Jelly Bombs – Hydrogels as Weapons against Microorganisms

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1. Introduction

The increased global thread of multi resistant microorganisms has become a major concern of mankind in recent years as more and more bacteria develop partly or complete resistence to all commonly used antibiotics. The overuse of antibiotic description and the reduced number of novel antimicrobial drugs in the pipelines of the pharmaceutical industries are a major cause for the worsening of this problem [1–3]. Despite an increased worldwide health standard, pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli* pose severe problems in many hospitals [1,4]. Especially in patients suffering from large scale wounds, infections can slow down the healing process or lead to sepsis of the affected site. Another major concern is the use of components that are in contact with the body: catheters are prone to bacterial infections and all biological components intended for the use within the body have to be carefully kept sterile as infections can lead to transplant rejection or even severe internal infections which are hard to detect and difficult to treat [5].

In this context, hydrogels are a unique class of materials which can represent novel functions to create a major weapon in the fight against pathogens [3,6,7]. They are a class of materials containing a very high amount of water and a specific 3D network structure. The term reaches back to more than 100 years, with the first major application described by Wichterle and Lim in 1960 for the use of hydrogels in contact lenses [8]. Since this breakthrough study, the number of publications per year has increased tremendously whith hydrogel applications in different fiels of science: as wound dressings [9–12], 3D cell culture systems [13–16], in tissue engineering [17,18] as well as for drug delivery purposes [19,20]. Due to their biocompatibility, their high water content and the possibility to design hydrogels to mimic extracellular structures or specific parts of tissues, they are also attractive candidates for the use within the body.

In antimicrobial treatment, it can be distinguished between two major groups of hydrogels: such with an inherent antimicrobial activity and such with encapsulated compounds which exhibit antimicrobial activity and can be delivered by the gel [3]. Hydrogels with inherent antimicrobial activity are divided in two major classes: natural and synthetic hydrogels. Among natural hydrogels sugars [11,21–23], DNA [24,25], and peptides / proteins [12,20,26–28] are the major building blocks while poly(vinylalcohol), polyethylene glycole, methacrylamide and acrylate are often used for the production of synthetic hydrogels [29–33]. For the encapsulation, several different approaches can be followed; in addition to traditionally used antibiotics, nanoparticles (e.g. silver) and antimicrobial peptides (AMP) can be encapsulated and released over defined periods [26,34–36].

In this chapter, we will highlight recent advances in the production and application of different types of hydrogels with a strong focus on novel antimicrobial hydrogel systems.

2. Hydrogels with inherent antimicrobial activity

2.1. Natural Components

Natural hydrogels are mainly used in fields such as drug delivery, wound dressing, tissue engineering or to cover the surface of orthopaedic implants [11,20,37–42]. For most of those applications, the antimicrobial activity of the used material is crucial to avoid a spoiling with microorganisms. The ideal hydrogel for biomedical application should also be biocompatible and favourably injectable [37]. Inherent natural antimicrobial hydrogels can be formed by either self-assembling of monomers (e.g. antimicrobial peptides) into a hydrogel network or by covalently attaching antimicrobial compounds to the hydrogel [7]. Natural hydrogels can be classified into three main groups: peptide based, sugar based and nucleotide based materials.

Peptide based hydrogels

A huge group of natural antimicrobial hydrogels is based on the discovery of antimicrobial peptides (AMP) [7,27,43,44]. They are generally amphiphilic and possess a cationic charge, enabling electrostatic interactions with anionic bacterial membranes, and thereby leading to membrane disruption and bacterial death. Because AMPs do not have the ability to self-assemble into a hydrogel network, new peptides resembling the properties of AMPs are in development which can self-assemble into hydrogels under the appropriate conditions [7].

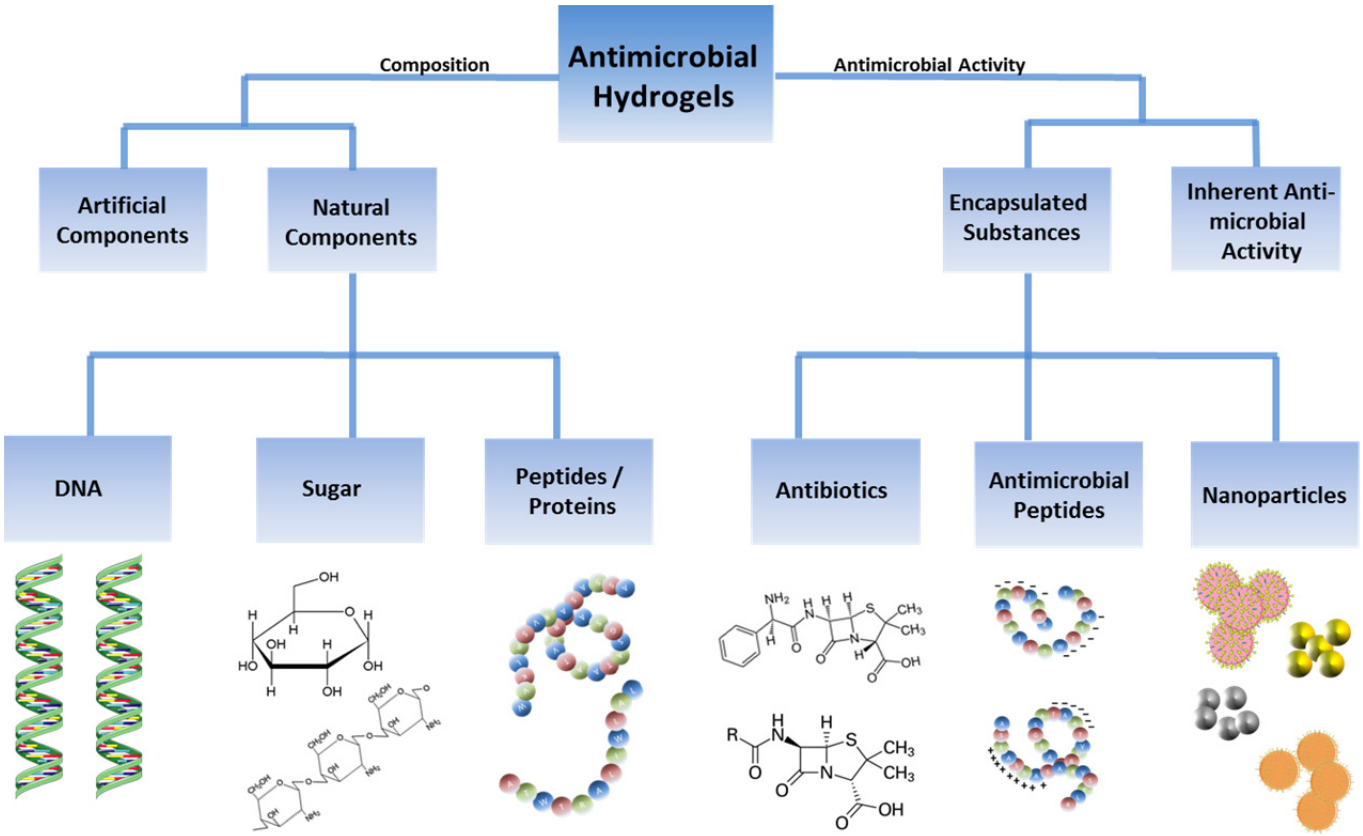

Figure 1. Schematic overview of hydrogel composition and acquisition of antimicrobial activity of hydrogels

In 2007, Salick *et al.* developed an inherent AMP based hydrogel, consisting of amphiphilic beta-hairpins. This AMP consists of a region of alternating valine and lysine residues which are connected by a tetra-peptide sequence [43]. The formation of the beta-hairpin conformation is triggered through the addition of cell culture media at a physiological pH, containing mono- and divalent salts. This results in a mechanically rigid hydrogel [26,43] with a high antimicrobial activity on the surface against bacterial strains commonly occurring in hospital environments, including Gram-negative bacteria, such as *E. coli* and *Klebsiella pneumoniae*, as well as Gram-positive bacteria such as *Staphylococcus epidermis*, *Staphylococcus aureus* and *Streptococcus pyogenes* [2,43,45]. Direct interaction between the hydrogel surface and the bacteria leads to cell membrane disruption and thus to cell death. The study showed that the antimicrobial effect of the hydrogel surface would be strong enough for the expected bacterial infection levels during surgery. Furthermore, the hydrogels surface was selectively cytotoxic for bacteria, however displaying no cytotoxicity toward mammalian cells. Therefore, the use of this hydrogel system may lead to improved tissue regeneration therapies due to the possibility to avoid bacterial infections [43].

Since this so-called MAX1 hydrogel has only a high antimicrobial activity against drug-susceptible bacteria, the same group developed a hydrogel against methicillin-resistant *S. aureus* [27]. By replacing two lysine residues in the sequence with arginine, the material provides a cationic surface charge for the interaction with the anionic components of bacterial cell membranes and the possibility of hydrogen bond-formation with oxygen atoms of negatively charged macromolecules of the outer bacterial surface, e.g. lipopolysaccharides [27,46]. Additionally, this hydrogel surface is also selectively cytotoxic for bacteria and has not shown any cytotoxic effect towards mammalian cells [27]. Furthermore, it can be shear-thin delivered by a syringe (decreased viscoelasticity with increasing mechanical stress), which makes it an attractive candidate for injection-based therapies [27]. In order to determine whether the replacement of even more lysine residues of MAX1 into arginine leads to an altered antimicrobial activity, new variants were developed containing four, six and eight arginine residues [44].

In 2016, Baral *et al.* have designed a simple, dipeptide based hydrogel which displays antimicrobial activity against Gram-negative bacteria like *E. coli* and *P. aeruginosa* [37]. The hydrogel consists of the dipeptide Boc-(11-aminoundecanoic acid)-Phe-COOH (P11) and was formed in phosphate buffer at physiological pH. Although P11 has shown to be effective against Gram-negative bacteria, no antimicrobial effect could be detected towards Gram-positive bacteria, e.g. *S. aureus* and *B. subtilis*. This can most likely be explained by the differences of cell walls of Gram-negative and Gram-positive bacteria [37]. The Gram-positive cell wall is composed of several layers of peptidoglycan with a lot of pores, allowing molecules to enter the bacterial cell. Contrarily, Gram-negative cell walls are composed of

only a thin layer of peptidoglycan, but two cell membranes. The outer cell membrane can therefore act as a barrier, preventing the entrance of foreign molecules [47]. Beside the biocompatibility of this hydrogel with human red blood cells and fibroblasts, it is further highly resistant against proteolytic enzymes. Furthermore, this hydrogel has shear-thinning properties allowing a local delivery by syringe [37]. Therefore, the research group has suggested a possible application of this hydrogel to combat biofilm formation in orthopedic implants, since these infections are highly resistant to commonly used antibiotics [37,48,49].

In addition to the use of antimicrobial hydrogels for chronic wounds or tissue engineering applications in general, antimicrobial hydrogels have also been useful as a contact lens like bandages for the treatment of corneal ulcers. For this purpose, a hydrogel was developed by Gallagher *et al.* consisting of poly-ε-lysine cross-linked with octanediodic acid [28]. This formation has several advantages: poly-ε-lysine is a non-toxic material, used in many food applications e.g. as emulsifier, and it gained the "general recognized as safe" status by the US Food and Drug Administration in 2004 [50,51]. Additionally, it is known to have inherent antimicrobial activity and good modifiability. It was shown that the hydrogel itself has an antimicrobial activity against *E. coli* and *S. aureus*. However, the antimicrobial efficacy against *S. aureus* could be further enhanced after the covalent attachment of either free poly-ε-lysine or penicillin G *via* ionic interactions. Since the hydrogel has shown similar properties as commercially available contact lenses e.g. the ability to adsorb high water volumes or the compatibility with HCE-T cells *in vitro* and along with the antimicrobial effects, this hydrogel is a promising candidate for antimicrobial bandage for ophthalmic applications [28].

Sugar

Sugar based hydrogels are mainly composed of chitin, chitosan, cellulose or derivatives of those compounds. Chitosan and cellulose occur in large amounts in nature and have suitable properties for biomedical applications. They are biocompatible, biodegradable, non-toxic, hydrophilic and are inexpensive [23,52–55]. Furthermore, chitosan or cellulose hydrogels can be formed easily by chemical or physical methods with suitable reagents [3]. In acidic medium, chitosan is, based on its protonated primary amino groups, positively charged and therefore possesses antimicrobial activity *per se* [3]. Moreover, antimicrobial compounds can be covalently ligated to the hydrogel network [56,57].

Since chitosan possesses several advantageous properties for biomedical applications, many different chitosan based materials have been developed [22,58,59]. In 2012, Mohamed *et al.* designed a chitosan based hydrogel where chitosan was crosslinked with varying concentrations of oxalyl-bis-4-(2,5-dioxo-2*H*-pyrrol-1(5H)-yl) benzamide [47]. The hydrogel inhibited the growth of Gram-positive bacteria (*B. subtilis, S. aureus and S. pneumoniae*), Gram-negative bacteria (*E. coli, S. thyphimurium*) and fungi (*A. niger* and *A. fumigatus*) effectively. However, the growth of Gram-positive bacteria was inhibited more effectively than the growth of Gram-negative bacteria, which could be explained by the different composition of the cell walls [47].

In addition to the treatment of infections, they can be applied in everyday life as for example in diapers. After the absorption of urine, harmful bacteria can grow within diapers, negatively affecting human health [60]. The surface of cellulose fibres could be modified by attaching several bactericidal compounds e.g. N-halamine siloxanes or beta-cyclodextrin to achieve antimicrobial effects [56,57]. Contrarily to these approaches, Peng *et al.* have incorporated quaternary ammonium into a cellulose hydrogel network, designing a novel material [60]. In subsequent tests, the hydrogel has shown a good biocompatibility towards the hepatic human cell line L02 and was able to inhibit the growth of *Saccharomyces cerevisiae* [60]. In this setup, microbial death is caused by membrane disruption which in turn is caused by the attraction of the anionic microbial membrane into internal pores of the material [59,60]. Additionally to the antimicrobial activity and the hydrogels biocompatibility, it exhibited superabsorbent properties and high mechanical strength, which would facilitate its application as a filling material in diapers in the future [60].

DNA

Since DNA hydrogels also have a wide potential for biomedical applications, antimicrobial DNA hydrogels have been developed. DNA hydrogels are mainly produced in two ways: DNA can either be chemically crosslinked or the network formation can be triggered enzymatically [24,25,61]. The antimicrobial activity of a DNA hydrogels is normally achieved by incorporating antimicrobial substances into the network [61].

In order to control the antimicrobial activity, Cao *et al.* (2013) designed and prepared a novel DNA hydrogel where the biocidal activity is driven by the addition of DNase [61]. The formation of the hydrogel is based on electrostatic interactions between negatively charged salmon sperm DNA hydrogel, which is used as a frame, and the positively charged oligo (phenylene ethynylene)s (OPEs). The addition of DNase leads to hydrolysis of the DNA frame into small fragments, which released the OPEs [62]. After the disintegration, OPEs are able to cross the membrane and cause damage within the cell by reactive oxygen species generation.

Apart from inherent antimicrobial activity, DNA based materials are mainly used for the controlled loading and delivery of substances which can be incorporated into the material. DNA hydrogels can be specifically designed to control pore sizes and mechanical properties. Furthermore, they can be used to bind drugs via electrostatic interaction, minor or major groove binding or intercalation. Current examples are highlighted in the appropriate part of this chapter.

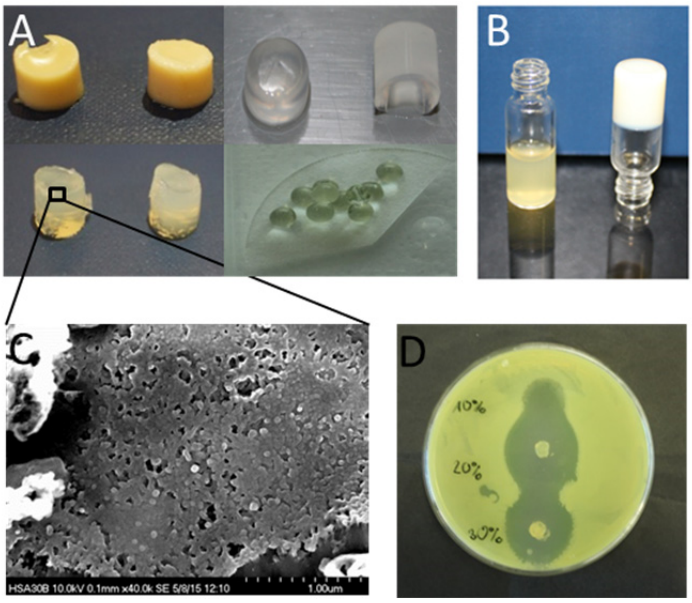

Figure 2. (A) Crosslinked yeast protein hydrogel (top left) Fibrillary phenylalanine hydrogel (top right) Crosslinked bovine serum albumin hydrogel (bottom left) and alginate hydrogel beads (bottom right) (B) Gel-to-sol transition of a crosslinked bovine serum albumin hydrogel (C) Electron microscopy of a nanoporous crosslinked bovine serum albumin hydrogel (D) Antimicrobial activity of a fibrillary phenylalanine hydrogel loaded with ampicillin against *P. aeruginosa.* Bodenberger *et al*., unpublished.

2.2 Artificial Components

Besides natural components as major building blocks, synthetic, cationic hydrogels with inherent antimicrobial characteristics are an emerging field and a promising alternative to conventional antimicrobial treatments. In contrast to hydrogel systems made from complex natural components, synthetic polymers are more simplified structures and the respective hydrogels show more reproducible physical and chemical characteristics that in turn are important criteria for most application purposes for novel materials [63]. Furthermore, no special modifications are necessary to enable antimicrobial properties as many synthetic materials have properties which repel or kill microorganisms on their own. Normally it is distinguished between antifouling and antimicrobial activity; antifouling represents materials which repel microorganisms and prevent infection while antimicrobial hydrogels kill microorganisms upon contact.

Antimicrobial and antifouling hydrogels usually show cationic properties by either bearing cationic side chains or by possessing intrinsically cationic or hydrophobic properties by themselves. These positively charged polymers can easily penetrate negatively charged bacterial membranes or interact with the membrane due to ultra-hydrophobic properties.

The synthetic polymers that are observed to exhibit the strongest antimicrobial effect as well as antifouling properties can be classified into a vast number of polymer groups: poly(acrylate) [64–69] and polycarbonates [70–75] are among the most potential antimicrobial polymers. Furthermore, zwitterionic materials often exhibit certain beneficial features; their charge often inhibits bacterial growth while an environmental parameter can often induce a switch in the materials charge to repel organisms. Further polymeric chemicals which exhibit antifouling properties are arylamides [76–78], beta-lactams [79–83] and norbonenes [78,84–86]. However, the main challenge is to produce hydrogels from those materials while maintaining the intrinsic antimicrobial characteristics. Different used hydrogel components and the strain they are used against are listed in table 1.

Polycarbonate

One major class of cationic materials that can be used to generate synthetic antimicrobial hydrogels are polycarbonates. A possible cationic polycarbonate hydrogel has been illustrated by Liu *et al*. in 2012 [33]. In this study, the researchers used ring opening polymerization to first create a PEG polymer as a di-block-copolymer and a cationic polycarbonate.

Thiol-functionalized PEG acts as a macro-initiator in this setup. A following step links the di-block-copolymers with the cationic polycarbonates [33]. This synthesis approach enabled the research group to obtain highly defined hydrogels with a controllable molecular weight, including a predefined polymerization degree, functionality and molecular composition. This material showed to be highly effective in eliminating *E. coli* and *C. albicans*, with an observed elimination efficacy of 99.99%. To further explore the behaviour of the gel when used as a coating, Liu *et al*. fixed the hydrogel to a rubber surface to mimic a possible application as coating material for catheters. In this setup, the hydrogel showed excellent antimicrobial as well as antifouling properties along with negligible skin irritation and hemolysis properties [33].

One year later, *Liu et al.* further improved the material to obtain shear-thinning behaviour, lower critical solution temperature behaviour and most important: enhanced biofilm disrupting capabilities. Results showed a complete elimination of *S. aureus*, *E. coli*, the fungi *candida albicans* and methicillin-resistant *S. aureus*, vancomycin resistant *enterococci*, *P. aeruginosa, A. baumannii*, *K. pneumonia* and *Candida neoformans*. Furthermore, the hydrogel did not show any toxicity *in vitro* and *in vivo* [87].

Zwitterionic materials

Another class of synthetic polymeric materials are zwitterionic polymers like poly(carboxybetaine) and poly(sulfobetaine) as presented by Cao *et al*. in 2013 [88]. Zwitterionic materials can change their conformation depending on the pH of the milieu. The team pursued the "capture and kill" principle where dead bacteria can be released after cell death by changing conditions, which is important to prevent possible biofilm formation and accumulation [89]. Preliminary work by Cao and co-workers in 2012 showed good results for the switching capabilities of a cationic six-membered *N,N*-dimethyl-2-morpholinone, which enabled an antimicrobial (kills microorganisms upon contact) and antifouling state (repels microorganisms) at the same time. Cao and colleagues were the first to create a switching hydrogel with enhanced mechanical strength [88] which exhibited antifouling properties in basic conditions and antimicrobial properties in acidic environment and eliminated 99.99996% of *E. coli* K12. [88].

Acrylate

Acrylate based polymers are another promising group of polymers to establish synthetic cationic hydrogels. Several acrylate based hydrogels that carry quaternary ammonium side chains were introduced by Perrault and Rouns. They can be used as a wound dressing material as they are non-irritating to the wound, exhibit antimicrobial activity against *P. aeruginosa*, *E. coli*, *K. pneumonia*, *S. aureus* and *E. faecalis,* absorb the wound exudate and moisturize the wound, which might be especially useful for large scale wounds like burns [90]. Furthermore, those materials support wound healing by facilitating wound clotting by binding clotting inhibitors, e.g. the anionic polysaccharide heparin due to their positive charge [90]. Furthermore, those polymers are radiation tolerant and can thus be kept sterile by applying radiation to the hydrogel.

Acrylates as well as their derivatives are a major group of hydrogel building blocks that have been promoted in several studies. Armes and colleagues proposed a poly-methacrylate hydrogel that is especially highly effective against *S. aureus* with confirmed non-toxicity towards mammalian cells [10,91]. While the discovery of poly(methacrylate) polymer hydrogels dates back to 1986, when Ikeda *et al*. first promoted a poly(methacrylate) polymer containing chlorhexidine-like side groups, they are still among the most effective antimicrobial cationic polymer against *S. aureus* [92]. When it comes to membranes and wound coatings, acrylate based PEG diacrylate polymers and ammonium functionalized methacrylate monomers showed the best results due to their excellent and widespread antifouling and antimicrobial properties [93].

Table 1 Antimicrobial hydrogels and the target microorganism they have been used against

Hydrogel components	**Target microorganism**
Chitosan-beta-poly(glutamic acid) polyelectrolyte	*S. aureus, E. coli* [58]
Dimethyldecylammonium chitosan (with high quaternization)-*graft*-poly(ethylene glycol) methacrylate and poly(ethylene glycol) diacrylate	*P. aeruginosa, E. coli, S. aureus, F. solani* [59]
Chitosan-dextran hydrogel	*S. aureus, S. pyogenes, C. perfringens, E. coli* [22]
Beta-hairpin arginine-lysine	*S. pyogenes , S. aureus, S. epidermis, E. coli, K. pneumoniae* [43]
Beta-hairpin arginine-lysine	*Methicillin-resistant S. aureus* [27]
PEP6R (peptide with 6 arginine residues)	*S. aureus, E. coli, P. aeruginosa* [44]
Oligo(phenylene ethynylene)-DNA	*E. coli* [61]
Quarternized cellulose	*S. cerevisiae* [60]
Chitosan with tetracycline	*E. coli, S. aureus* [21]
Poly (vinyl-alcohol)-alginate with ampicillin	*S. pyogenes, S. aureus, P .aeruginosa, P. vulgaris* [30]
Oligo(poly(ethylene glycol)fumarate) / sodium methacrylate hydrogel with vancomycin	*S. aureus* [94]
Alginate hydrogel with vancomycin	*S. aureus* [95]
Poly(2-hydroxyethyl methacrylate) and poly(ethylene–glycol diacrylate) with ciprofloxacin	*Methicillin resistant S. aureus* [96]
Tripeptide hydrogel with ciprofloxacin	*S. aureus, E.coli, K. pneumoniae* [97]
Poly(ethylene glycol) monomethyl ether with teicoplanin	*S. aureus* [98]

Alginate hydrogel with chitosan and amoxicillin	*H. pylori* [99]
Cyclodextrin	*S. aureus, E.coli* [100]
Cyclodextrin hydrogel with thiosemicarbazones	*P. aeruginosa, S. aureus* [101]
Carboxybetaine ester hydrogel with salicylate	*S. erpidimidis, E.coli* [102]
Poly(β-amino ester) hydrogel with vancomycin	*S. aureus* [103]
Polyethylene glycol with polymyxin B and nisin	*S. aureus, S. erpidimidis* [104]
Poly(2-hydroxyethyl methacrylate)	*S. erpidimids* [36]
Poly (ethylene glycol) hydrogel with several AMPS	*S. aureus* [12]
Alginate hydrogel with CM11 peptide	*Methicillin resistant S. aureus* [105]
Poly(ethylene glycol) polypeptide hydrogel	*E. coli, S. aureus* [106]
Alginate hydrogel with cateslytin	*P. gingivalis* [107]
Lysine-methacrylamide	*E. coli*, *P. aeruginosa*, *S. marcescens, S. aureus, C. albicans, Fusarium solani* [108]
Poly ($_{L}$-lactide) and poly($_{D}$-lactide) and cationic polycarbonate	*S. aureus, E. coli, C. albicans, methicillin-resistant S. aureus, vancomycin-resistant enterococci, P. aeruginosa, A. baumannii, K. pneumonia, C. neoformans* [87]
Thiol-functionalized PEG	*E. coli, C. albicans* [33]
Acrylate	*P. aeruginosa, E. coli, K. pneumonia, S. aureus and E. faecalis* [64,65,68]
Silk / nanohydroxyapatite composite	*S. aureus, S. epidermidis, E. coli and P. aeruginosa* [109]

3. Encapsulation of substances into hydrogels

Apart from hydrogels with inherent antimicrobial activity, there are different possibilities to generate or enhance the antimicrobial activity of a material by incorporation of antimicrobial agents. The main classes which have been used in this context are antibiotics, antimicrobial peptides and antimicrobial nanoparticles. When encapsulating substances, the release pattern can be swelling-dependant, stimuli responsive (pH, temperature etc.) or triggered by the addition of specific chemicals. Many groups work to achieve extended and precisely defined release times to create antimicrobial materials for wound dressing or as antimicrobial surfaces.

The release of antimicrobial agents from hydrogels also finds application is the development of coated medical devices and implants [36,104,107,108,110]. Infections in hospitals e.g. triggered by insufficient sterilization of devices or insufficient cleaning of premises, became alarmingly common. In 2011 / 2012 about 2.6 million cases of healthcare associated infections have been reported in the European Union [111]. The application of antibacterial coated medical implants and devices could be one right step to overcome this tremendous problem.

Antibiotics

The well-defined and precisely designed release times are the most important advantage for encapsulation of antibiotics compared to classical administration routes. While normal antibiotics are eliminated rather fast from an organism, hydrogels offer the possibility to release the drug over days or weeks to maintain constant and therapeutically effective concentrations at the site of infection, while the antibiotic is protected from degradation within the hydrogel. This helps to guarantee an elimination of all relevant pathogens, minimalizes possible mistakes in the treatment and / or administration of the drug and decreases the change of pathogen resistance [7,112]. Different types of antibiotics can be used in developing new antibacterial hydrogels like ampicillin, vancomycin or cyclosporine [9,21,30,94–103,113–115].

The use of vancomycin in combination with hydrogels has found a variety of application recently [94,95,103].

Vancomycin is used for the treatment of Gram-positive bacteria such as *Staphylococcus* species as well as a potential weapon against multi resistant bacteria. In 2014 a poly(β-amino ester) hydrogel with well-defined release properties and covalently bound vancomycin molecules on its surface was developed [103]. This biodegradable and tissue-like hydrogel is degraded over time in contact with biological materials due to hydrolysis of the covalent bonds. The degradation rate of the hydrogel also determines the release rate of the covalently bound vancomycin. It was shown that the material was fully degraded after approximately 20 days and the incorporated vancomycin was completely released over this period. The activity of released vancomycin was positively tested on *S. aureus.*

Antimicrobial Peptides

As bacteria evolve more and more resistance towards antibiotics, antimicrobial peptides become more and more attractive in the fight against multi resistant bacteria. Compared to antibiotics, many AMPs have the potential to entirely

eliminate microorganism which makes bacteria more unlikely to evolve resistance [116]. As a result, AMPs are intensively studied to develop novel antimicrobial hydrogels [36,43,104,105,107,108,117].
Song and coworkers have described a hydrogel with antibacterial activity with incorporated antibacterial peptides [106]. This material is made of a 100 amino acid polypeptides with alternating lysine and alanine crosslinked through a 6-armed PEG. Alanine and lysine have antimicrobial properties through their amphiphilic structure and inhibit bacterial growth by cell wall lysis [106].

In 2015, Babavalian *et al*. described a hydrogel to fight methicillin resistant *S. aureus* [105]. In this setup, the antimicrobial peptide is encapsulated into the material. They developed an alginate / sulfate hydrogel with an impregnated AMP called CM11. CM11 is a short cationic oligopeptide (WKLFKKILKVL) which has shown its antibacterial effects against Gram-positive and negative bacteria in previous studies [118]. They tested the hydrogels efficacy on mice wounds infected with *S. aureus*. CM11 was released from the matrix in a continuous manner over a period of three weeks. Mice wounds treated with this hydrogel were healed completely after 8 and 12 days, respectively.

In 2015, the group of Z. Xie provides another example for a wound healing hydrogel [12]. An *in situ* forming hydrogel was proposed which could cover every part of the wound through its shape-shifting properties. Furthermore, this hydrogel is not only biodegradable and highly biocompatible but has also several antimicrobial peptides conjugated to its surface. 24 h hours after wound treatment with the biodegradable gel in a rat skin model, wounds were exposed with *S. aureus*. Ampicillin and untreated wounds served as controls and no infection was observed when covering the wound with the antimicrobial hydrogel. However, the antimicrobial effects were still best for pure ampicillin.

Daphne and coworkers described an antibacterial hydrogel for medical implants [110]. The hydrogel itself consist of alternating linked valine and lysine. The surface of the hydrogel showed antibacterial activity against several Gram-positive *Staphylococcus* strains and Gram-negative strains like *E. coli* and *K. pneumoniae.* The gel showed the ability to disrupt inner and outer membrane, which leads to cell death while showing no hemolytic properties to human erythrocytes.

Another hydrogel for coatings of medical devices was proposed by Laverty *et al* [36]. An anti-adherent poly (2-hydroxyethyl methacrylate) hydrogel with incorporated antimicrobial peptides prevents the adhesion of bacteria on the surface of the covered implant. Furthermore, in case of contact of the medical device with bacteria, the integrated cationic antimicrobial peptide C_{12}-Orn-Orn-Trp-Trp-NH_2 kills the remaining microorganisms. A strong antibacterial effect was shown against *S. epidermidis,* which is known for serious infections through contact lenses, catheters etc.

Nanoparticles

Another important group of active compounds, which has successfully been used in antimicrobial therapy, are nanoparticles. Two different approaches can be applied in this context: either hydrogels themselves are produced as nanoparticles to increase their uptake by microorganisms or nanoparticles are directly encapsulated and / or loaded into the hydrogel. In both cases, the active substance is released from the material to reach higher concentrations at a specific target site.

Silver and gold nanoparticles have been known for a long time to exhibit antimicrobial activity; however, they are normally cytotoxic to mammalian cells as well. However, with the development of composite hydrogel materials and stronger investigations about the mechanism of metal nanoparticles, they become more and more attractive as antimicrobial agents.

Juby *et al.* developed a PVA hydrogel loaded with nanoparticles with antimicrobial activity [119]. Gel-to-sol transition takes place in a one pot reaction which is triggered by gamma irradiation. This technique also enables a sterilization of the material during formation along with a formation of nanoparticles in the range of 10 to 40 nm [119], while the loading with silver could be increased with rising crosslinking densities. Both the crosslinking density and the loading showed to have an influence on the toxicity of the hydrogel on *E. coli*.

In 2016, Zhang *et al.* produced an antimicrobial hydrogel inspired by the adhesion mechanisms of marine mussels [120]. One major challenge in antimicrobial treatment is the site-directed release of antimicrobial substances. By encapsulating the drug into nanoparticles, the properties of both platforms can be individually tailored; the nanoparticles are designed to enable sustainable, long-term release of the drug while the hydrogel is designed to enable a proper adhesion at the target site. The developed material shows high adhesion and strong mechanical properties on different substrates [120]. The hydrogel successfully eliminated all biofilm formation of *E. coli* and showed antimicrobial effects and non-toxicity in mice models.

Another promising application for hydrogels is their use in tissue engineering in biomedicine, where infections are a major concern. To address this challenge, Ribeiro *et al*. produced a hydrogel in 2017 for bone regeneration which has antimicrobial properties. This composite material made of silk fibroin and nanohydroxyapatite which has integrated silver and gold nanoparticles exhibits antimicrobial activity [109]. The hydrogel showed a good antimicrobial activity against *S. aureus, S. epidermidis, E. coli* and *P. aeruginosa*. The hydrogels were tested for cytotoxicity and the study revealed that mammalian cells were not affected at all by the hydrogel, independently of the used nanoparticle concentration, which makes the material a promising template for effective and save bone regeneration [109].

4. Capture and kill mechanism

Another innovative technique in the development of antimicrobial hydrogels, which has gained considerable attention in recent years, is the so-called "capture and kill" approach. The aim is to synthesize hydrogels which can both capture bacteria on a surface and eliminate them subsequently. These hydrogels could find application in the production of antibacterial surfaces for sterilization purposes, wound dressings or in medical devices such as coated catheters or tubing.

There is series of publications that showed the cell catching aspect of hydrogels [121–125]. Several ways to catch microorganisms are proposed: Shihui Li *et al*. , Zhaoyang Zhang *et al*. and Yanxia Xing *et al*. functionalized hydrogels with DNA aptamers to catch different cell types [121–123]. Aptamers consist of single stranded DNA which can bind specifically to their targets e.g. bacterial cells. However, this binding normally is highly specific. Xia Liu *et al*. developed a hydrogel functionalized with lectins to capture bacteria cells for analytical purposes [125]. Bacteria express carbohydrates and glycoproteins on the cell surface, which are bound by the lectins with high affinity. The immobilization of antibodies to the hydrogel is another possible method which was first described by Gloria Thomas *et al*. [124]. All those different materials highlight the efficient and sometimes reversible capture of microorganisms.

In 2013 Bin Cao *et al*. described the first hydrogel which can adhere, kill and release microorganism [126]. In their work they report that coated surfaces can efficiently capture *E. coli* and kill it through cell membrane damage.

Furthermore, the hydrogel can release dead cells through switching to a zwitterionic antifouling state triggered by hydrolyses. The disadvantage of this step is the irreversibility. Once switched to the antifouling state, cells cannot bind anymore to the surface.

Bin Xue and coworkers introduced a material which can change its physical properties through applied voltage [127]. It adsorbs high amounts of bacteria, which subsequently can be killed and removed through high voltage electric pulse.

Another promising approach are multi-component hydrogels as proposed by Li *et al*. in 2015 [128]. In their setup, the upper hydrogel layer can bind cancer cells specifically through immobilized aptamers while the lower layer releases toxic molecules in a consistent manner, which can diffuse through the hydrogels and kill the cells. Using different aptamers, this two-component system is also conceivable for catching and killing microorganisms.

5. Summary

Hydrogels with antimicrobial activity have been shown to be a potent tool in the fight against bacteria while many materials especially address multi resistant strains. They can be classified in hydrogels with or without inherent antimicrobial activity, while these groups are normally divided into natural and synthetic materials. While hydrogels composed of synthetic precursors normally have more defined structures and are easier to reproduce, natural materials offer a nearly unlimited range of possible starting materials and are generally biocompatible. The third option is to encapsulate antimicrobial agents like peptides or nanoparticles - covalently or diffusion controlled - to provide the hydrogel with antifouling or antimicrobial properties. Another development which has gained considerable attention lately is the so-called "capture and kill" approach where microorganisms can be - sometimes selectively - caught on a hydrogel surface and killed by the hydrogels properties, whereas especially regenerative materials possess promising features. The main application of antimicrobial hydrogels up to date is for wound dressing, the local delivery of drugs and the coating of medical devices like catheters or tubing in clinics.

The possible attachment and thus the high local dosage of a drug at a specific target site along with the modifiability and the vast number of possible antimicrobial hydrogel properties qualifies those "Jelly Bombs" as a novel class of materials and a potent and promising weapon in the fight against the emerging threat from pathogenic microorganisms.

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References

[1] Bush K, Pucci MJ. New antimicrobial agents on the horizon. Biochem. Pharmacol. 2011. p. 1528–1539.
[2] Weinstein RA. Nosocomial infection update. Emerg. Infect. Dis. 1998;4:416–420.
[3] Ng VWL, Chan JMW, Sardon H, Ono RJ, García JM, Yang YY, Hedrick JL. Antimicrobial hydrogels: A new weapon in the arsenal against multidrug-resistant infections. Adv. Drug Deliv. Rev. 2014;78:46–62.
[4] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 2010;74:417–433.
[5] Moellering RC. Discovering new antimicrobial agents. Int. J. Antimicrob. Agents. 2011. p. 2–9.
[6] Malmsten M. Antimicrobial and antiviral hydrogels. Soft Matter. 2011;7:8725.
[7] Veiga AS, Schneider JP. Antimicrobial hydrogels for the treatment of infection. Biopolymers. 2013;100:637–644.
[8] Wichterle O, Lím D. Hydrophilic Gels for Biological Use. Nature. 1960;185:117–118.

[9] Mi L, Xue H, Li Y, Jiang S. A Thermoresponsive Antimicrobial Wound Dressing Hydrogel Based on a Cationic Betaine Ester. Adv. Funct. Mater. 2011;21:4028–4034.
[10] Madsen J, Armes SP, Bertal K, Lomas H, MacNeil S, Lewis AL. Biocompatible Wound Dressings Based on Chemically Degradable Triblock Copolymer Hydrogels. Biomacromolecules. 2008;9:2265–2275.
[11] Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair S V., Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol. Adv. 2011;29:322–337.
[12] Xie Z, Aphale N V, Kadapure TD, Wadajkar AS, Orr S, Gyawali D, Qian G, Nguyen KT, Yang J. Design of antimicrobial peptides conjugated biodegradable citric acid derived hydrogels for wound healing. J. Biomed. Mater. Res. A. 2015;103:3907–3918.
[13] Bodenberger N, Kubiczek D, Paul P, Preising N, Weber L, Bosch R, Hausmann R, Gottschalk KE, Rosenau F. Beyond bread and beer: whole cell protein extracts from baker's yeast as a bulk source for 3D cell culture matrices. Appl. Microbiol. Biotechnol. 2016;pp 1-11.
[14] Bodenberger N, Paul P, Kubiczek D, Walther P, Gottschalk KE, Rosenau F.A novel cheap and easy to handle protein hydrogel for 3D cell culture applications : a high stability matrix with tunable elasticity and cell adhesion properties. Chemisrty Sel. 2016;1353–1360.
[15] Bodenberger N, Kubiczek D, Abrosimova I, Scharm A, Kipper F, Walther P, Rosenau F. Evaluation of methods for pore generation and their influence on physio-chemical properties of a protein based hydroge. Biotechnol. Reports. 2016;
[16] Raic A, Rödling L, Kalbacher H, Lee-Thedieck C. Biomimetic macroporous PEG hydrogels as 3D scaffolds for the multiplication of human hematopoietic stem and progenitor cells. Biomaterials. 2014;35:929–940. A
[17] Annabi N, Nichol JW, Zhong X, Ji C, Koshy S, Khademhosseini A, Dehghani F. Controlling the porosity and microarchitecture of hydrogels for tissue engineering. Tissue Eng. Part B. Rev. 2010;16:371–383.
[18] Mason BN, Califano JP, Reinhart-king C. Engineering Biomaterials for Regenerative Medicine. 2012;19–38.
[19] Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. Polymer. 2008;49:1993–2007.
[20] Chung HJ, Park TG. Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. Nano Today. 2009;4:429–437.
[21] Zhang W, Ren G, Xu H, Zhang J, Liu H, Mu S, Cai X, Wu T. Genipin cross-linked chitosan hydrogel for the controlled release of tetracycline with controlled release property, lower cytotoxicity, and long-term bioactivity. J. Polym. Res. 2016;23:156.
[22] Aziz M a., Cabral JD, Brooks HJL, Moratti SC, Hanton LR. Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use. Antimicrob. Agents Chemother. 2012;56:280–287.
[23] Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan - A versatile semi-synthetic polymer in biomedical applications. Prog. Polym. Sci. 2011;36:981–1014.
[24] Horkay F, Basser PJ. Osmotic observations on chemically cross-linked DNA gels in physiological salt solutions. Biomacromolecules. 2004;5:232–237.
[25] Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D. Enzyme-catalysed assembly of DNA hydrogel. Nat. Mater. 2006;5:797–801.
[26] Ozbas B, Kretsinger J, Rajagopal K, Schneider JP, Pochan DJ. Salt-Triggered Peptide Folding and Consequent Self-Assembly into Hydrogels with Tunable Modulus. Macromolecules. 2004;37:7331–7337.
[27] Salick D, Pochan DJ, Schneider JP. Design of an injectable ß-hairpin peptide hydrogel that kills methicillin-resistant staphylococcus aureus. Adv. Mater. 2009;21:4120–4123.
[28] Gallagher AG, Alorabi J, Wellings D, Lace R, Horsburgh MJ, Williams RL. A Novel Peptide Hydrogel for an Antimicrobial Bandage Contact Lens. Adv. Healthc. Mater. 2016;5:2013–2018.
[29] Stasko J, Kalniňš M, Dzene A, Tupureina V. Poly(vinyl alcohol) hydrogels. Proc. Est. Acad. Sci. 2009;58:63–66.
[30] Kamoun EA, Kenawy E-RS, Tamer TM, El-Meligy MA, Mohy Eldin MS. Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation. Arab. J. Chem. 2015;8:38–47.
[31] Lin CC, Anseth KS. Glucagon-like peptide-1 functionalized PEG hydrogels promote survival and function of encapsulated pancreatic cells. Biomacromolecules. 2009;10:2460–2467.
[32] Missirlis D, Spatz JP. Combined effects of PEG hydrogel elasticity and cell-adhesive coating on fibroblast adhesion and persistent migration. Biomacromolecules. 2014;15:195–205.
[33] Liu SQ, Yang C, Huang Y, Ding X, Li Y, Fan WM, Hedrick JL, Yang Y-Y. Antimicrobial and Antifouling Hydrogels Formed In Situ from Polycarbonate and Poly(ethylene glycol) via Michael Addition. Adv. Mater. 2012;24:6484–6489.
[34] Villanueva ME, Diez AMDR, González JA, Pérez CJ, Orrego M, Piehl L, Teves S, Copello GJ. Antimicrobial Activity of Starch Hydrogel Incorporated with Copper Nanoparticles. ACS Appl. Mater. Interfaces. 2016;8:16280–16288.
[35] Wong Po Foo CTS, Lee JS, Mulyasasmita W, Parisi-Amon A, Heilshorn SC. Two-component protein-engineered physical hydrogels for cell encapsulation. Proc. Natl. Acad. Sci. U. S. A. 2009;106:22067–22072.
[36] Laverty G, Gorman SP, Gilmore BF. Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of *Staphylococcus epidermidis*-associated biomaterial infections. J. Biomed. Mater. Res. Part A. 2012;100A:1803–1814.
[37] Baral A, Roy S, Ghosh S, Hermida-Merino D, Hamley IW, Banerjee A. A Peptide-Based Mechano-sensitive, Proteolytically Stable Hydrogel with Remarkable Antibacterial Properties. Langmuir. 2016;32:1836–1845.
[38] Campoccia D, Montanaro L, Arciola CR. A review of the clinical implications of anti-infective biomaterials andinfection-resistant surfaces. Biomaterials. 2013;34:8018–8029.
[39] Lee KY, Mooney DJ. Hydrogels for Tissue Engineering Applications. Chem. Rev. 2001;101:1869–1879.
[40] Li Y, Rodrigues J, Tomás H. Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. Chem. Soc. Rev. 2012;41:2193–2221.
[41] Hoffman AS. Hydrogels for biomedical applications. Adv. Drug Deliv. Rev. 2012. p. 18–23.
[42] Censi R, Di Martino P, Vermonden T, Hennink WE. Hydrogels for protein delivery in tissue engineering. J. Control. Release. 2012;161:680–692.

[43] Salick DA, Kretsinger JK, Pochan DJ, Schneider JP. Inherent antibacterial activity of a peptide-based beta-hairpin hydrogel. J. Am. Chem. Soc. 2007;129:14793–14799.
[44] Veiga AS, Sinthuvanich C, Gaspar D, Franquelim HG, Castanho MARB, Schneider JP. Arginine-rich self-assembling peptides as potent antibacterial gels. Biomaterials. 2012;33:8907–8916.
[45] Weber DJ, Raasch R, Rutala WA. Nosocomial infections in the ICU: the growing importance of antibiotic-resistant pathogens. Chest. 1999;115:34S–41S.
[46] Chan DI, Prenner EJ, Vogel HJ. Tryptophan- and arginine-rich antimicrobial peptides: Structures and mechanisms of action. Biochim. Biophys. Acta - Biomembr. 2006;1758:1184–1202.
[47] Mohamed NA, Fahmy MM. Synthesis and antimicrobial activity of some novel cross-linked chitosan hydrogels. Int. J. Mol. Sci. 2012;13:11194–11209.
[48] Song Z, Borgwardt L, Hoiby N, Wu H, Sorensen TS, Borgwardt A. Prosthesis infections after orthopedic joint replacement: the possible role of bacterial biofilms. Orthop. Rev. 2013;5:65–71.
[49] Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. *In vivo* pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. Antimicrob. Agents Chemother. 2012;56:2683–2690.
[50] Shih I-L, Shen M-H, Van Y-T. Microbial synthesis of poly(epsilon-lysine) and its various applications. Bioresour. Technol. 2006;97:1148–1159.
[51] Hyldgaard M, Mygind T, Vad BS, Stenvang M, Otzen DE, Meyer RL. The antimicrobial mechanism of action of epsilon-poly-L-lysine. Appl. Environ. Microbiol. 2014;80:7758–7770.
[52] Jayakumar R, Menon D, Manzoor K, Nair S V, Tamura H. Biomedical applications of chitin and chitosan based nanomaterials - A short review. Carbohydr. Polym. 2010;82:227–232.
[53] Larsson M, Huang WC, Hsiao MH, Wang YJ, Nydén M, Chiou SH, Liu DM. Biomedical applications and colloidal properties of amphiphilically modified chitosan hybrids. Prog. Polym. Sci. 2013;38:1307–1328.
[54] Tomšič B, Simončič B, Orel B, Vilčnik A, Spreizer H. Biodegradability of cellulose fabric modified by imidazolidinone. Carbohydr. Polym. 2007;69:478–488.
[55] Sannino A, Demitri C, Madaghiele M. Biodegradable cellulose-based hydrogels: Design and applications. Materials 2009;2:353–373.
[56] Dong C, Ye Y, Qian L, Zhao G, He B, Xiao H. Antibacterial modification of cellulose fibers by grafting β-cyclodextrin and inclusion with ciprofloxacin. Cellulose. 2014;21:1921–1932.
[57] Ren X, Kou L, Liang J, Worley SD, Tzou Y-M, Huang TS. Antimicrobial efficacy and light stability of N-halamine siloxanes bound to cotton. Cellulose. 2008;15:593–598.
[58] Tsao CT, Chang CH, Lin YY, Wu MF, Wang J-L, Lin J, Kuo HE, Hsieh H. Antibacterial activity and biocompatibility of a chitosan-poly(glutamic acid) polyelectrolyte complex hydrogel. Carbohydr. Res. 2010;345:1774–1780.
[59] Li P, Poon YF, Li W, Zhu H-Y, Yeap SH, Cao Y, Qi X, Zhou C, Lamrani M, Beuerman RW, Kang E-T, Mu Y, Li CM, Chang MW, Jan Leong SS, Chan-Park MB. A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. Nat. Mater. 2011;10:149–156.
[60] Peng N, Wang Y, Ye Q, Liang L, An Y, Li Q, Chang C. Biocompatible cellulose-based superabsorbent hydrogels with antimicrobial activity. Carbohydr. Polym. 2016;137:59–64.
[61] Cao A, Tang Y, Liu Y, Yuan H, Liu L. A strategy for antimicrobial regulation based on fluorescent conjugated oligomer–DNA hybrid hydrogels. Chem. Commun. 2013;49:5574.
[62] Tang Y, Feng F, He F, Wang S, Li Y, Zhu D. Direct visualization of enzymatic cleavage and oxidative damage by hydroxyl radicals of single-stranded DNA with a cationic polythiophene derivative. J. Am. Chem. Soc. 2006;128:14972–14976.
[63] Zhu J, Marchant RE. Design properties of hydrogel tissue-engineering scaffolds. Expert Rev. Med. Devices.
[64] Kuroda K, DeGrado WF. Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents. 2005. J. Am. Chem. Soc., 2005, 127 (12), pp 4128–4129
[65] Kuroda K, Caputo GA, DeGrado WF. The Role of Hydrophobicity in the Antimicrobial and Hemolytic Activities of Polymethacrylate Derivatives. Chem. - A Eur. J. 2009;15:1123–1133.
[66] Palermo EF, Kuroda K. Chemical Structure of Cationic Groups in Amphiphilic Polymethacrylates Modulates the Antimicrobial and Hemolytic Activities. Biomacromolecules. 2009;10:1416–1428.
[67] Oda Y, Kanaoka S, Sato T, Aoshima S, Kuroda K. Block versus Random Amphiphilic Copolymers as Antibacterial Agents. Biomacromolecules. 2011;12:3581–3591.
[68] Palermo EF, Vemparala S, Kuroda K. Cationic Spacer Arm Design Strategy for Control of Antimicrobial Activity and Conformation of Amphiphilic Methacrylate Random Copolymers. Biomacromolecules. 2012;13:1632–1641.
[69] Locock KES, Michl TD, Valentin JDP, Vasilev K, Hayball JD, Qu Y, Traven A, Griesser HJ, Meagher L, Haeussler M. Guanylated Polymethacrylates: A Class of Potent Antimicrobial Polymers with Low Hemolytic Activity. Biomacromolecules. 2013;14:4021–4031.
[70] Nederberg F, Zhang Y, Tan JPK, Xu K, Wang H, Yang C, Gao S, Guo XD, Fukushima K, Li L, Hedrick JL, Yang Y-Y. Biodegradable nanostructures with selective lysis of microbial membranes. Nat. Chem. 2011;3:409–414.
[71] Qiao Y, Yang C, Coady DJ, Ong ZY, Hedrick JL, Yang Y-Y. Highly dynamic biodegradable micelles capable of lysing Gram-positive and Gram-negative bacterial membrane. Biomaterials. 2012;33:1146–1153.
[72] Fukushima K, Tan JPK, Korevaar PA, Yang YY, Pitera J, Nelson A, Maune H, Coady DJ, Frommer JE, Engler AC, Huang Y, Xu K, Ji Z, Qiao Y, Fan W, Li L, Wiradharma N, Meijer EW, Hedrick JL. Broad-Spectrum Antimicrobial Supramolecular Assemblies with Distinctive Size and Shape. ACS Nano. 2012;6:9191–9199.
[73] Ng VWL, Ke X, Lee ALZ, Hedrick JL, Yang YY. Synergistic Co-Delivery of Membrane-Disrupting Polymers with Commercial Antibiotics against Highly Opportunistic Bacteria. Adv. Mater. 2013;25:6730–6736.
[74] Engler AC, Tan JPK, Ong ZY, Coady DJ, Ng VWL, Yang YY, Hedrick JL. Antimicrobial Polycarbonates: Investigating the Impact of Balancing Charge and Hydrophobicity Using a Same-Centered Polymer Approach. Biomacromolecules. 2013;14:4331–4339.

[75] Chin W, Yang C, Ng VWL, Huang Y, Cheng J, Tong YW, Coady DJ, Fan W, Hedrick JL, Yang YY. Biodegradable Broad-Spectrum Antimicrobial Polycarbonates: Investigating the Role of Chemical Structure on Activity and Selectivity. Macromolecules. 2013;46:8797–8807.
[76] Som A, Navasa N, Percher A, Scott RW, Tew GN, Anguita J. Identification of synthetic host defense peptide mimics that exert dual antimicrobial and anti-inflammatory activities. Clin. Vaccine Immunol. 2012;19:1784–1791.
[77] Tew GN, Liu D, Chen B, Doerksen RJ, Kaplan J, Carroll PJ, Klein ML, DeGrado WF. *De novo* design of biomimetic antimicrobial polymers. Proc. Natl. Acad. Sci. U. S. A. 2002;99:5110–5114.
[78] Gabriel GJ, Madkour AE, Dabkowski JM, Nelson CF, Nüsslein K, Tew GN. Synthetic Mimic of Antimicrobial Peptide with Nonmembrane-Disrupting Antibacterial Properties. Biomacromolecules. 2008;9:2980–2983.
[79] Dohm MT, Mowery BP, Czyzewski AM, Stahl SS, Gellman SH, Barron AE. Biophysical Mimicry of Lung Surfactant Protein B by Random Nylon-3 Copolymers. J. Am. Chem. Soc. 2010;132:7957–7967.
[80] Zhang J, Markiewicz MJ, Mowery BP, Weisblum B, Stahl SS, Gellman SH. C-Terminal Functionalization of Nylon-3 Polymers: Effects of C-Terminal Groups on Antibacterial and Hemolytic Activities. Biomacromolecules. 2012;13:323–331.
[81] Liu R, Chen X, Hayouka Z, Chakraborty S, Falk SP, Weisblum B, Masters KS, Gellman SH. Nylon-3 Polymers with Selective Antifungal Activity. J. Am. Chem. Soc. 2013;135:5270–5273.
[82] Chakraborty S, Liu R, Lemke JJ, Hayouka Z, Welch RA, Weisblum B, Masters KS, Gellman SH. Effects of Cyclic vs. Acyclic Hydrophobic Subunits on the Chemical Structure and Biological Properties of Nylon-3 Co-Polymers. ACS Macro Lett. 2013;2.
[83] Mowery BP, Lee SE, Kissounko DA, Epand RF, Epand RM, Weisblum B, Stahl SS, Gellman SH. Mimicry of Antimicrobial Host-Defense Peptides by Random Copolymers. J. Am. Chem. Soc. 2007;129:15474–15476.
[84] Ilker MF et al. Tuning the Hemolytic and Antibacterial Activities of Amphiphilic Polynorbornene Derivatives. 2004; J Am Chem. Soc. 126 (48), 15870-15875
[85] Lienkamp K, Madkour AE, Musante A, Nelson CF, Nüsslein K, Tew GN. Antimicrobial Polymers Prepared by ROMP with Unprecedented Selectivity: A Molecular Construction Kit Approach. J. Am. Chem. Soc. 2008;130:9836–9843.
[86] Colak S, Nelson CF, Nüsslein K, Tew GN. Hydrophilic modifications of an amphiphilic polynorbornene and the effects on its hemolytic and antibacterial activity. Biomacromolecules. 2009;10:353–359.
[87] Li Y, Fukushima K, Coady DJ, Engler AC, Liu S, Huang Y, Cho JS, Guo Y, Miller LS, Tan JPK, Ee PLR, Fan W, Yang YY, Hedrick JL. Broad-spectrum antimicrobial and biofilm-disrupting hydrogels: Stereocomplex-driven supramolecular assemblies. Angew. Chemie - Int. Ed. 2013;52:674–678.
[88] Cao B, Tang Q, Li L, Humble J, Wu H, Liu L, Cheng G. Switchable antimicrobial and antifouling hydrogels with enhanced mechanical properties. Adv. Healthc. Mater. 2013;2:1096–1102.
[89] Siedenbiedel F, Tiller JC. Antimicrobial polymers in solution and on surfaces: Overview and functional principles. Polymers. 2012;4:46–71.
[90] Perrault JJ, Rouns CG. Inherently antimicrobial quaternary amine hydrogel wound dressings. United States Pat. 2000;
[91] Bertal K, Shepherd J, Douglas CWI, Madsen J, Morse A, Edmondson S, Armes SP, Lewis A, MacNeil S. Antimicrobial activity of novel biocompatible wound dressings based on triblock copolymer hydrogels. J. Mater. Sci. 2009;44:6233–6246.
[92] Ikeda T, Hirayama H, Yamaguchi H, Tazuke S, Watanabe M. Polycationic biocides with pendant active groups: molecular weight dependence of antibacterial activity. Antimicrob. Agents Chemother. 1986;30:132–136.
[93] La Y-H, McCloskey BD, Sooriyakumaran R, Vora A, Freeman B, Nassar M, Hedrick J, Nelson A, Allen R. Bifunctional hydrogel coatings for water purification membranes: Improved fouling resistance and antimicrobial activity. J. Memb. Sci. 2011;372:285–291.
[94] Gustafson CT, Boakye-Agyeman F, Brinkman CL, Reid JM, Patel R, Bajzer Z, Dadsetan M, Yaszemski MJ. Controlled Delivery of Vancomycin via Charged Hydrogels. PLoS One. 2016;11:e0146401.
[95] Zhang LF, Yang DJ, Chen HC, Sun R, Xu L, Xiong ZC, Govender T, Xiong CD. An ionically crosslinked hydrogel containing vancomycin coating on a porous scaffold for drug delivery and cell culture. Int. J. Pharm. 2008;353:74–87.
[96] De Giglio E, Cometa S, Ricci MA, Cafagna D, Savino AM, Sabbatini L, Orciani M, Ceci E, Novello L, Tantillo GM, Mattioli-Belmonte M. Ciprofloxacin-modified electrosynthesized hydrogel coatings to prevent titanium-implant-associated infections. Acta Biomater. 2011;7:882–891.
[97] Marchesan S, Qu Y, Waddington LJ, Easton CD, Glattauer V, Lithgow TJ, McLean KM, Forsythe JS, Hartley PG. Self-assembly of ciprofloxacin and a tripeptide into an antimicrobial nanostructured hydrogel. Biomaterials. 2013;34:3678–3687.
[98] Peng K-T, Chen C-F, Chu I-M, Li Y-M, Hsu W-H, Hsu RW-W, Chang P-J. Treatment of osteomyelitis with teicoplanin-encapsulated biodegradable thermosensitive hydrogel nanoparticles. Biomaterials. 2010;31:5227–5236.
[99] Chang C-H, Lin Y-H, Yeh C-L, Chen Y-C, Chiou S-F, Hsu Y-M, Chen Y-S, Wang C-C. Nanoparticles Incorporated in pH-Sensitive Hydrogels as Amoxicillin Delivery for Eradication of Helicobacter pylori. Biomacromolecules. 2010;11:133–142.
[100] Concheiro A, Alvarez-Lorenzo C. Chemically cross-linked and grafted cyclodextrin hydrogels: From nanostructures to drug-eluting medical devices. Adv. Drug Deliv. Rev. 2013;65:1188–1203.
[101] Glisoni RJ, García-Fernández MJ, Pino M, Gutkind G, Moglioni AG, Alvarez-Lorenzo C, Concheiro A, Sosnik A. β-Cyclodextrin hydrogels for the ocular release of antibacterial thiosemicarbazones. Carb. Polym. 2013;93:449–457.
[102] Cheng G, Xue H, Li G, Jiang S. Integrated Antimicrobial and Nonfouling Hydrogels to Inhibit the Growth of Planktonic Bacterial Cells and Keep the Surface Clean. Langmuir. 2010;26:10425–10428.
[103] Lakes A, Peyyala R, Ebersole J, Puleo D, Hilt JZ, Dziubla T. Synthesis and Characterization of an Antibacterial Hydrogel Containing Covalently-bound Vancomycin. Biomacromolecules. 2014; 15 (8), pp 3009–3018
[104] Cleophas RTC, Sjollema J, Busscher HJ, Kruijtzer JAW, Liskamp RMJ. Characterization and Activity of an Immobilized Antimicrobial Peptide Containing Bactericidal PEG-Hydrogel. Biomacromolecules. 2014;15:3390–3395.
[105] Babavalian H, Latifi AM, Shokrgozar MA, Bonakdar S, Mohammadi S, Moosazadeh Moghaddam M. Analysis of Healing Effect of Alginate Sulfate Hydrogel Dressing Containing Antimicrobial Peptide on Wound Infection Caused by Methicillin-Resistant Staphylococcus aureus. Jundishapur J. Microbiol. 2015;8:e28320.

[106] Song A, Rane AA, Christman KL. Antibacterial and cell-adhesive polypeptide and poly(ethylene glycol) hydrogel as a potential scaffold for wound healing. Acta Biomater. 2012;8:41–50.
[107] Mateescu M, Baixe S, Garnier T, Jierry L, Ball V, Haikel Y, Metz-Boutigue MH, Nardin M, Schaaf P, Etienne O, Lavalle P. Antibacterial Peptide-Based Gel for Prevention of Medical Implanted-Device Infection. PLoS One. 2015;10:e0145143.
[108] Zhou C, Li P, Qi X, Sharif ARM, Poon YF, Cao Y, Chang MW, Leong SSJ, Chan-Park MB. A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-l-lysine. Biomaterials. 2011;32:2704–2712.
[109] Ribeiro M, Ferraz MP, Monteiro FJ, Fernandes MH, Beppu MM, Mantione D, Sardon H. Antibacterial silk fibroin/nanohydroxyapatite hydrogels with silver and gold nanoparticles for bone regeneration. Nanomedicine Nanotechnology, Biol. Med. 2017;13:231–239.
[110] Daphne A. Salick, Juliana K. Kretsinger, Darrin J. Pochan, Joel P. Schneider. Inherent Antibacterial Activity of a Peptide-Based β-Hairpin Hydrogel. 2007; 28;129(47):14793-9
[111] Cassini A, Plachouras D, Eckmanns T, Abu Sin M, Blank H-P, Ducomble T, Haller S, Harder T, Klingeberg A, Sixtensson M, Velasco E, Weiß B, Kramarz P, Monnet DL, Kretzschmar ME, Suetens C, Umscheid C, Mitchell M, Doshi J, et al. Burden of Six Healthcare-Associated Infections on European Population Health: Estimating Incidence-Based Disability-Adjusted Life Years through a Population Prevalence-Based Modelling Study. PLOS Med. 2016;13:e1002150.
[112] Calo E, Khutoryanskiy V V. Biomedical applications of hydrogels: A review of patents and commercial products. Eur. Polym. J. 2015;65:252–267.
[113] Li H, Yang J, Hu X, Liang J, Fan Y, Zhang X. Superabsorbent polysaccharide hydrogels based on pullulan derivate as antibacterial release wound dressing. J. Biomed. Mater. Res. Part A. 2011;98A:31–39.
[114] Sung JH, Hwang M-R, Kim JO, Lee JH, Kim Y Il, Kim JH, Chang SW, Jin SG, Kim JA, Lyoo WS, Han SS, Ku SK, Yong CS, Choi H-G. Gel characterisation and *in vivo* evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int. J. Pharm. 2010;392:232–240.
[115] Jones DS, Lorimer CP, McCoy CP, Gorman SP. Characterization of the physicochemical, antimicrobial, and drug release properties of thermoresponsive hydrogel copolymers designed for medical device applications. J. Biomed. Mater. Res. Part B Appl. Biomater. 2008;85B:417–426.
[116] Bahar AA, Ren D. Antimicrobial peptides. Pharmaceuticals. 2013;6:1543–1575.
[117] Lee ALZ, Ng VWL, Wang W, Hedrick JL, Yang YY. Block copolymer mixtures as antimicrobial hydrogels for biofilm eradication. Biomaterials. 2013;34:10278–10286.
[118] Moghaddam MM, Abolhassani F, Babavalian H, Mirnejad R, Azizi Barjini K, Amani J. Comparison of *in vitro* antibacterial activities of two cationic peptides CM15 and CM11 against five pathogenic bacteria: *Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae, Acinetobacter baumannii, and Escherichia coli*. Probiotics Antimicrob. Proteins. 2012;4:133–139.
[119] Juby KA, Dwivedi C, Kumar M, Kota S, Misra HS, Bajaj PN. Silver nanoparticle-loaded PVA/gum acacia hydrogel: Synthesis, characterization and antibacterial study. Carbohydr. Polym. 2012;89:906–913.
[120] Zhang Y, Zhang J, Chen M, Gong H, Thamphiwatana S, Eckmann L, Gao W, Zhang L. A Bioadhesive Nanoparticle-Hydrogel Hybrid System for Localized Antimicrobial Drug Delivery. ACS Appl. Mater. Interfaces. 2016;8:18367–18374.
[121] Zhang Z, Chen, Niancao, Li S, Battig MR, Wang Y. Programmable Hydrogels for Controlled Cell Catch and Release Using Hybridized Aptamers and Complementary Sequences. J. Am. Chem. Soc. 2012, 134 (38), pp 15716–15719
[122] Li S, Chen N, Zhang Z, Wang Y. Endonuclease-responsive aptamer-functionalized hydrogel coating for sequential catch and release of cancer cells. Biomaterials. 2013;34:460–469.
[123] Xing Y, Guo J, Chen Q, Li F, Yang Y. The application of aptamer functionalized hydrogel to capture circulating tumor cells. Int J Clin Exp Med. 2016;9:12931–12936.
[124] Thomas G, El-Giar EM, Locascio LE, Tarlov MJ. Hydrogel-Immobilized Antibodies for Microfluidic Immunoassays Hydrogel Immunoassay. Methods Mol Biol. 2006;321:83-95.
[125] Liu X, Lei Z, Liu F, Liu D, Wang Z. Fabricating three-dimensional carbohydrate hydrogel microarray for lectin-mediated bacterium capturing. Biosens. Bioelectron. 2014;58:92–100.
[126] Cao B, Tang Q, Li L, Humble J, Wu H, Liu L, Cheng G. Switchable Antimicrobial and Antifouling Hydrogels with Enhanced Mechanical Properties. Adv. Healthc. Mater. 2013;2:1096–1102.
[127] Xue B, Qin M, Wu J, Luo D, Jiang Q, Li Y, Cao Y, Wang W. Electroresponsive Supramolecular Graphene Oxide Hydrogels for Active Bacteria Adsorption and Removal.
[128] Li S, Chen N, Gaddes ER, Zhang X, Dong C, Wang Y. A Drosera-bioinspired hydrogel for catching and killing cancer cells. Sci. Rep. 2015;5:14297.