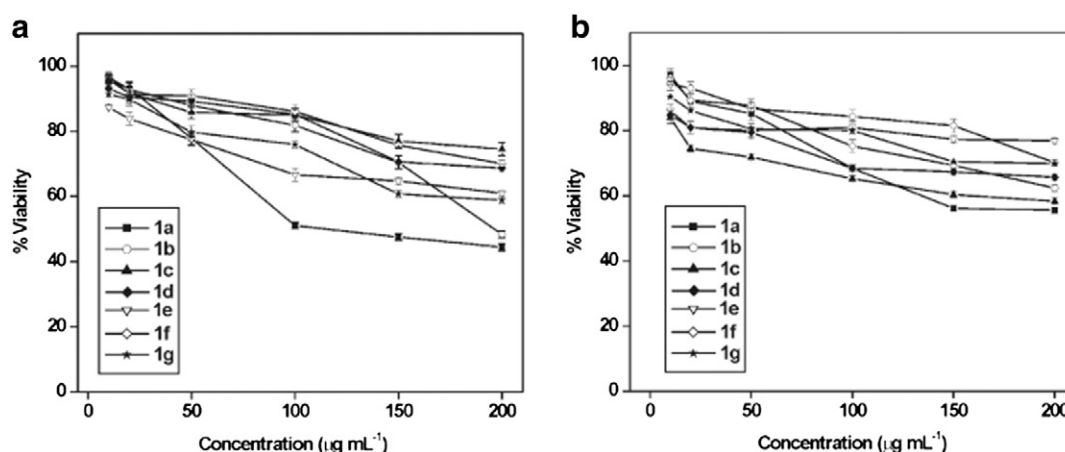


Fig. 15. Viability (%) of (a) HepG2 and (b) NIH3T3 cells using a variety of different counterions (1a is chloride, 1d corresponds to *n*-hexanoate anion). Reprinted from Ref. [133] with permission from the American Chemical Society, Copyright (2011).

P. chrysogenum before and after treatment and showed that the fungal hyphae was damaged in the presence of AgNPs and as well as the spores (Fig. 14). The authors hypothesize that the AgNPs modulate tyrosine phosphorylation once inside the cell, leading to the inhibition of cell division.

5.2. Reducing toxicity of silver nanoparticles in hydrogels

In addition to solving problems associated with nanoparticle dispersion, additives within the hydrogel matrix can greatly reduce AgNP toxicity to mammalian cells while maintaining antimicrobial activity. For example, the counterion of positively-charged hydrophilic hydrogelators affected antimicrobial activity in AgNP hydrogels [134]. Das et al. found that exchanging the counterion of a positively-charged amino acid-based hydrogelator from chloride to a hydrophobic carboxylate influenced the MIC against Gram-positive bacterial and fungal strains. The MIC for Gram-positive *B. subtilis* decreased from 10.0 to 2.0 $\mu\text{g/mL}$ when the chloride was exchanged for *n*-hexanoate and the fungus *S. cerevisiae* was less sensitive to the counterion effect, however the MIC still decreased from 10.0 to 8.0 $\mu\text{g/mL}$. Additionally, they found that the toxicity towards HepG2 and NIH3T3 mammalian cells was diminished, as determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assays (Fig. 15).

The toxic effects of silver nanoparticles (AgNP) were addressed by Grade et al. through the use of the addition of bovine serum albumin (BSA) [135]. BSA is a protein that has been identified for its silver ion binding properties, and a solution of 1% BSA was used in conjunction with AgNPs to reduce the toxicity to human gingival fibroblasts

(HGFib) while maintaining antimicrobial activity against *S. aureus*, *S. salivarius*, *E. coli*, and *P. aeruginosa* (Fig. 16).

5.3. Additives for enhancing hydrogel properties

Silver-containing hydrogels synthesized with salt additives were found to be effective treatments for chronic wounds [136]. The term “chronic” is meant to differentiate common injuries from wounds that cause prolonged inflammation and have poor healing rates. Antimicrobial hydrogels are ideally suited for chronic wound treatment, since they keep the wound hydrated while also delivering treatment. McBain et al. explored the use of adding a known preservative and blood clotting agent, sodium hexametaphosphate, to a hydrogel containing silver ions as an additional defense against *P. aeruginosa*, *S. aureus*, *Salmonella typhimurium* and *Clostridium spp.* bacteria. Hydrogels containing mixtures of silver with the polyphosphate additive were evaluated for bacteriostatic activity against *S. aureus*, *P. aeruginosa*, and *C. albicans* cultures. In most cases, the hydrogels that contained the additive decreased the MIC, although some strains were less influenced by the presence of the salt additive. For example, the MIC for *S. aureus* for a solution of silver nitrate was 30 mg/L and decreased to 9 mg/L with sodium hexametaphosphate present, whereas *C. albicans* showed a slight increase in MIC from 70 to 80 mg/L. The treatment was also effective towards biofilms albeit at higher minimum biofilm eradication concentrations (MBECs). For comparison, the MBEC for *S. aureus* to a solution of silver nitrate was 7500 mg/L, which reduced to 3130 mg/L, and the MBEC for *C. albicans* decreased from 40,000 mg/L in a silver nitrate solution to 1250 mg/L with the polyphosphate salt present. The blend of the polyphosphate and silver salts in the gel

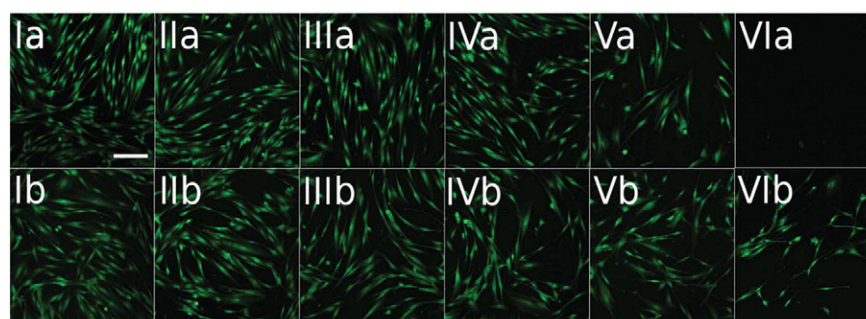


Fig. 16. Calcein-stained human gingival fibroblasts treated with different concentrations of silver nanoparticles; I) control, II) 5 mg mL^{-1} , III) 10 mg mL^{-1} , IV) 20 mg mL^{-1} , V) 35 mg mL^{-1} , VI) 70 mg mL^{-1} ; (a) without BSA, (b) with 1% (w/v) BSA. Pictures obtained by confocal laser scanning microscopy (20 \times objective), scale bar = 200 nm. Reprinted from Ref. [134] with permission from the Royal Society of Chemistry, Copyright (2013).

were speculated to improve tissue health during treatment due to the acceleration of blood clotting, as well as reducing fibrinolysis and elastase and collagenase activity [137,138]. Other studies have focused on the use of metal ions additives as a method for enhancement of Ag-containing gels for antibacterial properties, including cerium, which can be added for the potential application as a bone substitute [139]. Zinc organometallic complexes have been used as crosslinkers for antibacterial polymeric materials consisting of poly(acrylic acid) [140] and Au–Ag mixtures have been found to increase the effectiveness of antimicrobial gels compared to Ag alone [141].

5.4. Other metal-based nanoparticles as antimicrobial hydrogels

Jonas et al. explored a hydrogel containing zinc oxide nanoparticles distributed within a poly(*N*-isopropylacrylamide) (PNIPAAm) matrix in a two-step process which involved the synthesis of zinc nanocrystals in a preliminary step [142]. The separate synthesis allowed for control over particle size and uniformity, as well as characterization of the particles by TEM, HRTEM and XRD prior to incorporation into the gel. Antimicrobial activity of the 20-nm ZnO nanoparticles-impregnated hydrogels were then tested against *E. coli* by immersion of films in a solution and then harvested and tested for their colony-growing ability after exposure to the hydrogels with varying concentrations of the ZnO nanoparticles (NPs). They observed that with ZnO NP concentrations as low as 1% in the hydrogel there was an appreciable difference in the growth ability of the bacteria. Additionally, inductively coupled plasma optical emission spectrometry (ICP) measurements were performed on the films and the MIC was determined to be 0.74–1.25 $\mu\text{g}/\text{cm}^2$ and extrapolated to be 4.7–7.8 $\mu\text{g}/\text{mL}$ for ZnO solutions. Compared to silver antimicrobial activity, this MIC is quite low, and as a result the authors suggested that research in ZnO NPs should be considered as a possible alternate antimicrobial technology to silver NPs. Toxicity to mammalian cells was evaluated with NIH/3T3 Swiss mouse fibroblasts through fluorescence activated cell sorting (FACS) with fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining to differentiate between damaged and viable cells. The conclusion of these studies was that there was no effect of the ZnO NPs on cell viability. However, they noted that the hydrogel controls lacking NPs caused cell apoptosis and necrosis (13.6–20.4% and 29.8–44.8% respectively for PNIPAAm).

6. Conclusion and perspectives

The recent advances in natural and synthetic hydrogels having either intrinsic antimicrobial properties or as carriers for antibiotics, macromolecular antimicrobials, or metal nanoparticles, are reviewed. Significant progress has been made in the chemical modification of natural polymers such as chitosan to impart broad-spectrum antimicrobial activity. However, with the latest developments in polymer chemistry, synthetic polymer-based antimicrobial hydrogels offer considerable latitude in tuning antimicrobial activity, processability, mechanical and rheological behavior, and sensitivity toward external stimuli such as temperature and pH. Both covalent and non-covalent gel-forming reactions have been discussed for both biodegradable and non-biodegradable polymers. The antimicrobial activity of these intrinsically antimicrobial hydrogels depends on crosslinking degree, hydrophobicity, and cationic charge density. The higher the crosslinking degree, the stronger is the antimicrobial activity. The presence of hydrophobic components is essential to render the hydrogels effective against Gram-negative bacteria and fungi. Chemically-crosslinked gels make promising wound dressing candidates for the prevention of infections. Non-covalent polymer- and peptide-based gels can be designed to possess unique shear-thinning capabilities that allow these materials to be injected or spread on a surface, significantly broadening their range of applications. In addition to having broad-spectrum activity against clinically relevant pathogens, many of the non-covalent gels possess the ability to disperse mature biofilms, further expanding the

application scope to medical devices and implants, antimicrobial creams, cleaning supplies, and surface sterilization in hospitals. Although the exact functional mechanisms of intrinsically antimicrobial hydrogels still remain unclear, the hydrogels are known to break down microbial membranes based on SEM studies and kill microbes upon contact. This membrane-lytic function not only allows the hydrogels to kill multidrug-resistant microbes effectively, but also prevents/delays the onset of drug resistance.

For treating microbial infections, it is crucial that antimicrobial components can be released from gels to enter immune cells and kill the pathogenic microbes from within. Hydrogels loaded with antibiotics, metal nanoparticles, antimicrobial polymers, and peptides can release the antimicrobial agents in a sustained manner, which is important to treat infections effectively and prevent biofilm formation. Biodegradable antimicrobial polymer- or peptide-loaded gels are more attractive than gels encapsulated with antibiotics or metal nanoparticles as antibiotics easily develop drug resistance and it is relatively more difficult to mitigate toxicity of metal nanoparticles due to their non-degradability. Particularly, when hydrogels are used to simultaneously co-deliver antimicrobial polymers/peptides and conventional antimicrobial agents, a strong synergistic effect can be achieved. This co-delivery approach significantly mitigates antimicrobial polymer/peptide-associated toxicity toward human tissue and normal human flora by allowing for smaller amounts of polymer/peptide to be used. The polymer/peptide in turn enhances the potency of the conventional antimicrobial agents, and together, they can reduce the incidence of drug resistance.

For future clinical applications, it is important to test hydrogels against clinically-isolated microbes, especially multidrug-resistant strains, and evaluate the *in vitro* and *in vivo* biocompatibility of hydrogels and encapsulated cargo. With rational design, synthetic polymer chemistry, and comprehensive *in vitro* and *in vivo* evaluation, hydrogel systems having broad-spectrum antimicrobial activity against multidrug-resistant microbes, high selectivity and negligible toxicity, would find great potential in the prevention and treatment of infections.

Acknowledgments

This work was funded by IBM Almaden Research Center (USA) and Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore).

References

- [1] C. Mathers, D.M. Fat, J. Boerma, The Global Burden of Disease: 2004 Update, World Health Organization, 2008.
- [2] J. Berdy, Thoughts and facts about antibiotics: where we are now and where we are heading, *J. Antibiot.* 65 (2012) 385–395.
- [3] G.L. French, The continuing crisis in antibiotic resistance, *Int. J. Antimicrob. Agents* 36S3 (2010) S3–S7.
- [4] J.-H. Song, What's new on the antimicrobial horizon? *Int. J. Antimicrob. Agents* 32 (2008) S207–S213.
- [5] D.E. Karageorgopoulos, M.E. Falagas, New antibiotics: optimal use in current clinical practice, *Int. J. Antimicrob. Agents* 34S (2009) S55–S62.
- [6] S. Pournaras, E. Iosifidis, E. Roilides, Advances in antibacterial therapy against emerging bacterial pathogens, *Semin. Hematol.* 46 (2009) 198–211.
- [7] L.D. Högberg, A. Heddi, O. Cars, The global need for effective antibiotics: challenges and recent advances, *Trends Pharmacol. Sci.* 31 (2010) 509–515.
- [8] R.C. Moellering Jr., Discovering new antimicrobial agents, *Int. J. Antimicrob. Agents* 37 (2011) 2–9.
- [9] J.-P.S. Powers, R.E.W. Hancock, The relationship between peptide structure and antibacterial activity, *Peptides* 24 (11) (2003) 1681–1691.
- [10] K.V.R. Reddy, R.D. Yedery, C. Aranha, Antimicrobial peptides: premises and promises, *Int. J. Antimicrob. Agents* 24 (6) (2004) 536–547.
- [11] A.K. Marr, W.J. Gooderham, R.E.W. Hancock, Antibacterial peptides for therapeutic use: obstacles and realistic outlook, *Curr. Opin. Pharmacol.* 6 (5) (2006) 468–472.
- [12] R.E.W. Hancock, H.G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, *Nat. Biotechnol.* 24 (12) (2006) 1551–1557.
- [13] N.K. Brogden, K.A. Brogden, Will new generations of modified antimicrobial peptides improve their potential as pharmaceuticals? *Int. J. Antimicrob. Agents* 38 (3) (2011) 217–225.
- [14] C.D. Fjell, et al., Designing antimicrobial peptides: form follows function, *Nat. Rev. Drug Discov.* 11 (1) (2012) 37–51.

- [15] M.-D. Seo, et al., Antimicrobial peptides for therapeutic applications: a review, *Molecules* 17 (10) (2012) 12276–12286.
- [16] J.L. Fox, Antimicrobial peptides stage a comeback, *Nat. Biotechnol.* 31 (5) (2013) 379–382.
- [17] G.N. Tew, et al., De novo design of antimicrobial polymers, foldamers, and small molecules: from discovery to practical applications, *Acc. Chem. Res.* 43 (1) (2009) 30–39.
- [18] A.C. Engler, et al., Emerging trends in macromolecular antimicrobials to fight multi-drug-resistant infections, *NanoToday* 7 (2012) 201–222.
- [19] A. Muñoz-Bonilla, M. Fernández-García, Polymeric materials with antimicrobial activity, *Prog. Polym. Sci.* 37 (2012) 281–339.
- [20] A.J. Huh, Y.J. Kwon, “Nanoantibiotics”: a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era, *J. Control. Release* 156 (2) (2011) 128–145.
- [21] M.J. Hajipour, et al., Antibacterial properties of nanoparticles, *Trends Biotechnol.* 30 (10) (2012) 499–511.
- [22] S. Parveen, R. Misra, S.K. Sahoo, Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, *Nanomedicine: NBM* 8 (2) (2012) 147–166.
- [23] K.A. Brogren, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* 3 (3) (2005) 238–250.
- [24] R.M. Epanand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, *Biochim. Biophys. Acta Biomembr.* 1462 (1) (1999) 11–28.
- [25] K. Matsuzaki, Magainins as paradigm for the mode of action of pore forming polypeptides, *Biochim. Biophys. Acta* 1376 (3) (1998) 391.
- [26] J.M. van Bemmelen, L’hydrogel et l’hydrate cristallin de l’oxyde de cuivre, *Recl. Trav. Chim. Pays-Bas* 13 (8) (1894) 271–274.
- [27] S.C. Lee, I.K. Kwon, K. Park, Hydrogels for delivery of bioactive agents: a historical perspective, *Adv. Drug Deliv. Rev.* 65 (1) (2013) 17–20.
- [28] O. Wichterle, D. Lim, Hydrophilic gels for biological use, *Nature* 185 (4706) (1960) 117–118.
- [29] A.S. Hoffman, Hydrogels for biomedical applications, *Adv. Drug Deliv. Rev.* 64 (Supplement(0)) (2012) 18–23.
- [30] N.A. Peppas, et al., Hydrogels in biology and medicine: from molecular principles to bionanotechnology, *Adv. Mater.* 18 (11) (2006) 1345–1360.
- [31] Y. Li, J. Rodrigues, H. Tomas, Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications, *Chem. Soc. Rev.* 41 (6) (2012) 2193–2221.
- [32] T. Vermonden, R. Censi, W.E. Hennink, Hydrogels for protein delivery, *Chem. Rev.* 112 (5) (2012) 2853–2888.
- [33] R. Censi, et al., Hydrogels for protein delivery in tissue engineering, *J. Control. Release* 161 (2) (2012) 680–692.
- [34] H.J. Chung, T.G. Park, Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering, *Nano Today* 4 (5) (2009) 429–437.
- [35] K.Y. Lee, D.J. Mooney, Hydrogels for tissue engineering, *Chem. Rev.* 101 (7) (2001) 1869–1880.
- [36] M.W. Tibbitt, K.S. Anseth, Hydrogels as extracellular matrix mimics for 3D cell culture, *Biotechnol. Bioeng.* 103 (4) (2009) 655–663.
- [37] C.A. DeForest, K.S. Anseth, Advances in bioactive hydrogels to probe and direct cell fate, *Annu. Rev. Chem. Biomol. Eng.* 3 (1) (2012) 421–444.
- [38] R. Jayakumar, et al., Biomaterials based on chitin and chitosan in wound dressing applications, *Biotechnol. Adv.* 29 (3) (2011) 322–337.
- [39] D. Campoccia, L. Montanaro, C.R. Arciola, A review of the biomaterials technologies for infection-resistant surfaces, *Biomaterials* 34 (34) (2013) 8533–8554.
- [40] D. Campoccia, L. Montanaro, C.R. Arciola, A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces, *Biomaterials* 34 (33) (2013) 8018–8029.
- [41] D.Y. Ko, et al., Recent progress of in situ formed gels for biomedical applications, *Prog. Polym. Sci.* 38 (3–4) (2013) 672–701.
- [42] M. Malmsten, Antimicrobial and antiviral hydrogels, *Soft Matter* 7 (19) (2011) 8725–8736.
- [43] A. Salomé Veiga, J.P. Schneider, Antimicrobial hydrogels for the treatment of infection, *Pept. Sci.* 100 (6) (2013) 637–644.
- [44] M. Dash, et al., Chitosan—a versatile semi-synthetic polymer in biomedical applications, *Prog. Polym. Sci.* 36 (8) (2011) 981–1014.
- [45] R. Jayakumar, et al., Biomedical applications of chitin and chitosan based nanomaterials—a short review, *Carbohydr. Polym.* 82 (2) (2010) 227–232.
- [46] M. Larsson, et al., Biomedical applications and colloidal properties of amphiphilically modified chitosan hybrids, *Prog. Polym. Sci.* 38 (9) (2013) 1307–1328.
- [47] I. Wedmore, et al., A special report on the chitosan-based hemostatic dressing: experience in current combat operations, *J. Trauma. Acute Care Surg.* 60 (3) (2006) 655–658, <http://dx.doi.org/10.1097/01.ta.0000199392.91772.44>.
- [48] C.T. Tsao, et al., Antibacterial activity and biocompatibility of a chitosan- γ -poly(glutamic acid) polyelectrolyte complex hydrogel, *Carbohydr. Res.* 345 (12) (2010) 1774–1780.
- [49] C.T. Tsao, et al., Evaluation of chitosan/ γ -poly(glutamic acid) polyelectrolyte complex for wound dressing materials, *Carbohydr. Polym.* 84 (2) (2011) 812–819.
- [50] P. Li, et al., A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability, *Nat. Mater.* 10 (2) (2011) 149–156.
- [51] M.A. Aziz, et al., Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use, *Antimicrob. Agents Chemother.* 56 (1) (2012) 280–287.
- [52] G. Liu, et al., Synthesis and characterization of chitosan/ dextran-based hydrogels for surgical use, *Macromol. Symp.* 279 (1) (2009) 151–157.
- [53] N.A. Mohamed, M.M. Fahmy, Synthesis and antimicrobial activity of some novel cross-linked chitosan hydrogels, *Int. J. Mol. Sci.* 13 (9) (2012) 11194–11209.
- [54] N.A. Mohamed, N.Y. Al-mehbad, Novel terephthaloyl thiourea cross-linked chitosan hydrogels as antibacterial and antifungal agents, *Int. J. Biol. Macromol.* 57 (2013) 111–117.
- [55] Q. Jiang, et al., Synthesis and antibacterial activities of quaternary ammonium salt of gelatin, *J. Macromol. Sci. B* (2013) 133–141.
- [56] K. Kuroda, W.F. DeGrado, Amphiphilic polymethacrylate derivatives as antimicrobial agents, *J. Am. Chem. Soc.* 127 (12) (2005) 4128–4129.
- [57] K. Kuroda, G.A. Caputo, W.F. DeGrado, The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives, *Chem. Eur. J.* 15 (5) (2009) 1123–1133.
- [58] E.F. Palermo, K. Kuroda, Chemical structure of cationic groups in amphiphilic polymethacrylates modulates the antimicrobial and hemolytic activities, *Biomacromolecules* 10 (6) (2009) 1416–1428.
- [59] Y. Oda, et al., Block versus random amphiphilic copolymers as antibacterial agents, *Biomacromolecules* 12 (10) (2011) 3581–3591.
- [60] E.F. Palermo, S. Vempala, K. Kuroda, Cationic spacer arm design strategy for control of antimicrobial activity and conformation of amphiphilic methacrylate random copolymers, *Biomacromolecules* 13 (5) (2012) 1632–1641.
- [61] M. Mizutani, et al., Design and synthesis of self-degradable antibacterial polymers by simultaneous chain- and step-growth radical copolymerization, *Biomacromolecules* 13 (5) (2012) 1554–1563.
- [62] K.E.S. Locock, et al., Guanidylated polymethacrylates: a class of potent antimicrobial polymers with low hemolytic activity, *Biomacromolecules* 14 (11) (2013) 4021–4031.
- [63] M.F. Ilker, et al., Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives, *J. Am. Chem. Soc.* 126 (48) (2004) 15870–15875.
- [64] K. Lienkamp, et al., Antimicrobial polymers prepared by ROMP with unprecedented selectivity: a molecular construction kit approach, *J. Am. Chem. Soc.* 130 (30) (2008) 9836–9843.
- [65] K. Lienkamp, et al., “Doubly selective” antimicrobial polymers: how do they differentiate between bacteria? *Chem. Eur. J.* 15 (43) (2009) 11710–11714.
- [66] S. Colak, et al., Hydrophilic modifications of an amphiphilic polynorbornene and the effects on its hemolytic and antibacterial activity, *Biomacromolecules* 10 (2) (2009) 353–359.
- [67] G.J. Gabriel, et al., Synthetic mimic of antimicrobial peptide with nonmembrane-disrupting antibacterial properties, *Biomacromolecules* 9 (11) (2008) 2980–2983.
- [68] B. Gao, X. Zhang, Y. Zhu, Studies on the preparation and antibacterial properties of quaternized polyethyleneimine, *J. Biomater. Sci. Polym. Ed.* 18 (2007) 531–544.
- [69] N. Pasquier, et al., From multifunctionalized poly(ethylene imine)s toward antimicrobial coatings, *Biomacromolecules* 8 (9) (2007) 2874–2882.
- [70] N. Pasquier, et al., Amphiphilic branched polymers as antimicrobial agents, *Macromol. Biosci.* 8 (2008) 903–915.
- [71] A. Som, et al., Identification of synthetic host defense peptide mimics that exert dual antimicrobial and anti-inflammatory activities, *Clin. Vaccine Immunol.* 19 (2012) 1784–1791.
- [72] G.N. Tew, et al., De novo design of biomimetic antimicrobial polymers, *Proc. Natl. Acad. Sci.* 99 (8) (2002) 5110–5114.
- [73] H.D. Thaker, et al., Synthetic mimics of antimicrobial peptides with immunomodulatory responses, *J. Am. Chem. Soc.* 134 (27) (2012) 11088–11091.
- [74] B.P. Mowery, et al., Mimicry of antimicrobial host-defense peptides by random copolymers, *J. Am. Chem. Soc.* 129 (50) (2007) 15474–15476.
- [75] M.T. Dohm, et al., Biophysical mimicry of lung surfactant protein B by random nylon-3 copolymers, *J. Am. Chem. Soc.* 132 (23) (2010) 7957–7967.
- [76] J. Zhang, et al., C-Terminal functionalization of nylon-3 polymers: effects of c-terminal groups on antibacterial and hemolytic activities, *Biomacromolecules* 13 (2) (2011) 323–331.
- [77] R. Liu, et al., Nylon-3 polymers with selective antifungal activity, *J. Am. Chem. Soc.* 135 (14) (2013) 5270–5273.
- [78] S. Chakraborty, et al., Effects of cyclic vs acyclic hydrophobic subunits on the chemical structure and biological properties of nylon-3 copolymers, *ACS Macro Lett.* 2 (8) (2013) 753–756.
- [79] A.C. Engler, et al., Effects of side group functionality and molecular weight on the activity of synthetic antimicrobial polypeptides, *Biomacromolecules* 12 (5) (2011) 1666–1674.
- [80] C. Zhou, et al., High potency and broad-spectrum antimicrobial peptides synthesized via ring-opening polymerization of α -amino acid-N-carboxyanhydrides, *Biomacromolecules* 11 (1) (2009) 60–67.
- [81] F. Norderberg, et al., Biodegradable nanostructures with selective lysis of microbial membranes, *Nat. Chem.* 3 (5) (2011) 409–414.
- [82] Y. Qiao, et al., Highly dynamic biodegradable micelles capable of lysing Gram-positive and Gram-negative bacterial membrane, *Biomaterials* 33 (2012) 1146–1153.
- [83] K. Fukushima, et al., Broad spectrum antimicrobial supramolecular assemblies with distinctive size and shape, *ACS Nano* 6 (2012) 9191–9199.
- [84] V.W.L. Ng, et al., Synergistic co-delivery of membrane-disrupting polymers with commercial antibiotics against highly opportunistic bacteria, *Adv. Mater.* 25 (2013) 2730–2736.
- [85] A.C. Engler, et al., Antimicrobial polycarbonates: investigating the impact of balancing charge and hydrophobicity using a same-centered polymer approach, *Biomacromolecules* 14 (2013) 4331–4339.
- [86] W. Chin, et al., Biodegradable broad-spectrum antimicrobial polycarbonates: investigating the role of chemical structure on activity and selectivity, *Macromolecules* 46 (22) (2013) 8797–8807.
- [87] S.Q. Liu, et al., Antimicrobial and antifouling hydrogels formed in situ from polycarbonate and poly(ethylene glycol) via Michael addition, *Adv. Mater.* 24 (48) (2012) 6484–6489.

- [88] K. Lohner, E.J. Prenner, Differential scanning calorimetry and X-ray diffraction studies of the specificity of the interaction of antimicrobial peptides with membrane-mimetic systems, *Biochim. Biophys. Acta Biomembr.* 1462 (1–2) (1999) 141–156.
- [89] T.J. Beveridge, Structures of Gram-negative cell walls and their derived membrane vesicles, *J. Bacteriol.* 181 (16) (1999) 4725–4733.
- [90] Y. Li, et al., Broad-spectrum antimicrobial and biofilm-disrupting hydrogels: stereocomplex-driven supramolecular assemblies, *Angew. Chem. Int. Ed.* 52 (2) (2013) 674–678.
- [91] S. Jiang, Z. Cao, Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications, *Adv. Mater.* 22 (9) (2010) 920–932.
- [92] Z. Cao, S. Jiang, Super-hydrophilic zwitterionic poly(carboxybetaine) and amphiphilic non-ionic poly(ethylene glycol) for stealth nanoparticles, *Nano Today* 7 (5) (2012) 404–413.
- [93] Z. Cao, et al., Reversibly switching the function of a surface between attacking and defending against bacteria, *Angew. Chem. Int. Ed.* 51 (11) (2012) 2602–2605.
- [94] B. Cao, et al., Switchable antimicrobial and antifouling hydrogels with enhanced mechanical properties, *Adv. Healthc. Mater.* 2 (8) (2013) 1096–1102.
- [95] B. Cao, et al., The impact of structure on elasticity, switchability, stability and functionality of an all-in-one carboxybetaine elastomer, *Biomaterials* 34 (31) (2013) 7592–7600.
- [96] Perrault, J.J. and C.G. Rouns, *Inherently antimicrobial quaternary amine hydrogel wound dressings*. United States Patent, 2000. **6039940A**.
- [97] K. Bertal, et al., Antimicrobial activity of novel biocompatible wound dressings based on triblock copolymer hydrogels, *J. Mater. Sci.* 44 (23) (2009) 6233–6246.
- [98] J. Madsen, et al., Biocompatible wound dressings based on chemically degradable triblock copolymer hydrogels, *Biomacromolecules* 9 (8) (2008) 2265–2275.
- [99] Y.-H. La, et al., Bifunctional hydrogel coatings for water purification membranes: improved fouling resistance and antimicrobial activity, *J. Membr. Sci.* 372 (1–2) (2011) 285–291.
- [100] M.A. Andrews, et al., Antimicrobial hydrogels formed by crosslinking polyallylamine with aldaric acid derivatives, *J. Appl. Polym. Sci.* 119 (6) (2011) 3244–3252.
- [101] D.A. Salick, et al., Inherent antibacterial activity of a peptide-based β -hairpin hydrogel, *J. Am. Chem. Soc.* 129 (47) (2007) 14793–14799.
- [102] D.A. Salick, D.J. Pochan, J.P. Schneider, Design of an injectable β -hairpin peptide hydrogel that kills methicillin-resistant *Staphylococcus aureus*, *Adv. Mater.* 21 (41) (2009) 4120–4123.
- [103] A.S. Veiga, et al., Arginine-rich self-assembling peptides as potent antibacterial gels, *Biomaterials* 33 (35) (2012) 8907–8916.
- [104] Y. Liu, et al., Stimuli-responsive self-assembling peptides made from antibacterial peptides, *Nanoscale* 5 (14) (2013) 6413–6421.
- [105] S. Debnath, et al., Hydrogelation through self-assembly of fmoc-peptide functionalized cationic amphiphiles: potent antibacterial agent, *J. Phys. Chem. B* 114 (13) (2010) 4407–4415.
- [106] Z. Yang, et al., Intracellular hydrogelation of small molecules inhibits bacterial growth, *Angew. Chem. Int. Ed.* 46 (43) (2007) 8216–8219.
- [107] M. Hughes, et al., Antimicrobial properties of enzymatically triggered self-assembling aromatic peptide amphiphiles, *Biomater. Sci.* 1 (11) (2013) 1138–1142.
- [108] C. Zhou, et al., A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-L-lysine, *Biomaterials* 32 (11) (2011) 2704–2712.
- [109] A. Song, A.A. Rane, K.L. Christman, Antibacterial and cell-adhesive polypeptide and poly(ethylene glycol) hydrogel as a potential scaffold for wound healing, *Acta Biomater.* 8 (1) (2012) 41–50.
- [110] Y. Li, et al., Broad-spectrum antimicrobial and biofilm-disrupting hydrogels: stereocomplex-driven supramolecular assemblies, *Angew. Chem.* 52 (2) (2013) 674–678.
- [111] G. Laverty, S.P. Gorman, B.F. Gilmore, Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of *Staphylococcus epidermidis*-associated biomaterial infections, *J. Biomed. Mater. Res. A* 100A (7) (2012) 1803–1814.
- [112] A.L.Z. Lee, et al., Block copolymer mixtures as antimicrobial hydrogels for biofilm eradication, *Biomaterials* 34 (38) (2013) 10278–10286.
- [113] B. Hamad, The antibiotics market, *Nat. Rev. Drug Discov.* 9 (9) (2010) 675–676.
- [114] J.H. Sung, et al., Gel characterisation and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan, *Int. J. Pharm.* 392 (1–2) (2010) 232–240.
- [115] A. Concheiro, C. Alvarez-Lorenzo, Chemically cross-linked and grafted cyclodextrin hydrogels: from nanostructures to drug-eluting medical devices, *Adv. Drug Deliv. Rev.* 65 (9) (2013) 1188–1203.
- [116] A. Zumbuehl, et al., Antifungal hydrogels, *Proc. Natl. Acad. Sci.* 104 (32) (2007) 12994–12998.
- [117] R.J. Glisoni, et al., β -Cyclodextrin hydrogels for the ocular release of antibacterial thiosemicarbazones, *Carbohydr. Polym.* 93 (2) (2013) 449–457.
- [118] D.S. Jones, et al., Characterization of the physicochemical, antimicrobial, and drug release properties of thermoresponsive hydrogel copolymers designed for medical device applications, *J. Biomed. Mater. Res. B Appl. Biomater.* 85B (2) (2008) 417–426.
- [119] G. Cheng, et al., Integrated antimicrobial and nonfouling hydrogels to inhibit the growth of planktonic bacterial cells and keep the surface clean, *Langmuir* 26 (13) (2010) 10425–10428.
- [120] L. Mi, et al., A thermoresponsive antimicrobial wound dressing hydrogel based on a cationic betaine ester, *Adv. Funct. Mater.* 21 (21) (2011) 4028–4034.
- [121] C. Zhao, et al., Dual functionality of antimicrobial and antifouling of poly(n-hydroxyethylacrylamide)/salicylate hydrogels, *Langmuir* 29 (5) (2013) 1517–1524.
- [122] S.P. Tan, et al., Development of a novel antimicrobial seaweed extract-based hydrogel wound dressing, *Int. J. Pharm.* 456 (1) (2013) 10–20.
- [123] P.-L. Lai, D.-W. Hong, K.-L. Ku, Z.-T. Lai, I.-M. Chu, Novel thermosensitive hydrogels based on methoxy polyethylene glycol-co-poly(lactic acid-co-aromatic anhydride) for cefazolin delivery, *Nanomedicine: Nanotechnology, Biology and Medicine* 10 (2014) 553–560.
- [124] J.L. Clement, P.S. Jarrett, Antibacterial silver, *Metal-Based Drugs* 1 (5–6) (1994) 467–482.
- [125] A.Y. Sung, T.-H. Kim, Optical application of poly(HEMA-co-MMA) containing silver nanoparticles and N, N-dimethylacrylamide, *Korean J. Chem. Eng.* 29 (5) (2012) 686–691.
- [126] R. Singh, D. Singh, Radiation synthesis of PVP/alginate hydrogel containing nanosilver as wound dressing, *J. Mater. Sci. Mater. Med.* 23 (11) (2012) 2649–2658.
- [127] B. Obradovic, et al., Novel alginate based nanocomposite hydrogels with incorporated silver nanoparticles, *J. Mater. Sci. Mater. Med.* 23 (1) (2012) 99–107.
- [128] S. Agnihotri, S. Mukherji, S. Mukherji, Antimicrobial chitosan-PVA hydrogel as a nanoreactor and immobilizing matrix for silver nanoparticles, *Appl. Nanosci.* 2 (3) (2012) 179–188.
- [129] T. Jayaramudu, et al., Iota-Carrageenan-based biodegradable Ag₀ nanocomposite hydrogels for the inactivation of bacteria, *Carbohydr. Polym.* 95 (1) (2013) 188–194.
- [130] H. El-Sherif, M. El-Masry, A. Kansoh, Hydrogels as template nanoreactors for silver nanoparticles formation and their antimicrobial activities, *Macromol. Res.* 19 (11) (2011) 1157–1165.
- [131] K. Varaprasad, et al., Synthesis and characterization of hydrogel-silver nanoparticle-curcumin composites for wound dressing and antibacterial application, *J. Appl. Polym. Sci.* 121 (2) (2011) 784–796.
- [132] H.-R. Cha, et al., Fabrication of amino acid based silver nanocomposite hydrogels from PVA-poly(acrylamide-co-acryloyl phenylalanine) and their antimicrobial studies, *Bull. Kor. Chem. Soc.* 33 (10) (2012) 3191–3195.
- [133] S.V. Otari, et al., A novel microbial synthesis of catalytically active Ag-alginate biohydrogel and its antimicrobial activity, *Dalton Trans.* 42 (27) (2013) 9966–9975.
- [134] S. Dutta, et al., Counterion-induced modulation in the antimicrobial activity and biocompatibility of amphiphilic hydrogelators: influence of in-situ-synthesized Ag-nanoparticle on the bactericidal property, *Langmuir* 27 (8) (2011) 5000–5008.
- [135] S. Grade, et al., Serum albumin reduces the antibacterial and cytotoxic effects of hydrogel-embedded colloidal silver nanoparticles, *RSC Adv.* 2 (18) (2012) 7190–7196.
- [136] G. Humphreys, et al., Combinatorial activities of ionic silver and sodium hexametaphosphate against microorganisms associated with chronic wounds, *J. Antimicrob. Chemother.* 66 (11) (2011) 2556–2561.
- [137] S.A. Smith, J.H. Morrissey, Polyphosphate enhances fibrin clot structure, *Blood* 112 (7) (2008) 2810–2816.
- [138] J.V. Edwards, P.S. Howley, Human neutrophil elastase and collagenase sequestration with phosphorylated cotton wound dressings, *J. Biomed. Mater. Res. A* 83A (2) (2007) 446–454.
- [139] D.S. Morais, et al., Biological evaluation of alginate-based hydrogels, with antimicrobial features by Ce(III) incorporation, as vehicles for a bone substitute, *J. Mater. Sci. Mater. Med.* 24 (9) (2013) 2145–2155.
- [140] C. James, et al., An antimicrobial zinc based molecule for cross linking poly-acrylic acid, *Eur. Polym. J.* 47 (6) (2011) 1338–1345.
- [141] P. Ranga Reddy, et al., Fabrication of Au and Ag Bi-metallic nanocomposite for antimicrobial applications, *J. Appl. Polym. Sci.* 125 (2) (2012) 1357–1362.
- [142] V.B. Schwartz, et al., Antibacterial surface coatings from zinc oxide nanoparticles embedded in poly(N-isopropylacrylamide) hydrogel surface layers, *Adv. Funct. Mater.* 22 (11) (2012) 2376–2386.