Review

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Posted Date: 30 July 2024

doi: 10.20944/preprints202407.2265.v1

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Insights into the Preparation and Evaluation of the Bactericidal Effects of Phage-Based Hydrogels

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Abstract: The rise of antibiotic-resistant strains demands new alternatives in antibacterial treatments. Bacteriophages, with their precise host specificity and ability to target and eliminate bacteria safely, present a valuable option. Meanwhile, hydrogels, known for their excellent biodegradability and biocompatibility, serve as ideal carriers for bacteriophages. The combination of bacteriophages and hydrogels ensures heightened phage activity, concentration, controlled release, and strong antibacterial properties, making it a promising avenue for antibacterial treatment. This article provides a comprehensive review of different cross-linking methods for phage hydrogels and focuses on their application in treating infections caused by various drug-resistant bacteria.

Keywords: bacteriophage (phage); hydrogel; antibacterial; wound healing

1. Introduction

The human body hosts numerous benign colonizing bacteria in areas such as gut and skin, which play a crucial role in external communication. Nevertheless, when these bacteria migrate to sites like the lungs or bladder, they can readily lead to bacterial infections [1]. Antibiotics, traditional drugs used for treating or preventing bacterial infections [2], have significantly contributed to improving human health. However, their excessive use and misuse have led to bacterial resistance, resulting in the emergence of multidrug-resistant (MDR) strains [3]. Infections caused by MDR microorganisms can be exceptionally challenging to treat, leading to prolonged treatment times [4], mortality rates among patients, and a heightened economic burden on treatment[5], posing a significant threat to the global economy[6]. While new antibiotics hold the potential for controlling multidrug-resistant bacteria [7], their development has slowed due to cost and market profitability pressures, creating a pressing need for new antibacterial therapies [8]. In recent years, bacteriophage therapy has successfully treated life-threatening multidrug-resistant bacterial infections [9], providing a potential alternative to antibiotics for treating bacterial infections [10]. Phage therapy offers significant advantages, including more universal applications, host specificity, broader antibacterial potential, and causes less harm to the human body compared to traditional antibiotics [11]. Bacteriophage therapy also encounters several limitations, including the development of bacteriophage-resistant strains, concerns about the host immune response, and a narrow host spectrum. Current research indicates that hydrogels can serve as effective carriers for local bacteriophage delivery. Phage-based hydrogels not only ensure the activity, concentration, and release efficiency of bacteriophages but also effectively reduce bacterial presence at the infection site, thereby enhancing the overall antibacterial effect [12]. These hydrogels possess excellent bacteriophage controlled release capabilities and strong antibacterial properties, sparking significant research interest. Studies by Stijn Gerard Rotman and his colleagues have shed light on the method of encapsulating bacteriophages in hydrogels and elucidated the potential and obstacles of bacteriophage hydrogels in treating bacterial infections [13]. Previously, Hyun Young Kim [14], Haoran Bai[15] and other researchers delved into

 doi:10.20944/preprints202407.2265.v1

the diverse construction methods and benefits of phage hydrogels, with a focus on their applications and effects at various infection sites. Fatemeh Shafigh Kheljan *et al.* developed a phage-infused wound dressing to effectively combat wound infections caused by *P. aeruginosa.* The phage cocktail was carefully encapsulated in a hydrogel composed of sodium alginate and CMC. The study's results demonstrated that this novel phage hydrogel exhibited strong antibacterial properties and effectively facilitated wound healing[16]. In a similar vein, Baixing Chen and his team employed phage therapy as a means of managing infections associated with fractures. They devised a strategy that involved blending phage cocktails with antibiotics and encapsulating them within hydrogel beads. This ingenious approach not only minimized the risk of phage resistance emergence but also exhibited robust antibacterial properties[17]. The team led by Farzaneh Moghtader improved the traditional hydrogel and created a mixed alginate and chitosan hydrogel formula, which was loaded with phage T4 to control *E. coli* infection, achieving good control release and antibacterial performance[18]. Recently reported on skin and soft tissue infections, L Vacek innovatively used gum Karaya (GK)-based injection hydrogel loaded with staphylococcus phage 812K1/420 to treat *S. aureus* (MRSA） infection. This method significantly reduces bacterial load and local inflammation and has good application potential[19]. Mahshid Khazani Asforooshani *et al.* developed hydrogel based on phage in response to the resistance of *E. fecal* antibiotics, which proved its great potential in transmitting phages and promoting wound healing[20]. Kannan Badri Narayanan and his colleagues used marine polysaccharide carrageenan (carr-vB_Eco2571-YU1) to make hydrogel wound dressings. After loading *E. coli* phages, they evaluated their antibacterial effect, which can cause a sharp drop in bacteria in a short time and effectively prevent bacteriophage resistance[21]. This review centers on site-specific infections caused by diverse drug-resistant bacteria and delves into key aspects of phage hydrogel therapy, including preparation, research model construction, and therapeutic effects under various bacterial infections.

2. Various Types of Hydrogels with Different Crosslinking Methods

Hydrogels are a type of three-dimensional network structure gel that swiftly expands in water and retains a substantial volume of water without dissolving. Hydrogels are commonly cross-linked using two strategies: physical cross-linking and chemical cross-linking [22].

2.1. Utilizing Physical Mechanisms for Crosslinking

Physical crosslinking methods for developing hydrogels include ionic and coordination bond crosslinking, hydrogen bonding crosslinking, host-guest interactions crosslinking, and self-assembling peptide crosslinking. Polymers capable of forming hydrogels through ionic crosslinking include Alginate, PVA-SA, PolyHIPE/Nanocellulose, Hydroxyapatite (HA), and beta tricalcium phosphate (β-TCP). Alginate is a widely used polymer for ion crosslinking. Yongsheng Ma and colleagues created a phage K-containing hydrogel by combining 2% (w/v) sodium alginate with 10^8 PFU/mL phage K and crosslinking it in a 100 mM calcium chloride solution, which effectively boosts the survival of free bacteriophages in simulated gastric conditions [23]. Using a parallel approach, Leah H. Cobb and colleagues vigorously blended a 2% (w/v) alginate solution loaded with therapeutic drugs with calcium sulfate for 1 minute to produce an alginate gel, followed by the addition of 3×10^7 PFU /mL of phage into the alginate hydrogel. The resulted cross-linked alginate gel displayed antibacterial characteristics and biocompatibility [24]. Furthermore, Prabhjot Kaur's team blended 10% PVA with 3% SA resulting mixture onto sterile 5×5 cm cotton yarn and crosslinking it with saturated boric acid alongside a 2% calcium chloride solution. Subsequently, the cross-linked blend was frozen overnight at -60 ℃, lyophilized, and infused with 1.0×10^8 PFU/mL of phage. It was then frozen overnight at -60 ℃ to form the PVA-SA the highest swelling index (~850%), gel fraction (~52%), protein adsorption capacity, blood compatibility, and superior mechanical properties after assessments [25].

An alternative method for creating hydrogels through physical crosslinking involves utilizing thermal gelation. Polymers that can create hydrogels via thermal gelation encompass Poloxamer 407, HPMC, Agarose/Hyaluronan Hydrogel Matrices (HAMA) and Poly N-isopropylacrylamide co-

 doi:10.20944/preprints202407.2265.v1

allylamine (PNIPAMCo-ALA). Poloxamer 407 (P407), also known as Pluronic F-127, is a tri-block copolymer composed of polyethylene oxide（70%) and polypropylene oxide (30%). The most notable characteristic of P407 is its reversible thermoresponsive nature, enabling it to undergo gelation near body temperature (approximately 37℃) and remain at the site of implantation as a sustainable carrier. At lower temperatures (typically <15–25℃, depending on the polymer weight fraction), P407 remains in a liquid state, enabling the loading of therapeutics for subsequent release when it transitions into a gel state. Previously, P407 has been explored as a carrier for E. faecalis phages, A. baumannii phages, and antibiotics such as vancomycin, levofloxacin, and metronidazole. Combining P407 with other polymers or adding salt-form molecules like chitosan salts can modify its polymer matrix, affecting drug release kinetics through changes in matrix structure, erosion, swelling, polymer degradation, and drug release rate.

Hydroxypropyl methylcellulose (HPMC) is widely utilized in controlled release applications for its thickening, gelling, and swelling as well as its non-toxicity, ease of compression, and capability to accommodate high drug levels. The bioadhesive attribute of HPMC is a result of its rich -OH functional groups, which are capable of forming hydrogen bonds with water and other HPMC molecules.Due to its strong biocompatibility, HPMC can serve as a thermosensitive natural polymer, enabling the creationless hydrogel with exceptional stability, viscosity, and texture modification capabilities. Furthermore, HPMC exhibits minimal drug interactions, primarily, and has shown effectiveness in enhancing bioadhesion and local drug delivery by improving retention. Seema Kumari's team dissolved HPMC in warm water, stirred it to form a gel, and then loaded the 3% HPMC hydrogel with a 1.0 mL phage sample to reach a concentration of 10^8 PFU/mL in the phage-loaded HPMC hydrogel. This hydrogel effectively prevents excessive body fluid loss, forms a reliable anti-corrosion and particle barrier, and efficiently absorbs wound exudate, thereby establishing itself as an optimal wound dressing [26]. PNIPAM, a thermally responsive polymer, exhibits a sol-gel transition from liquid to gel state in response to temperature changes. At a lower critical solution temperature (32–36℃), PNIPAM forms a hydrophobic globule structure by expelling water. When co-polymerized with allylamine, PNIPAM nanospheres create an interactive surface for phage binding and regulate the lower critical solution temperature to 37℃. In a study led by Hollie Hathaway [27], a blend of 0.96g N-isopropylacrylamide and 65.5 μL allylamine was cross-linked with 20.8 μL of ethylene glycol diacrylate. Harnessing the beneficial temperature-responsive properties of the hydrogel, the use of PNIPAM-co-ALA hydrogel for encapsulating phage K was discovered to significantly enhance the release of phages and the eradication of S. aureus.

Additionally, the freeze-thaw method acts as a physical crosslinking technique for the formation of hydrogels, and Polyvinyl Alcohol (PVA) is frequently chosen as the polymer for this process. Polyvinyl Alcohol (PVA) is a hydrogel feedstock polymer, known for its semi crystalline structure and the repetition of isomers (CH2CHOH). Its high content of hydroxyl groups promotes water absorption and expansion of the polymeric network, facilitating drug release. In the pharmaceutical industry, it is widely used for controlled drug release due to its biocompatibility, non-toxicity, chemical stability, low cost, and excellent mechanical resistance. Scarlet Milo and her colleagues created a 10% w/v PVA solution containing phages by dissolving and cooling 20% w/v Poly(vinyl alcohol), and then adding bacteriophage lysate at a 1:1 ratio. The resulting PVA solution was applied to the catheter, then frozen overnight at -20 ℃ to form the phage-containing PVA gel, effectively extending the blockage time and successfully eliminating bacterial colonization on the catheter [28]. Structured hydrogels made of self-organized M13 bacteriophage bundles, composed of hundreds of M13 Nano filaments, were visible in electron micrographs in their cross-linked state, capable of absorbing up to 16 times their weight in water. These hydrogels showed advanced properties at room temperature, such as self-healing under biological conditions, auto fluorescence in three channels with decay through biodegradation, allowing non-destructive imaging, and bioactivity towards host bacteria in their cross-linked state. This bioactivity is particularly powerful, enabling the development of hydrogels with adjustable bioactivity when combined with phage display and/or recombinant DNA technology [29]. In 2021, Peivandi's team successfully created a composite phage hydrogel using M13 phages and 0.25% w/v bovine serum albumin at a lower phage

 doi:10.20944/preprints202407.2265.v1

concentration, demonstrating remarkable properties including high water absorption, biological activity, adjustable mechanical properties, and self-repair capabilities [30].

2.2. Employing Chemical Agents for Crosslinking

Chemical crosslinking connects the network structure of hydrogels through chemical bonds, typically initiating polymerization primarily through crosslinking agents in an aqueous solution, which allows monomer molecules in the hydrogel to form a cross-linked structure. Chemical crosslinking methods for developing hydrogels encompass Michael-type addition, bulk polymerization, and glutaraldehyde cross-linking. PEG-4-MAL is commonly utilized in Michael-type addition, Wroe et al. [31] first combined an adhesive peptide and a crosslinking agent in a buffer, followed by the addition of 1.2×10^8 PFU/mL of active phages. The resulting phage mixture, combined with 4.0% (w/v) PEG-4-MAL macromers (20 kDa) at a pH range of 6.0-6.5, forms a phage-hydrogel capable of effectively controlling orthopedic-related infections. Susan M. Lehman and her team utilized PEG-polyurethane-coated catheters derived through bulk polymerization and subsequently pretreated the hydrogel catheters with phages. Following assessment with a multi-day continuous flow in vitro model in urine, it was observed that the phage-treated hydrogel catheters demonstrated a significant reduction in bacteria and biofilm formation. Under the leadership of Mayhar Bassi [32], the team blended a 2% chitosan (CS) solution with a 5% starch solution and 2.5% glutaraldehyde, after which the mixture was transferred to a mold and incubated at -80 ℃ for 24 hours. This hydrogel demonstrates outstanding biocompatibility, biodegradability, and effective slow controlled release properties. Similarly, Samar S. Mabrouk employed a chemical crosslinking technique to create a phase-CMC hydrogel. With a pH of 7.7 and a diffusion coefficient of 25, they carefully incorporated a small amount of CMC powder into the phage lysate, which had a concentration of 10^8 PFU/mL, while continuously stirring until a homogeneous hydrogel formed. The resulting phage-CMC hydrogel exhibited exceptional water absorption and viscoelasticity, rendering it highly effective for treating wound infections caused by burns [33].

Table 1. The various type and characteristics of hydrogel crosslinking.

Crosslinking type	Plomer	Preparation method	Characteristic	Ref.
Physical crosslinking	Alnigate	Ionic crosslinking	Excellent biocompatible, low viscosity	[23,24]
	PVA	Freezing and thawing	Biocompatible, film-forming ability, Chemical stability	[25,34]
	PCL-Col I nanofibers	Thermal gelation	Adjustable mechanical strength, Well hemostatic	[35]
	HA/ β-TCP	Ionic crosslinking	Similar in structure and composition to bone minerals	[36,37]
	Eudragit® S100 and Alnigate	Physical crosslinking	Excellent compactness	[28,38]
	PolyHIPE /Nanocellulose	Ionic crosslinking	/	[39]
	PVA-SA	Ionic crosslinking	Strong hydrophilicity, Painless removal	[25]
	Agarose/HAMA	Thermal gelation	Thermal reversibility, Low cell adhesion	[40]
	PNIPAMco-ALA	Thermal gelation	Thermal reversibility, Hardly degradable	[27]
	HPMC	Thermal gelation	Thermal reversibility, Biodegradability	[26,41,42]

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	QCS poly (xylitol sebacate)–APP	Freeze-thawing	Biodegradability Low toxicity, Biocompatibility	[43]
	Agarose	Physical crosslinking	Withstand acidic conditions, Biodegradability	[44]
	Chitosan	Ionic crosslinking	Biocompatibility, Biodegradability, non-toxicity	[45]
	Poloxamer P407	Thermal gelation	Good bactericidal effect, Thermo-reversible properties	[46]
	Pluronic® F-127 /HPMC	Thermal gelation	Thermal responsiveness,	[47]
Chemical crosslinking	PEG-polyurethane	Bulk polymerization	Heat reactivity Anti-biological pollution, Hardly degradable	[48–51]
	CMC	/	Odorless, non-toxic	[33]
	PEG-4MAL	Michael-type addition	Biodegradability	[31]
	CS-NP	Coercavation method	/	[52]

3. Enhancing Phage- Hydrogel Therapy for Treating Infections Caused by a Range of Bacterial Strains

3.1. Phage Hydrogel Therapy for Infections Caused by E. coli

E. coli is a conditional pathogenic bacterium that can cause local tissue or organ infections in humans or animals under certain conditions. With the use of antibiotics, appearing large numbers of drug-resistant bacteria, and phage hydrogel is an effective antibacterial material that can be used as a reliable therapeutic method. The following describes the corresponding phage hydrogel therapy for the different serotypes of *E. coli* (Table 1). Han-Yu Shen et al. [53] targeted the local infection of *E. coli* DH5α by embedding the targeting phage HZJ into the alginate hydrogel sample through physical crosslinking, creating a phage-based hydrogel wound dressing. The hydrogel released 10% of the phage within 24 hours, and the number of bacteria killed reached 57% to 67% ($p<0.001$) within 2 hours, with antibacterial effects lasting at least 24 hours. Tricalcium phosphate (TCP) is frequently utilized as a prosthetic material for bone substitution in the treatment of osteoarticular diseases and injuries. R.Ismail and his colleagues found that the incorporation of alginate hydrogels loaded with phage λvir as a coating on TCP ceramic bone substitutes can effectively delay the process of phage desorption, resulting in a prolonged release of the phage. The results demonstrate that incorporating an alginate hydrogel over the TCP ceramic pellets increases the initial phage concentration on the material and extends the release time of phages to two weeks compared to control pellets. Moreover, these alginate-coated biomaterials exhibit accelerated bacterial lysis kinetics, making them a promising choice for practical prosthetic devices in bone and joint surgeries by facilitating localized phage therapy for bacterial infections over an extended duration [36].

Chitosan has also garnered significant attention in recent years as a delivery carrier for phage therapy. The group led by Adamu Ahmad K [52], utilized chitosan nanoparticles to prepare chitosan-phage ΦKAZ14 -loaded nanoparticles. These nanoparticles were then evaluated for their potential to effectively protect the bacteriophage from gastric acids and enzymes within the chicken gastrointestinal tract. The phage ΦKAZ14 encapsulated in chitosan nanoparticles was effectively shielded from enzymatic degradation, as evidenced by gel electrophoresis analysis, whereas the naked phage ΦKAZ1 experienced degradation.

Antibacterialatic materials that are both effective and affordable have garnered significant interest within clinical wound care practices. Cheng's team utilized electrospinning to combine

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phageT4 with polycaprolactone /collagen I (PCL-ColI) nanofibers, with the purpose of eliminating Escherichia coli infection while concurrently facilitating hemostasis.The PCL-ColI membrane incorporating T4 phage demonstrated exceptional antibacterial efficacy, with a rate of above 90%. In vivo testing revealed that the PCL-ColI B membrane fully degraded within 8 weeks, and no apparent pathological reactions were observed in the muscle or subcutaneous layer tissues at the back of the rabbit [35].

Table 2. Phage hydrogel therapy for *E. coli* infection**.**

E. coli	**Phages**	**Ploymer**	**Characteristic**	**preparation method**	**Type of infection**	**Effect**	**Ref.**
E. coli DH5α	HZJ	Alnigate	Biocompatibility, Biodegradability, Ease of gelation	Ion crosslinking	Wound infection	Reducing bacterial numbers by 59.3-68.5%	[53]
E. coli O157:H7	UFV-AREG1	PVA	Biocompatibility, Chemical stability	Freezing and thawing	Skin wound infection	Increasing bacterial inhibition zone	[54]
E. coli XL-1	T4	PCL-Col I nanofibers	Biocompatibility, Biodegradability, Good flexibility	Thermal gelation	Wound infection	Improving antibacterial effect by 90%	[35]
E. coli K12	*λvir*	Hydroxyapatite/ *β*-TCP	Osteoinduction, Biodegradability	Ion crosslinking	Infection by bone reconstruction surgery	Enhancing the release of phages	[36] [37]
E. coli	ΦKAZ14	CS-NP	Biocompatibility, Biodegradability, non-toxicity	Coercavation	Alimentary infection	Providing strong protection against ΦKAZ14 phages	[52]
E. coli EV36	K1F	Eudragit® S100/ Alnigate	Dissoluble, Excellent compactness	Physical crosslinking	Alimentary infection	/	[38]
E. coli K-12 MG1655	T7	PolyHIPE /Nanocellulose	/	Ion crosslinking	Alimentary infection	Shielding phages in acidic conditions and releasing them in alkaline conditions	[39]
E. coli ATCC 11303	T4 Coli-proteus	PEG-polyurethane	Heat reactivity, Anti-biological pollution	Chemical crosslinking	Urinary catheter infection	Reducing the biofilm formation by 90%	[48]
E. coli O104:H4	H4	Alnigate	Biocompatibility, Biodegradability, Ease of gelation	Ion crosslinking	Food pollution	Reducing *E. coli* count by 1.3 $\log_{10}$ CFU/g	[55]

 doi:10.20944/preprints202407.2265.v1

3.2. Bacteriophage Hydrogel Therapy for Infections Caused by S. aureus

Phage-based hydrogels have recently emerged as a promising therapy option for various infections caused by *S. aureus*, highlighting their exceptional efficacy in addressing this pathogen. Prabhjot Kaur *et al.* [25] utilized a PVA-SA mixed hydrogel membrane that incorporated phage MR10 as a wound dressing for effectively targeting and treating burn wound infections caused by *S. aureus*. Using a mouse burn wound model, this study demonstrated that the phage-enhanced PVA-SA mixed hydrogel membrane not only developed a protective barrier for the burn wound but also created an essential moist environment for optimal tissue regeneration. Additionally, the membrane exhibited remarkable antibacterial efficacy, as evidenced by a significant reduction of approximately 6 $\log_{10}$ in *S. aureus* biomass. These findings underscore the membrane's effectiveness in lowering bacterial levels and highlight its potential as a potent antibacterial protective shield.

Diabetic populations are more prone to developing wound infections, leading to poor and delayed wound healing, exacerbated by drug-resistant organisms, prompting exploration of alternative treatments like phage therapy. To tackle this issue, Sanjay Chhibber's group developed a phage cocktail encapsulated in liposomes (LCP) to enhance wound healing [56]. In a diabetic mouse model with cut wounds, the untreated control group showed consistently high bacterial load (8-9 log CFU/mL) that gradually decreased from the seventh day, while those treated with LCP demonstrated the most significant reduction in bacterial load by the third day, reaching approximately 4 log CFU/mL. Additionally, the encapsulation of bacteriophages by liposomes led to a significant 2-log increase in bacteriophage titer.

Orthopedic implant infections are a prevalent concern in medical practice. Sandeep Kaur and his colleagues formulated MR-5 phages and Linazolamide-coated HPMC wires (double coated wires) to target infections caused by *S. aureus* ATCC 43300 [41]. In their research, an animal model was established, wherein infection was induced in the mouse femoral joint, followed by the implantation of K-wires coated with both phage and linezolid (dual coated wires) into the femoral medullary canal. Mice implanted with dual coated wires showed the most substantial decrease in bacterial adhesion, joint inflammation, and faster recovery of limb movement and motor function. Furthermore, none of the treatments led to the emergence of resistant mutants.

Beyond its impact on healthcare, a *S. aureus* infection also represents a substantial food safety threat. Using freeze-thaw technology, Reuben Wang's team designed a positively charged Quaternized Chitosan (QCS) hydrogel that integrates bacteriophage 44AHJD and is cross-linked with a multivalent agent, aiming to improve its effectiveness [43]. The hydrogel-phage controlled release model can release up to 60% of phage particles within a 6-hour timeframe, effectively combating the proliferation of bacteria and preventing the development of phage-resistant strains.

Table 3. Phage hydrogel therapy for *S. aureus* infection.

S. aureus	Phage	Plomyer	Characteristic	Preparation method	Type of infection	Effect	Ref.
MRSA	MR10	PVA-SA	Strong hydrophilicity	Chemical/ionic crosslinking	Burn wound infection	Decreasing bacteria number from 8 to 2 $\log_{10}$ CFU/mL	[25]
S. aureus H560	ΦK	Agarose/ HAMA	Thermal reversibility, Low cell adhesion	Thermal gelation	Wound infection	Enhancing bacteria-killing capability.	[40]
S. aureus ST228	ΦK	PNIPA Mco-ALA	Thermal reversibility, Not readily degradable	Thermal gelation	Skin and soft tissue infection	Effectively lysing *S. aureus* at 37 ℃	[27]

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MRSA	MR5 MR10	Liposomes	Biodegradable, Not eliciting an immune response.	/	Wound infection	Reducing bacterial load by 4 log CFU/mL	[56]
S. aureus ATCC 43300	MR-5	HPMC	Thermal reversibility, Biodegradability	Thermal gelation	Orthopedic implant infection	No bacterial burden found on the wire	[41]
S. aureus	Phage K	Alginate	Withstand acidic conditions, Biodegradability	Ion crosslinking	Alimentary infection	Improving the survival of free phages	[23]
S. aureus BCRC 13077	44AHJD	QCS/poly (xylitol sebacate) –co-APP	Biodegradabilit, Low toxicity, Biocompatibility	Freeze-thawing	Food contamination	Releasing up to 60% of phage particles within 6 hours	[43]
S. aureus	Modified phage	Alginate	Biodegradability	Ion crosslinking	Bone-related infection	Reducing soft tissue infection	[24]
S. aureus	Phage K	Agarose/ HAMA	Thermal reversibility, Nondegradable,Low cell adhesion	Thermal gelation	Bone-related infection	/	[57]

3.3. Bacteriophage Hydrogel Therapy for Infections Caused by P. aeruginosa

P. aeruginosa has the potential to induce infections in multiple areas of the body, encompassing the respiratory tract, urinary tract soft tissues, and is linked with severe conditions such as pneumonia, bloodstream infections, and wound infections. WA. Sarhan's team added bee venom (BV) to honey/polyvinyl alcohol/ chitosan (HPCS) nanofibers to produce HPCS-BV, and then loaded phage PS1 into HPCS-BV for the treatment of multidrug-resistant *P. aeruginosa* wound infection. The HPCS-BV/PS1 nanofibers demonstrated significant antibacterial efficacy against drug-resistant *P. aeruginosa*, reducing the initial count from 7×10^8 CFU/mL to nearly 0 within 24 hours, highlighting their effectiveness against resistant strains [58].

G.R. Abdellatif and colleagues conducted a study exploring the implementation of phage vB_Pae_SMP1/SMP5 combined with carboxymethylcellulose hydrogel for managing burn wound infections induced by carbapenem-resistant *P. aeruginosa* (CRPA) [33]. Inhibition zones were detected around the cups containing either the tested hydrogel of SMP1 or SMP5, whereas no inhibition zone was observed around the cups with the control hydrogel. To evaluate the therapeutic efficacy of phage-incorporated hydrogel against CRPA, they established a mouse burn wound model infected with CRPA. The findings revealed a 60% survival rate in the control group that received the hydrogel treatment, while the phage-containing hydrogel treatment group achieved a 100% survival rate, demonstrating a superior anti-CRPA infection effect.

James A. Wroe and his team created an injectable hydrogel that can encapsulate phages and transport them to the area of bone infections. The release rates of phages from the hydrogel can be controlled by adjusting the gel formulation. This engineered hydrogels containing phages successfully eradicate their target bacteria in both planktonic and biofilm states without affecting the metabolic function of human mesenchymal stromal cells. This engineered hydrogels demonstrated a significant 4.7-fold reduction in live *P. aeruginosa* counts in murine radial segmental defects infected with *P. aeruginosa*, affirming their potential for the treatment of local bone infections [31].

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Lehman S. M. and colleagues introduced a method where PEG-polyurethane hydrogel-coated catheters, paired with a mixed bacteriophage cocktail, were combined to combat urinary catheter-induced *P. aeruginosa* infection. This approach led to a remarkable reduction of *P. aeruginosa* biofilm by 4 $\log_{10}$ CFU/cm^2 within 48 hours ($P<0.01$), demonstrating excellent antibacterial efficacy [50].

Table 4. Phage hydrogel therapy for *P. aeruginosa* infection.

P. aeruginosa	**Phage**	**Plomyer**	**Characteristic**	**Preparation method**	**Type of infection**	**Effect**	**Ref.**
P. aeruginosa	PS1	HPCS	/	/	Wound infection	Reducing the bacterial count from 7×10^8 CFU/mL to 0	[58]
CRPA	vB_Pae_SMP1/SMP5	CMC	Odorless , non-toxic	Chemical crosslinking	Burn wound infection	100% survival rate for mice	[33]
P. aeruginosa	KT28,KTN and LUZ19	Agarose	Temperature response	Physical crosslinking	Wound infection	Effectively hindering biofilm formation	[44]
P. aeruginosa	ΦPaer4/14/22 and ΦW2005A	PEG-4MAL	Biodegradability	Michael-type addition	Orthopedic implant infection	Reducing the CFU amount of bacteria by 16.9 times	[31]
P. aeruginosa isolate (Paer09)	FJK,R9–30,KR3–15	Alginate	Biocompatibiliy, Biodegradability, Hypotoxicity	Thermal gelation	Infection by fracture	Decreasing bacteria in the soft tissue by 6.5-fold	[17]
P. aeruginosa	PA5	PVA-SA	Strong hydrophilicity	Chemical/ionic crosslinking	Burn wound infection	Reducing *P. aeruginosa* biomass by 4.6 $\log_{10}$	[25]
P. aeruginosa	Phage cocktail	PEG-polyurethane	Heat reactivity, Anti-biological pollution	Bulk polymerization	Urinary catheter infection	Reducing the number of *p. aeruginosa* biofilm by 4 $\log_{10}$ CFU/cm^2	[49]
P. aeruginosa	M4	PEG-polyurethane	Heat reactivity, Anti-biological pollution	Bulk polymerization	Urinary catheter infection	Reducing biofilm cells from 7.13 to 4.13 $\log_{10}$ CFU/cm^2	[50]

3.4. Bacteriophage Hydrogel Therapy for Infections Caused by K. pneumoniae

K. pneumoniae, a prevalent and highly antibiotic-resistant gram-negative bacillus, frequently emerges as a leading pathogen in hospital-acquired infections, particularly causing pneumonia and

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respiratory illnesses. Remarkably, multiple encouraging results from clinical trials underscore the potential efficacy of using phage-containing hydrogel as an effective treatment for *K. pneumoniae* infections, providing optimism for addressing the challenges posed by antibiotic resistance. Seema Kumari *et al.* [42] conducted a study in this field, wherein they developed a phage kpn5-HPMC hydrogel by encapsulating bacteriophage Kpn5 within a hot-melted hydrogel composed of the natural polymer, HPMC. Seema Kumari and her team demonstrated the significant therapeutic potential of phage kpn5-hydrogel in treating *K. pneumoniae* infection in mice, with a notable 63.33% survival rate .in the group treated with phage kpn5-containing hydrogel compared to 0% in untreated counterparts by day 7 post-infection. Subsequently, the same team incorporated bacteriophages into the hydrogel composed of PVA-SA, aiming to evaluate the excipient's therapeutic potential in treating wound infections caused by *K. pneumoniae*. Prabhjot Kaur *et al.* proposed an innovative approach by combining phage with hydrogels to effectively combat *K. pneumoniae* wound infection [25]. In their study, they utilized a PVA-SA mixed polymer ions cross-linked with targeted phage Kpn5 to create a phage-based PVA-SA hydrogel. The bacterial growth inhibition tests *in vitro* reveal a substantial 6.37 $\log_{10}$ reduction in *K. pneumoniae* biomass when exposed to Kpn5 phage PVA-SA hydrogel treatment, emphasizing the encouraging potential of this groundbreaking therapeutic strategy.

3.5. Bacteriophage Hydrogel Therapy for Infections Caused by P. mirabilis

P. mirabilis is a common pathogen in urinary tract infections, especially in individuals with urinary catheters or structural urinary tract abnormalities, as its swarming motility facilitates ascension and colonization of the bladder and kidneys. Moreover, the emergence of drug-resistant bacteria has presented substantial obstacles in managing *P. mirabilis* infections. In response to this challenge, a team led by Scarlet Milo has devised a dual-coated catheter system [28], in which a PVA hydrogel envelops the phage in the lower layer and EUDRAGIT®S 100 in the upper layer on the catheter surface. Due to the production of the bacterial urease enzyme by *P. mirabilis*, the urinary pH is elevated above 7, causing the upper gel to dissolve and subsequently releasing bacteriophages from the lower gel into the environment. The in vitro bladder model system demonstrated that the double-coated catheter effectively decreased the concentration of *P. mirabilis* by 6 log within 2 hours and extended the catheter obstruction time from 13 hours to 26 hours, effectively delaying catheter blockage. Similarly, Lehman, S.M, *et al.* [49] engineered a catheter with a hydrogel embedding multiple bacteriophages tailored to target *P. mirabilis*. Subsequently immersed in an artificial urine medium (AUM), the resultings exhibited a notable reduction of over 2 $\log_{10}$ CFU/cm^2 in the *P. mirabilis* biofilm within 48 hours.

3.6. Bacteriophage Hydrogel Therapy for Infections Caused by A. baumannii

A. baumannii, a Gram-negative coccobacillus commonly found in the environment, exhibits resistance to key antibiotics like colistin, tigecycline, and carbapenems, making it a significant nosocomial ESKAPE pathogen. The WHO has identified it as a critical research priority, while the CDC considers it an urgent public health threat. In a prevous research, Wei Yan and colleagues assessed the effectiveness of a phage-loaded thermosensitive hydrogel in treating wound infections caused by MDR *A. baumannii* [59], with IME-AB2 phage and MDR-AB2 serving as the model phage and bacteria, respectively.The IME-AB2 phage exhibited excellent storage stability in a ~18 wt% Poloxamer 407 hydrogel solution, with negligible titer loss over 24 months at 4 ℃, and demonstrated sustained release with a cumulative release of 60% within the first 24 hours. Their findings revealed that the IME-AB2 Phage-embedded hydrogel significantly decreased *A. baumannii* levels by more than 5 $\log_{10}$ CFU/mL at 37 ℃ and effectively eliminated biofilms by 59%. Furthermore, in an in vitro wound infection model on pig skin, the IME-AB2 Phage-embedded hydrogel demonstrated a remarkable ability to reduce bacterial counts by 90%. In 2023, the phage IME-AB2 combined with colistin was incorporated into a composite hydrogel composed of Pluronic® F-127 and HPMC [47].The engineered hydrogel infused with phage IME-AB2, demonstrates excellent antibacterial efficacy by effectively eliminating bacteria in both planktonic (by 5.66 log) and biofilm (by 3 log)

 doi:10.20944/preprints202407.2265.v1

states while also inhibiting bacterial regrowth. Furthermore, this phage-loaded hydrogel proves highly effective in reducing 4.65 log of *A. baumannii* in a pig skin wound model.

Table 5. Phage hydrogel therapy for *K. pneumonia*, *P. mirabilis* and *A. baumannii* infection**.**

Strain	Phages	Ploymer	Characteristic	Preparation method	Type of infection	Antibacterial effect	Ref.
K. pneumoniae B5055	Kpn5	HPMC	Thermal reversibility, Biodegradability	Thermal gelation	Burn wound infection	The survival rate was 63.33% for mice	[42]
Carbapenem-resistant *K. pneumoniae*	Kpn5	PVA-SA	Strong hydrophilicity	Ion crosslinking	Wound infection	Decreasing the biomass of *K. pneumoniae* by 6.37 log_{10}	[25]
K. pneumoniae B5055	Kpn5	HPMC	Thermal reversibility, Biodegradability	Thermal gelation	Burn wound infection	The survival rate was 66.66% for mice	[26,60]
P. mirabilis	B4	PVA-Eudragit ®S 100	Poor biodegradable, Poor cell adhesion	Freezing and thawing	Urinary catheter infection	Reducing *P. mirabilis* biofilm by 6-log	[28]
P. mirabilis	ΦPmir1/32/34/37	PEG-polyurethane	Heat reactivity, Anti-biological pollution, Poor biodegradable	Bulk polymerization	Urinary catheter infection	Reducing the number of *P. mirabilis* biofilm by ＞2 log_{10} CFU/cm^2	[49]
P. mirabilis 13 HER1094	T4	PEGpolyurethane	Heat reactivity	Chemical crosslinking	Urinary catheter infection	Reducing the *P. mirabilis* biofilm formation by 90%	[48]
A. baumannii MDR-AB2	IME-AB2 phage	P407	Thermo-reversible properties	physical crosslinking	Wound infection	Reducing *A. baumannii* >5 log_{10} CFU/mL, eliminating biofilm by 59%	[59]
A. baumannii	AB140/50	Chitosan	Biocompatibility, Biodegradability, non-toxicity	Ionic crosslinking	Wound infection	Completely eliminating *A. baumannii*	[45]
A. baumannii	Phage 53	Poloxamer P407	Thermo-reversible properties	Thermal gelation	Wound infection	Resulting *A. baumannii by* 3.62 log $_{10}$	[46]
A. baumannii	vB_AbaM-IME-AB2	Pluronic ® F-127/HPMC	Thermal responsiveness	Thermal gelation	Wound infection	Killing 5.66 log of *A. baumannii*	[47]

 doi:10.20944/preprints202407.2265.v1

4. Conclusions

Phage therapy exhibits robust antibacterial potential while minimizing adverse effects on the human body. Hydrogel, as a dependable carrier for phages, efficiently regulates their release during local applications, thus optimizing their activity and concentration. Consequently, this approach elevates the antibacterial efficacy and has been successfully employed in addressing diverse bacterial infections, yielding impressive outcomes. In this review, we explored a variety of hydrogel polymers and highlighted the effectiveness of phage hydrogels in the treatment of diverse bacterial infections. Moreover, beyond the environmentally sensitive hydrogels discussed earlier, recent findings illustrate the development of pH sensitive, photosensitive, enzyme-sensitive, magnetic-sensitive, and redox hydrogels, among others. Xiaoliang Qi *et al.* prepared pH sensitive hydrogels using grafting copolymerization reaction, which can adjust the release rate of loaded insulin according to the pH value in the environment. The release rate of the hydrogel was 26.1% within 24 hours at pH 1.2, and exceeded 50% within 6 hours at pH 7.4 [61]. The team led by Xun Tong used halogen bonds and visible light sensitive parts to jointly prepare a photosensitive hydrogel. The hydrogel can achieve a transition from solution to gel under blue light irradiation, while the opposite process occurs under green light irradiation [62]. Shalini V. Gohil *et al*. prepared injectable and biodegradable enzyme sensitive hydrogel by enzymatic crosslinking. This hydrogel can be degraded by lysozyme to release the loaded protein, and the release rate can be adjusted by changing the degree of acetylation [63]. Magnetic sensitive hydrogels carrying paramagnetic iron oxide nanoparticles (SPIONS) can be remotely heated under the effect of external magnetic field, and it is expected to improve the tumor treatment effect by combining hyperthermia, radiotherapy and chemotherapy [64]. The magnetic sensitive gel loaded with SPIONS designed by Samantha A. Meenach *et al.* has achieved good application results and can selectively kill M059K glioblastoma cells [65]. Oxidation-reduction sensitive hydrogels can undergo a liquid gel transition based on changes in the external redox environment [66]. Itsuro Tomatsu's team constructed an oxidation-reduction sensitive hydrogel using ferrocenecarboxylic acid (FCA), which appears gel like in the reduced state and solution like in the oxidized state [67]. Recent studies have highlighted the various potential applications of hydrogels, extending beyond traditional uses as wound dressings and oral particles to innovative new methods of application. Jeong Yeon Choi *et al.* have developed a spray-type alginate hydrogel for burned wounds, which can facilitate and rapid wound treatment, effectively promote wound healing and greatly reduce pain [68]. On the basis of microneedle as a new and powerful drug transmission system, Ming Ji has developed a hydrogel microneedle, which can maintain drug activity while having a good curative effect in wound healing [69].

References

1. Deusenbery C, Wang Y, Shukla A. Recent Innovations in Bacterial Infection Detection and Treatment. ACS INFECT DIS **2021** 2021-04-09;7(4):695-720.
2. Peng B, Li H, Peng X. Proteomics approach to understand bacterial antibiotic resistance strategies. EXPERT REV PROTEOMIC **2019** 2019-10-01;16(10):829-39.
3. Liu X, Xiao J, Wang S, et al. Research Progress on Bacterial Membrane Vesicles and Antibiotic Resistance. INT J MOL SCI **2022** 2022-09-30;23(19).
4. Chinemerem ND, Ugwu MC, Oliseloke AC, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J CLIN LAB ANAL **2022** 2022-09-01;36(9):e24655.
5. Makabenta J, Nabawy A, Li CH, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. NAT REV MICROBIOL **2021** 2021-01-01;19(1):23-36.
6. Melo L, Oliveira H, Pires DP, Dabrowska K, Azeredo J. Phage therapy efficacy: a review of the last 10 years of preclinical studies. CRIT REV MICROBIOL **2020** 2020-02-01;46(1):78-99.
7. Crunkhorn S. Prospecting for new antibiotics. NAT REV DRUG DISCOV **2019** 2019-07-01;18(8):581.
8. Hatfull GF, Dedrick RM, Schooley RT. Phage Therapy for Antibiotic-Resistant Bacterial Infections. ANNU REV MED **2022** 2022-01-27;73:197-211.
9. Strathdee SA, Hatfull GF, Mutalik VK, Schooley RT. Phage therapy: From biological mechanisms to future directions. CELL **2023** 2023-01-05;186(1):17-31.

10. Chang R, Wallin M, Lin Y, et al. Phage therapy for respiratory infections. ADV DRUG DELIVER REV **2018** 2018-08-01;133:76-86.
11. Dave R, Banerjee D. Bacteriophage therapy- a refurbished age-old potential strategy to treat antibiotic and multidrug resistant bacterial infections in future. BRAZ J MICROBIOL **2024** 2024-07-01.
12. Shiue SJ, Wu MS, Chiang YH, Lin HY. Bacteriophage-cocktail hydrogel dressing to prevent multiple bacterial infections and heal diabetic ulcers in mice. J BIOMED MATER RES A **2024** 2024-05-06.
13. Rotman SG, Sumrall E, Ziadlou R, et al. Local Bacteriophage Delivery for Treatment and Prevention of Bacterial Infections. FRONT MICROBIOL **2020** 2020-01-20;11:538060.
14. Kim HY, Chang R, Morales S, Chan HK. Bacteriophage-Delivering Hydrogels: Current Progress in Combating Antibiotic Resistant Bacterial Infection. ANTIBIOTICS-BASEL **2021** 2021-01-29;10(2).
15. Bai H, Borjihan Q, Li Z, et al. Phage-Based antibacterial hydrogels for bacterial targeting and Ablation: Progress and perspective. EUR J PHARM BIOPHARM **2024** 2024-05-01;198:114258.
16. Shafigh KF, Sheikhzadeh HF, Aminifazl MS, Skurnik M, Gholadze S, Zarrini G. Design of Phage-Cocktail-Containing Hydrogel for the Treatment of Pseudomonas aeruginosa-Infected Wounds. VIRUSES-BASEL **2023** 2023-03-21;15(3).
17. Chen B, Benavente LP, Chitto M, et al. Alginate microbeads and hydrogels delivering meropenem and bacteriophages to treat Pseudomonas aeruginosa fracture-related infections. J CONTROL RELEASE **2023** 2023-12-01;364:159-73.
18. Moghtader F, Solakoglu S, Piskin E. Alginate- and Chitosan-Modified Gelatin Hydrogel Microbeads for Delivery of E. coli Phages. GELS-BASEL **2024** 2024-04-02;10(4).
19. Vacek L, Polastik KD, Lipovy B, et al. Phage therapy combined with Gum Karaya injectable hydrogels for treatment of methicillin-resistant Staphylococcus aureus deep wound infection in a porcine model. INT J PHARMACEUT **2024** 2024-07-20;660:124348.
20. Khazani AM, Elikaei A, Abed S, et al. A novel Enterococcus faecium phage EF-M80: unveiling the effects of hydrogel-encapsulated phage on wound infection healing. FRONT MICROBIOL **2024** 2024-01-20;15:1416971.
21. Narayanan KB, Bhaskar R, Choi SM, Han SS. Development of carrageenan-immobilized lytic coliphage vB_Eco2571-YU1 hydrogel for topical delivery of bacteriophages in wound dressing applications. INT J BIOL MACROMOL **2024** 2024-02-01;259(Pt 2):129349.
22. Bashir S, Hina M, Iqbal J, et al. Fundamental Concepts of Hydrogels: Synthesis, Properties, and Their Applications. POLYMERS-BASEL **2020** 2020-11-16;12(11).
23. Yongsheng Ma A B, Pacan JC, Qi WBPM, Yongping X. Enhanced alginate microspheres as means of oral delivery of bacteriophage for reducing Staphylococcus aureus intestinal carriage. FOOD HYDROCOLLOID **2010**;26（2012）:434-40.
24. CobbID LH, JooYoun P, Elizabeth AS, et al. CRISPR-Cas9 modified bacteriophage for treatment of Staphylococcus aureus induced osteomyelitis and soft tissue infection. PLOS ONE **2019**:258-73.
25. Kaur P, Gondil VS, Chhibber S. A novel wound dressing consisting of PVA-SA hybrid hydrogel membrane for topical delivery of bacteriophages and antibiotics. INT J PHARMACEUT **2019** 2019-12-15;572:118779.
26. Kumari S, Harjai K, Chhibber S. Topical treatment of Klebsiella pneumoniae B5055 induced burn wound infection in mice using natural products. J INFECT DEV COUNTR **2010** 2010-06-30;4(6):367-77.
27. Hathaway H, Alves DR, Bean J, et al. Poly(N-isopropylacrylamide-co-allylamine) (PNIPAM-co-ALA) nanospheres for the thermally triggered release of Bacteriophage K. EUR J PHARM BIOPHARM **2015** 2015-10-01;96:437-41.
28. Milo S, Hathaway H, Nzakizwanayo J, et al. Prevention of encrustation and blockage of urinary catheters by Proteus mirabilis via pH-triggered release of bacteriophage. J MATER CHEM B **2017** 2017-07-21;5(27):5403-11.
29. Azadeh Peivandi LTRM. Bioactive hydrogels with self-organizing bundles of phage nanofilaments. CHEM MATER **2019**;31(2019):5442-9.
30. Peivandi A, Jackson K, Tian L, et al. Inducing Microscale Structural Order in Phage Nanofilament Hydrogels with Globular Proteins. ACS BIOMATER SCI ENG **2022** 2022-01-10;8(1):340-7.
31. Wroe JA, Johnson CT, Garcia AJ. Bacteriophage delivering hydrogels reduce biofilm formation in vitro and infection in vivo. J BIOMED MATER RES A **2020** 2020-01-01;108(1):39-49.
32. Bassi M, Kalpana R, Kumar V. Starch Glutaraldehyde Cross-Linked Hydrogel for Drug Release Properties. J PHARM BIOALLIED SC **2024** 2024-04-01;16(Suppl 2):S1198-200.

33. S MS, Abdellatif GR, Abu ZA, Aziz RK, Aboshanab KM. In Vitro and Pre-Clinical Evaluation of Locally Isolated Phages, vB_Pae_SMP1 and vB_Pae_SMP5, Formulated as Hydrogels against Carbapenem-Resistant Pseudomonas aeruginosa. VIRUSES-BASEL **2022** 2022-12-11;14(12).
34. Delaine Meireles Gouvˆea Boggione A, Igor JEBS, Saymon MDSB, Mendonça RCES. Preparation of polyvinyl alcohol hydrogel containing bacteriophage and its evaluation for potential use in the healing of skin wounds. J DRUG DELIV SCI TEC **2021**;63（2021）:102484.
35. Cheng W, Zhang Z, Xu R, et al. Incorporation of bacteriophages in polycaprolactone/collagen fibers for antibacterial hemostatic dual-function. J BIOMED MATER RES B **2018** 2018-10-01;106(7):2588-95.
36. Ismail R, Dorighello CN, Hornez JC, Bouchart F. A Localized Phage-Based Antimicrobial System: Effect of Alginate on Phage Desorption from beta-TCP Ceramic Bone Substitutes. ANTIBIOTICS-BASEL **2020** 2020-08-31;9(9).
37. Meurice E, Rguiti E, Brutel A, et al. New antibacterial microporous CaP materials loaded with phages for prophylactic treatment in bone surgery. J MATER SCI-MATER M **2012** 2012-10-01;23(10):2445-52.
38. Vinner GK, Richards K, Leppanen M, Sagona AP, Malik DJ. Microencapsulation of Enteric Bacteriophages in a pH-Responsive Solid Oral Dosage Formulation Using a Scalable Membrane Emulsification Process. PHARMACEUTICS **2019** 2019-09-14;11(9).
39. Kopac T, Lisac A, Mravljak R, Rucigaj A, Krajnc M, Podgornik A. Bacteriophage Delivery Systems Based on Composite PolyHIPE/Nanocellulose Hydrogel Particles. POLYMERS-BASEL **2021** 2021-08-09;13(16).
40. Jessica E. Bean DRAM, Mark C. Enright AATA. Triggered Release of Bacteriophage K from Agarose/Hyaluronan Hydrogel Matrixes by Staphylococcus aureus Virulence Factors. CHEM MATER **2014**;26:7201-8.
41. Kaur S, Harjai K, Chhibber S. In Vivo Assessment of Phage and Linezolid Based Implant Coatings for Treatment of Methicillin Resistant S. aureus (MRSA) Mediated Orthopaedic Device Related Infections. PLOS ONE **2016** 2016-01-20;11(6):e0157626.
42. Kumari S, Harjai K, Chhibber S. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by Klebsiella pneumoniae B5055. J MED MICROBIOL **2011** 2011-02-01;60(Pt 2):205-10.
43. Wang R, Yeh YJ, An YN, Virly. Engineering pH-sensitive erodible chitosan hydrogel composite containing bacteriophage: An interplay between hydrogel and bacteriophage against Staphylococcus aureus. INT J BIOL MACROMOL **2023** 2023-12-31;253(Pt 7):127371.
44. Dorotkiewicz-Jach A, Markwitz P, Rachuna J, Arabski M, Drulis-Kawa Z. The impact of agarose immobilization on the activity of lytic Pseudomonas aeruginosa phages combined with chemicals. APPL MICROBIOL BIOT **2023** 2023-02-01;107(2-3):897-913.
45. Ilomuanya MO, Enwuru NV, Adenokun E, Fatunmbi A, Adeluola A, Igwilo CI. Chitosan-Based Microparticle Encapsulated Acinetobacter baumannii Phage Cocktail in Hydrogel Matrix for the Management of Multidrug Resistant Chronic Wound Infection. TURK J PHARM SCI **2022** 2022-04-29;19(2):187-95.
46. Li C, Nyaruaba R, Zhao X, et al. Thermosensitive Hydrogel Wound Dressing Loaded with Bacteriophage Lysin LysP53. VIRUSES-BASEL **2022** 2022-09-03;14(9).
47. Mukhopadhyay S, To K, Liu Y, Bai C, Leung S. A thermosensitive hydrogel formulation of phage and colistin combination for the management of multidrug-resistant Acinetobacter baumannii wound infections. BIOMATER SCI-UK **2023** 2023-12-19;12(1):151-63.
48. Carson L, Gorman SP, Gilmore BF. The use of lytic bacteriophages in the prevention and eradication of biofilms of Proteus mirabilis and Escherichia coli. FEMS Immunol Med Microbiol **2010** 2010-08-01;59(3):447-55.
49. Lehman SM, Donlan RM. Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model. ANTIMICROB AGENTS CH **2015** 2015-02-01;59(2):1127-37.
50. Fu W, Forster T, Mayer O, Curtin JJ, Lehman SM, Donlan RM. Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system. ANTIMICROB AGENTS CH **2010** 2010-01-01;54(1):397-404.
51. Guoying Zhou ACMA. Synthesis of polyurethane-g-poly(ethylene glycol) copolymers by macroiniferter and their protein resistance. Polymer Chemistry **2011**;2:1409-14.

52. Adamu AK, Sabo MA, Abas F. Chitosan Nanoparticles as Carriers for the Delivery of PhiKAZ14 Bacteriophage for Oral Biological Control of Colibacillosis in Chickens. MOLECULES **2016** 2016-03-14;21(3):256.
53. Shen HY, Liu ZH, Hong JS, Wu MS, Shiue SJ, Lin HY. Controlled-release of free bacteriophage nanoparticles from 3D-plotted hydrogel fibrous structure as potential antibacterial wound dressing. J CONTROL RELEASE **2021** 2021-03-10;331:154-63.
54. Chahardoli F, Pourmoslemi S, Soleimani AS, Tamri P, Haddadi R. Preparation of polyvinyl alcohol hydrogel containing chlorogenic acid microspheres and its evaluation for use in skin wound healing. J BIOMATER APPL **2023** 2023-04-01;37(9):1667-75.
55. Lone A, Anany H, Hakeem M, et al. Development of prototypes of bioactive packaging materials based on immobilized bacteriophages for control of growth of bacterial pathogens in foods. INT J FOOD MICROBIOL **2016** 2016-01-18;217:49-58.
56. Chhibber S, Kaur J, Kaur S. Liposome Entrapment of Bacteriophages Improves Wound Healing in a Diabetic Mouse MRSA Infection. FRONT MICROBIOL **2018** 2018-01-20;9:561.
57. Brendan L. Roach ABNG, Hung ACT. Agarose hydrogel characterization for regenerative medicine applications: Focus on engineering cartilage. In Biomaterials from Nature for Advanced Devices and Therapies **2016**;2016:258-73.
58. Sarhan WA, Azzazy HM. Apitherapeutics and phage-loaded nanofibers as wound dressings with enhanced wound healing and antibacterial activity. NANOMEDICINE-UK **2017** 2017-09-01;12(17):2055-67.
59. Yan W, Banerjee P, Liu Y, et al. Development of thermosensitive hydrogel wound dressing containing Acinetobacter baumannii phage against wound infections. INT J PHARMACEUT **2021** 2021-06-01;602:120508.
60. Cheng Y, Xiaolei S, Xuepeng J, Xiaohui WAHQ. Printability of a cellulose derivative for extrusion-based 3D printing: The application on a biodegradable support material. FRONT MATER;2020.
61. Qi X, Wei W, Li J, et al. Salecan-Based pH-Sensitive Hydrogels for Insulin Delivery. MOL PHARMACEUT **2017** 2017-02-06;14(2):431-40.
62. Tong X, Qiu Y, Zhao X, et al. Visible light-triggered gel-to-sol transition in halogen-bond-based supramolecules. SOFT MATTER **2019** 2019-08-21;15(31):6411-7.
63. Gohil SV, Padmanabhan A, Kan HM, Khanal M, Nair LS. Degradation-Dependent Protein Release from Enzyme Sensitive Injectable Glycol Chitosan Hydrogel. TISSUE ENG PT A **2021** 2021-07-01;27(13-14):867-80.
64. Milcovich G, Lettieri S, Antunes FE, et al. Recent advances in smart biotechnology: Hydrogels and nanocarriers for tailored bioactive molecules depot. ADV COLLOID INTERFAC **2017** 2017-11-01;249:163-80.
65. Meenach SA, Hilt JZ, Anderson KW. Poly(ethylene glycol)-based magnetic hydrogel nanocomposites for hyperthermia cancer therapy. ACTA BIOMATER **2010** 2010-03-01;6(3):1039-46.
66. He J, Zhang A, Zhang Y, Guan Y. Novel Redox Hydrogel by in Situ Gelation of Chitosan as a Result of Template Oxidative Polymerization of Hydroquinone. MACROMOLECULES **2011** 2011-01-01;44(7):2245-52.
67. Tomatsu I, Hashidzume A, Harada A. Redox-Responsive Hydrogel System Using the Molecular Recognition ofβ -Cyclodextrin. MACROMOL RAPID COMM **2006** 2006-02-24;27(4):238-41.
68. Choi JY, Joo YJ, Kang RJ, Jeon HK, Hong GS. Effect of Spray-Type Alginate Hydrogel Dressing on Burn Wounds. GELS-BASEL **2024** 2024-02-19;10(2).
69. Ji M, Zhan F, Qiu X, et al. Research Progress of Hydrogel Microneedles in Wound Management. ACS BIOMATER SCI ENG **2024** 2024-07-10.