



# Nanogels: A novel approach in antimicrobial delivery systems and antimicrobial coatings

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## ABSTRACT

The implementation of nanotechnology to develop efficient antimicrobial systems has a significant impact on the prospects of the biomedical field. Nanogels are soft polymeric particles with an internally cross-linked structure, which behave as hydrogels and can be reversibly hydrated/dehydrated (swollen/shrunken) by the dispersing solvent and external stimuli. Their excellent properties, such as biocompatibility, colloidal stability, high water content, desirable mechanical properties, tunable chemical functionalities, and interior gel-like network for the incorporation of biomolecules, make them fascinating in the field of biological/biomedical applications. In this review, various approaches will be discussed and compared to the newly developed nanogel technology in terms of efficiency and applicability for determining their potential role in combating infections in the biomedical area including implant-associated infections.

## 1. Introduction

Bacterial infections are the major causes of death worldwide. Reducing the mortality rate caused by bacterial infections is considered one of the most critical challenges. The infections are mostly occurring due to the bacterial biofilms formed by initial bacterial attachment and subsequent proliferation and colonization of bacterial cells [1–4]. Bacterial biofilm and infection complications are not affecting only the biomedical field; it creates problems also in the textile and food packaging industries, marine equipment, and water purification systems. When microorganisms colonize on textile materials, this leads to undesired hygienic problems [5]. For food packaging materials, biofilm formation creates a health risk for the consumer and also reduces the production efficacy of the industry itself [6]. For the marine industry, biofilm can be the cause of corrosion and biofouling of marine equipment, resulting in its degradation and high economic costs [7]. Bacteria accumulation in water purification systems decreases the quality of home drinking water creating health threats [8]. Medical implants and devices, such as hip and knee joints, pacemakers, intraocular lenses, and

vascular catheters, are crucial in health care. Infections can develop in or around these devices, which impairs the function of the device and can even lead to device failure. Besides, these complications place the health of the patient at high risk, sometimes even leading to death [9–11]. Over the past decades, the situation is becoming even worse due to increasing antibiotic resistance [12]. Antibiotic resistance occurs when bacteria develop the capability to become unresponsive towards the drug, which then results in infections that are complicated to treat [13,14]. Therefore, novel antibacterials, delivery systems and surface coatings are urgently needed to fight infections arising within the biomedical context in which the implant-associated infections take a prominent place but certainly is not the only urgent type of infection.

Recently, the development of hydrogels has become an attractive strategy and the design of tissue engineering scaffolds, antifouling coatings on implant surfaces, drug delivery, and wound dressing systems have been widely studied [15,16]. Hydrogels are soft materials with cross-linked networks of hydrophilic polymers with high water content, which contribute to their biocompatibility and make them highly useful for biomedical applications; hence the development of hydrogels are one

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of the most studied areas at the interface of engineering and medicine [17–19]. The translation and usage of hydrogels into the clinical applications can be seen in many examples such as contact lenses, dermal fillers in soft tissue augmentation for aesthetic products, encapsulation and release systems for cancer products, mechanical support and scaffolding materials for spinal fusion applications [20]. While many applications involve hydrogels, these are often of macroscopic nature and for many interesting applications, particularly when interacting with tissue cells and bacterial cells, nano- and microstructures are of high interest because these interact with cells on a different level and even have the capability of being internalized. Hence, many efforts have been directed towards the development of nano- and microgels.

Nanogels are newly emerging nano-sized hydrogel-like polymeric materials and have overlapping properties of both nanoparticles and hydrogels [21]. Nanoparticles, both inorganic (gold, silver, etc.) and organic (liposomes), are already present in the clinic as well as many natural and synthetic hydrogels [22]. To this end, nanogels provide a cross-over for combinations of hydrogels and nanomaterials. They have advantages over macro-sized networks because their size enables them to interact with cells in a more specific fashion and even be internalized and due to their soft nature behave differently from solid and self-assembled polymer-based drug delivery systems, including polymer-based nanoparticles, micelles, and liposomes [23–25]. Nanogels consist of physically or chemically cross-linked three-dimensional polymer networks that can also be functionalized and integrated easily with pharmaceutical agents. Up to now, various nanogels have been widely used in the delivery of pharmaceuticals, mainly antitumor agents and proteins [26]. More recently, increasing attention has been paid to enhance their biocompatibility as well as using them as antimicrobial

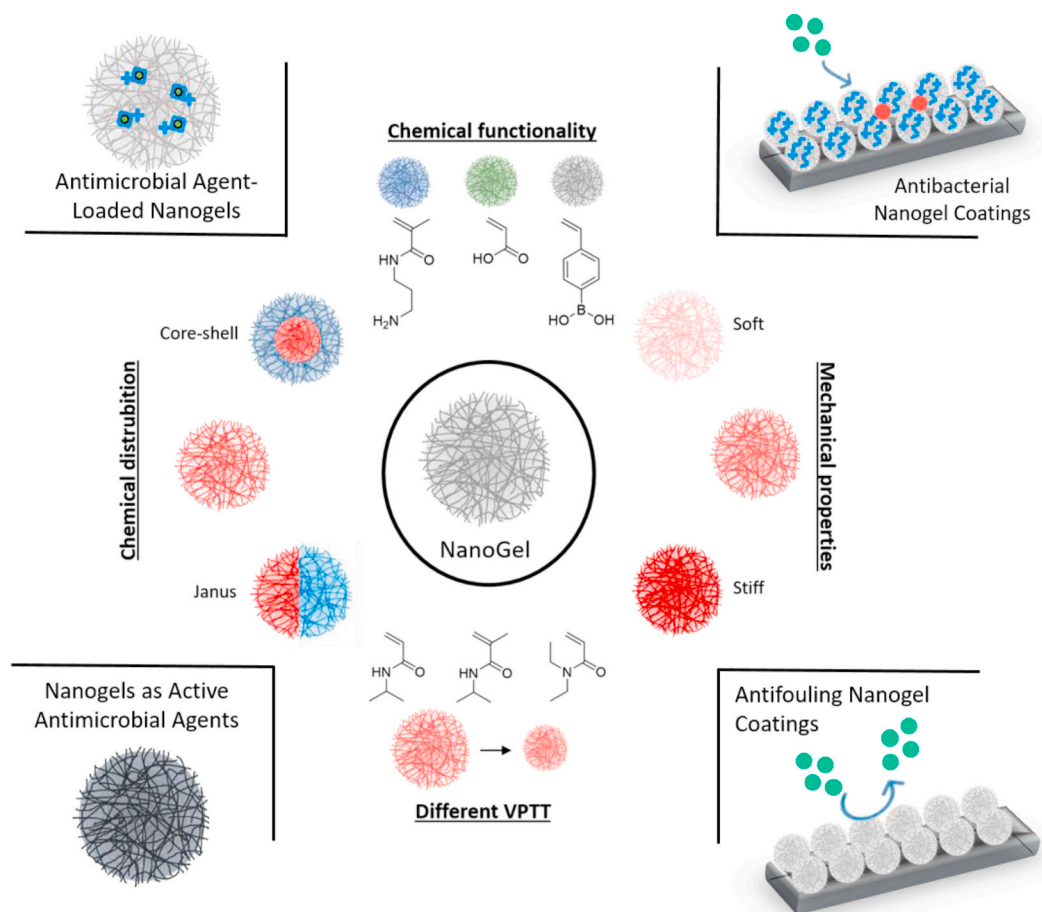
and antifouling surface coatings. In this context, different approaches have recently been followed in the biomedical field; for example, the antifouling performance to inhibit protein, macrophage, and bacteria adhesion to the surface, the enzyme uptake capability for biosensors, the capacity to control the cell proliferation and cell adhesion of nanogel coatings have been investigated [27–32]. Their features such as size, charge, porosity, amphiphilicity, and softness can be tuned by changing the chemical composition during the synthesis or post-modification [33]. Moreover, the properties of nanogels can be modified by adding stimuli-responsive functional groups, which respond to external stimuli such as temperature and pH by swelling or collapsing [34]. The responsiveness can be ideal for a combination with drug release, in order to create a combined antimicrobial and antifouling coating [35]. Different physicochemical properties of nanogels and their applications are represented in Scheme 1.

Despite the promising progress made in the past years, much development is still needed. This review will describe the recent advances of highly biocompatible nanogel particles with particular emphasis as antimicrobial delivery carriers and surface coatings, including antifouling coatings, for biological and biomedical applications.

## 2. Synthesis, properties and biocompatibility of nanogels

### 2.1. Synthesis

Nanogels can be fabricated by numerous techniques that have been summarized recently in various excellent reviews [36–43]. Generally, the synthesis methods can be divided into three major categories: 1)



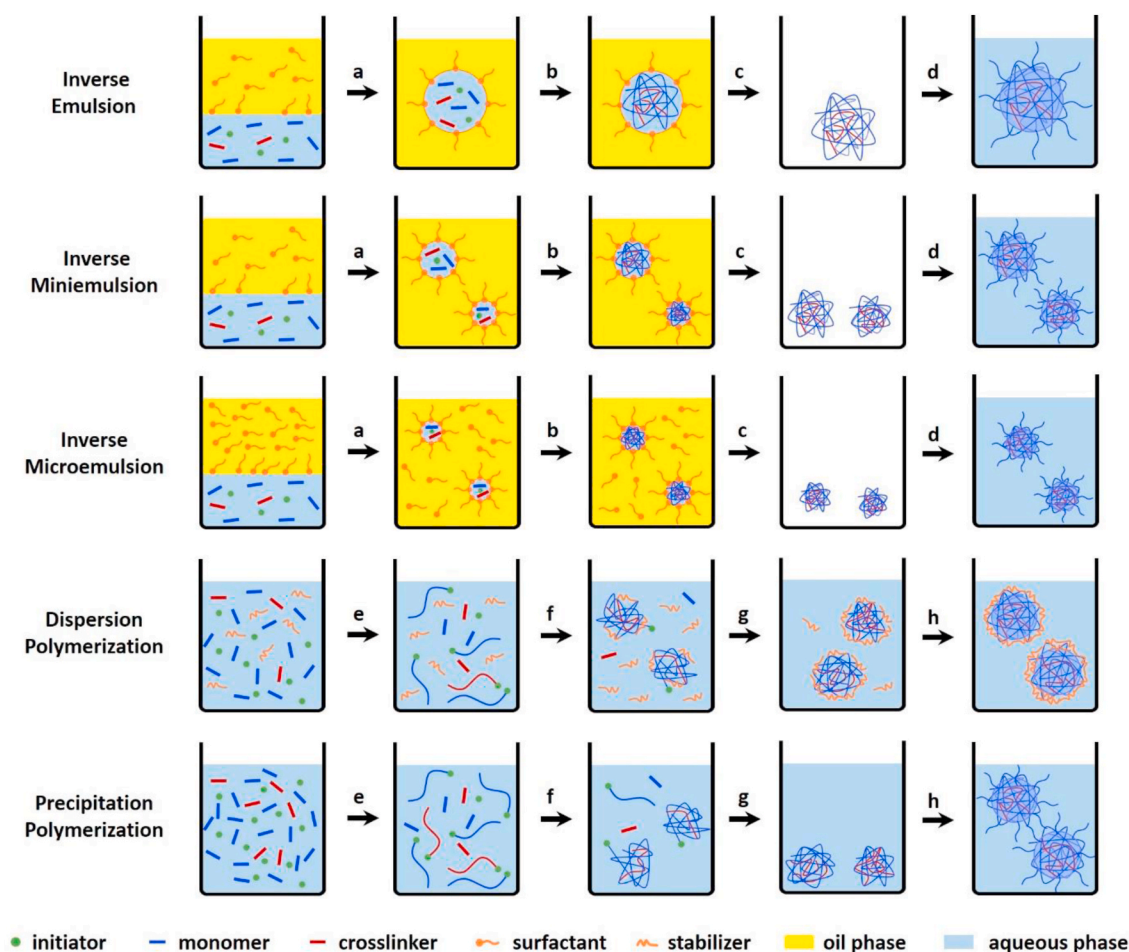
**Scheme 1.** Schematic representation of the different strategies of nanogel design and their applications in biomedical field.

polymerization of monomers, 2) physical or chemical cross-linking of polymer precursors or natural polymers, 3) template-assisted nanofabrication. A brief overview of the commonly used techniques for the preparation of nanogels that have been studied in the context of antimicrobial applications will be provided.

Heterogeneous polymerization is the most extensively employed method used to synthesize stable nanogels by free radical polymerization of monomers with the aid of cross-linkers and functional comonomers. The polymerization is generally performed in aqueous matrix since most of the monomers and cross-linkers are hydrophilic. Approaches for such heterogeneous polymerization reactions include inverse emulsion, inverse miniemulsion and inverse microemulsion polymerization [44–47], dispersion polymerization [48–50], and precipitation polymerization [42,51,52], as shown in Fig. 1.

The most appropriate polymerization processes depend on the desired properties of nanogels and the nature of the monomers. The inverse emulsion, inverse miniemulsion, and inverse microemulsion polymerization methods proceed via the polymerization of hydrophilic (ionic) monomers and cross-linkers within “(nano/micro) reactors” (the aqueous droplets), where the polymerization occurs upon the addition of the polymerization initiator [53]. One main difference between inverse emulsion and inverse miniemulsion processes is the initial size of the dispersed aqueous phase. In the case of inverse emulsion polymerizations, monomer droplets are formed by mechanical stirring and the size range from 1 to 20  $\mu\text{m}$  [54]. In contrast, inverse miniemulsion

polymerization has been broadly used to synthesize poly (N-isopropylacrylamide) (p (NIPAM)) nanogels and the nanogel size is around 150–300 nm [55–57]. The aqueous droplets are generated by applying high shear stress, e.g., by ultrasonication or high-pressure homogenizer, and stabilized with surfactants in a continuous organic phase. The used surfactant concentration is below or near its critical micellar concentration (CMC), so the miniemulsions are only kinetically stable where the stability greatly depends on the formation conditions. Thermodynamic stability is obtained by a further addition of surfactant above the CMC leading to inverse microemulsions. The inverse microemulsion is used to produce p (NIPAM), polyacrylamide (P (AAM)), and poly (vinylpyrrolidone) (PVP) nanogels with a diameter usually less than 100 nm [45,58–63]. In contrast to the heterogeneous inverse emulsion polymerizations, dispersion and precipitation polymerizations are initiated by a homogeneous nucleation mechanism that contains all ingredients, including the monomers, cross-linker, initiator and/or surfactants that are initially dissolved in the solvent. Following the polymerization, the polymer chains start growing until an insoluble critical chain length is reached and ultimately leads to the formation of stable distributed polymeric nanogels. Dispersion polymerization is mainly used to synthesize nanospheres of hydrophobic polymers such as polystyrene (PS) [64]. Recently, the efforts have been made towards the synthesis of nanogels in aqueous systems (water or water/ethanol mixture) using reversible addition-fragmentation chain transfer (RAFT) dispersion polymerization [49,50,65–67]. Dispersion polymerization



**Fig. 1.** Schematic description of nanogel formation by heterogeneous polymerization. The inverse emulsion, inverse miniemulsion and inverse microemulsion polymerization are considered to proceed as follows: (a) emulsification and homogenization, (b) polymerization, (c) removal of excess surfactant, and (d) transfer to good solvent. The dispersion and precipitation polymerization are considered to proceed as follows: (e) initiation and chain growth, (f) precipitation and nucleation by polymer chains collapse when the temperature is far above the lower critical solution temperature or by colloidal stabilizer on the surface of the unstable particles, (g) particle growth and (h) transfer to good solvent or decrease of temperature below the volume phase transition temperature.

generally yields smaller nanogels because of the presence of a colloidal stabilizer [68]. Differently, precipitation polymerization is frequently used to synthesize thermosensitive p (NIPAM) and poly (N-vinyl-caprolactam) (PVCL) nanogels utilizing the phase separation behavior from swollen to a collapsed state since the reaction temperature (50–80 °C) is far above the volume phase transition temperature (VPTT). The detailed polymerization mechanism of precipitation polymerization has been addressed previously [42,69,70]. Briefly, initiators produce free radicals at the polymerization temperature, which attack water-soluble monomers followed by radical propagation and chain-growth to form oligoradicals. The growing polymer chains subsequently collapse when they reach a critical length and form precursor particles because the temperature is far above the lower critical solution temperature (LCST) of the formed polymers. The formed precursor particles can grow by aggregation with other precursor particles to form larger colloidal particles, by deposition onto the surface of existing particles, or by further addition of monomers. Once the nanogel particles reach the critical size, they are stabilized by the use of surfactants or electrostatic stabilization originated from charges of the initiator. When the reaction stops and cools down to room temperature (below VPTT), the nanogels swell to a “fuzzy” morphology and are stabilized by steric mechanisms due to the formation of hydrogen bonds between polymer segments and water molecules. There are some advantages using precipitation polymerization for the preparation of aqueous nanogels: 1) controllable nanogel size by the use of surfactants or co-monomers; 2) narrow nanogel size distribution; 3) desired properties and complex architectures can be obtained by integration of different co-monomers or even encapsulation of nanoparticles during the polymerization process.

Apart from the heterogeneous polymerization of monomers, nanogels prepared by cross-linking of synthetic or natural polymer precursors provides opportunities for producing more biocompatible nanogels [41, 71]. Synthetic polymers with the desired function can be first synthesized by controlled polymerization to finely tune the composition, molecular weight, functional groups, and architecture. Functionalized natural polymers, such as polypeptides, alginate, pullulan, chitosan, dextran, and hyaluronic acid contribute to the improvement of biocompatibility and biodegradability of nanogels [72–77]. There is a variety of cross-linking approaches, including “click” chemistry, thiol-disulfide exchange, Schiff-base reactions, photo-induced cross-linking have been developed for the synthesis of nanogels from the polymer precursors [21,75,78–83]. Complementary to chemical cross-linking, physical self-assembly offers a reversible property since they are stabilized by relatively weak interactions between polymer chains such as hydrogen bonding, hydrophobic interactions, van der Waals interactions, and electrostatic interactions. Usually, the physical self-assembly is conducted under mild conditions in an aqueous medium. The size of nanogels is controlled by the polymer concentration, amphiphilic character, functional groups, pH, ionic strength, and temperature [84].

Different from the other previously mentioned polymerization approaches where templates were used such as emulsion droplets, another template-assisted nanofabrication, such as photolithography [85,86], is a new top-down particle lithographic technique called PRINT (Particle Replication In Non-wetting Templates), which provides the opportunity to fabricate a stable nanogel system with precise control over size, shape, deformability, and surface functionality [84,87]. Step and Flash Imprint Lithography (S-FIL) is a method that was recently developed as a nanofabrication method that is able to directly harvest nanogels from a silicon wafer substrate into aqueous buffers using a simple and biocompatible process in a high-throughput manner [88]. With this technique, the shape and surface chemistry of nanogels can be finely controlled, which is difficult using other bottom-up methods.

## 2.2. Properties

Polymer nanogels, also known as microgels -since the first usage of the term “Microgel” in 1949 by W. O. Baker, have a sub-micron confined network resulting from chemical cross-linking between polymer strands and have attracted considerable attention in theoretical studies regarding soft matter [89–91]. At low concentrations, a nanogel solution acts like a dilute colloidal system, and the size of these well-dispersed nanogel particles and the local concentration of the cross-linked polymer chains in each particle can be adjusted by changing the cross-link density and surfactant amount during the polymerization [92,93]. By the time, the term microgel gave its place to the term “nanogels” since smaller microgels were started to be synthesized. Thanks to the unique behaviors of nanogels, such as their three-dimensional, macromolecular polymer network of colloidal size swollen by the dispersing solvent, and their versatile features like biocompatibility, degradability, high colloidal stability, and high loading capacity make them important candidates for biomedical applications for drug delivery, cell imaging, and tissue engineering [94–96]. Moreover, the ability to rapidly respond to environmental changes such as temperature, light, pH, ionic strength offers them distinct advantages over other types of nanomaterials in the biomedical field [21,97]. Through the advanced chemical design and synthesis methods mentioned above, the important parameters as size, shape, swelling degree, and chemical, as well as the topological composition, can be tuned to achieve the unique properties of nanogels [94]. Various reviews have summarized the fundamental research of nanogel structure, properties and applications in materials science and the biomedical field [21,38,40,41,43,83,94,95,98]. Within this review, the focus lies on the selective properties of nanogels specific for antimicrobial applications.

First of all, aqueous colloidal stability under physiological conditions is the most important requirement for any type of nanomaterials to be applied in antimicrobial applications, which will ultimately decide the biological efficiency, *in vivo* distribution, and toxicity of nanoparticles. Nanogels show better stability compared to macromolecules, micelles, inorganic nanoparticles, and other nanoparticles, which is derived from intra-particle cross-linking of the polymer chains and their hydrophilic nature [39,94,95].

To apply nanogels as a delivery system to combat bacterial infections, the versatility of the architecture and the tunable pore size of nanogels allows for the incorporation of numerous guest molecules ranging from inorganic nanoparticles to biomacromolecules such as proteins and DNA or small therapeutic molecules without compromising their hydrogel-like behavior and colloidal stability [53,99–103]. To achieve long circulation half-life of their cargo and improve the retention at the target site *in vivo*, meanwhile preventing the degradation of enzymes or genetic material, the size, softness, charge, and surface properties of nanogels play crucial roles. As compared to the size of biomolecules, the nanoscale size of nanogels helps to avoid rapid renal segregation, enhances bloodstream transport, easily enables tissue permeation, and increases passive targeting by the enhanced permeation and retention (EPR) effect [21]. Another unique characteristic that helps nanogels prevent fast renal clearance is their softness and deformability, which help them easily pass through the splenic filtration bed leading to longer circulation half-life and lower splenic accumulation [104–107]. Further, nanogels can be designed to be stimuli-responsive and react to external stimuli to help achieve a controlled, triggered response at the infection region. The external stimuli can be the physiological environmental change within the body such as temperature, pH, enzyme presence, and redox conditions, or the stimuli can be applied externally such as light, electrochemical signal, pressure, and magnetic field. These stimuli can cause conformational or structural changes and then alter the hydrophilicity and/or hydrophobicity of the nanogels, subsequently resulting in swelling or collapse of the nanogel network [108,109]. The extent of swelling depends on the chemical composition, hydrophilicity of cross-linkers, ionization of



functional groups, and the degree of cross-linking of the nanogel network, which controls the polymer mobility and the interaction of the polymer chains with water [110–112]. Depending on the desired stimulus and the utilized antimicrobial agents and target ligands, the cargo can be either chemically conjugated to nanogels or physically loaded into the network to achieve an on-demand release nanogel delivery system.

The hydrophilic nature of the hydrogel facilitates uses *in vivo* due to the presence of numerous polar groups such as –OH, –COOH, –CONH<sub>2</sub> and –SO<sub>3</sub>H distributed along the polymer chain [41,113]. Besides, to facilitate the renal clearance of the final degraded products, many labile cross-links have been introduced in the chemical design of nanogels such as hydrazone, disulfide, carbonate ester, and siloxane to develop degradable nanogels [82,99,114,115]. Besides, nanogels composed of natural biodegradable polymers such as chitosan, pullulan, dextran, hyaluronic acid, alginate, and gelatin can be degraded by biological microenvironments [99].

### 2.3. Biocompatibility of nanogels

Nanogels coatings and particles have great potential in the development of smart biomaterials and are gaining interest in the biomedical field [116]. Because nanogels may interact with tissues and cells, they must be non-cytotoxic, non-apoptotic, and non-necrotic. A biomaterial is said to be biocompatible when it optimally performs its desired function without eliciting any local or systemic harmful effects [117]. Introduction of a biomaterial into a host organism triggers the foreign body response (FBR), which is preceded by the formation of a provisional matrix as a result of blood-biomaterial interactions, acute and chronic inflammation and coupled with the formation of foreign body giant cells [118]. The aim of the FBR is to enclose the biomaterial in a fibrotic capsule, isolating it from the surrounding tissue [119]. This inevitable enclosure in a fibrotic capsule has led to a revision of the definition of biocompatibility [117] where a distinction has been made between biotolerability and biocompatibility. The former is the ability of a biomaterial to reside in the host for a long period of time while eliciting a low degree of inflammation and the latter is defined as the ability of a material to locally trigger and guide non-fibrotic wound healing, reconstruction, and tissue integration [120]. Biocompatibility, and specifically cytotoxicity is often tested *in vitro*. The present *in vitro* assays are insufficient to allow *in vitro* assessment of biotolerability. Therefore, information on biotolerability is obtained from *in vivo* studies.

Nanogels are often found to be non-cytotoxic, although sometimes it is a dose and time dependent phenomenon. Temperature sensitive poly (N-vinylprolactam)-based nanogels were found to be non-cytotoxic to both 9-day and 16-day neurons from the cerebral cortex of rat embryos for up to 24 h [131] based on the outcome of both MTT and LDH assays. Rosmarinic nanogel particles, cross-linked products of Rosmarinic acid, showed improved cytotoxicity of COS-1 cells lines at concentrations below 100 µg/mL when compared to rosmarinic acid, a non-biocompatible antioxidant and bioflavonoid. Also, Rosmarinic acid nanogels were blood compatible only at concentrations below 50 µg/mL as it showed a dose dependent stimulation of blood clotting and hemolysis at 100 µg/mL [127]. Similarly, hybrid core shell nanogels based on poly (NIPAM-co-3-acrylamidophenylboronic acid-co-dextran-maleic acid) coated nanoparticles with a great potential for temperature-controlled insulin release, showed higher cell viability of A549 cells from 2 to 4 days of exposure at 400 µg/mL with respect to 3-(Methacryloxy) propyltrimethoxysilane coated silica nanoparticles according to the results of MTT assay. Even increasing the concentration of the nanogels was accompanied with a slight increase in cytotoxicity after 2 and 4 days [132].

Conjugation of nanogels to biomaterials are often applied in order to modulate their biocompatibility. Khan et al. [133] developed gold nanorod-nanogel (Au NRs-nanogels) composite particles by connecting

gold nanorods to pNIPAM based nanogels via electrostatic interactions. The Au NRs-nanogels showed reduced cytotoxicity to MCF-7 cells based on MTT and LDH. In addition, the nanogel almost eliminated the hemolytic activity of the gold nanorods on blood agar [133]. Ketoprofen, an anti-inflammatory drug, inhibits cyclooxygenase and shows high gastrointestinal toxicity. Encapsulation by nanogels based on cellulose acetate phthalate and hydroxyethyl methacrylate (CAP-co-poly (HEMA)), reduced the cytotoxicity of ketoprofen against Vero cells after 24 h incubation at a concentration range of 1–20 µg/mL [130]. 2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959) is an often used photoinitiator for water borne photo-curing of biomaterials because of its excellent water solubility and low toxicity [134]. However, Irgacure 2959 is characterized by a high migration of the photolysis fragments into the liquid environment due to its low molecular weight, which is potentially harmful for tissue cells. Incorporation of Irgacure 2959 into nanogels based on oligo (ethylene glycol) monomethyl ether methacrylate (OEOMA) with a molecular weight of 950 g/mol and polyethylene glycol dimethacrylate (PEGDMA) enhanced its thermostability and also reduced the migration of the photolysis fragments of the photo-cured film when compared with Irgacure 2959. Furthermore, MTT assay was used to demonstrate excellent cell viability of HeLa Cells after 24 h exposure to the nanogels in comparison to Irgacure 2959 [134].

Besides the reduction of cytotoxicity of compounds used in biomedical applications through encapsulation in nanogels, the biocompatibility of nanogels themselves can be affected by further functionalization [130]. Modification of the zwitterionic betaine of polyethyleneimine (PEI) via treatment with 1,3-propane sultone to improve the antifouling properties of PEI, reduced its proapoptotic and pronecrotic properties as well as its cytotoxicity to Vero79 cells in comparison with bare PEI after 48 h incubation. However, as a result, the antimicrobial ability of bare PEI was lost after modification [135]. Gum Arabic is widely employed as a thickening and emulsifying agent in the food industry. Nanogels of gum arabic crosslinked with gelatin is cytotoxic at concentration 0.125–1 mg/mL and hemocompatible at concentrations 0.2–1 mg/mL under ISO/TR 7406 [136]. Biocompatibility of Gum Arabic gel particles conjugated with diethylenediamine (GA-DETA) and taurine (GA-TA) in an attempt to tune the properties of the nanogels was studied. On one hand, DETA introduced positive charges which improved protein adsorption and in turn contributed to its improved cytotoxicity on fibroblast L929 cells in comparison with unmodified Gum Arabic nanogels and on the other hand, taurine resulted in increased cytotoxicity on fibroblast L929 cells at 0–200 µg/mL. While both GA-DETA and GA-TA nanogels were found to induce blood clotting, GA-TA showed a higher capacity to induce apoptosis by reducing intracellular calcium concentrations [129].

On one hand, hydrophilic nanogel drug carriers resist opsonization and protein adhesion in a host but are poor transporters of hydrophobic drugs. On the other hand, the blood circulation time and therapeutic potential of hydrophobic nanogels is low because of their removal by the reticuloendothelial system. Amphiphilic nanogels are a new family of nanogels that combine resistance to opsonization and removal with increased uptake of hydrophobic drugs due to the presence of both hydrophilic and hydrophobic groups respectively [137]. The cytotoxicity of amphiphilic nanogels is important since they can induce toxicity through interaction with cellular lipids. Combining 80 mol% of hydrophilic 2-hydroxypropylamin (HPA) groups with 20 mol% of either benzyl (BENZA-20), hexyl (HEXA-20), cholesteryl (CHOIA-20) or dodecyl (DODA-20) showed similar cell viability (circa 100%) of monocytic-like THP-1 cells at 20–100 µg/mL after 2 h incubation in comparison to the biocompatible pentafluorophenyl methacrylate (PFPMA) as determined by WST-1 assay. Additionally, opsonization and protein adhesion was dependent on the hydrophobicity and structure of the nanogels while the former determined the corona composition of the nanogels, which will in turn determine their circulation time [137]. Magnetic nanogels can be guided to areas of interest to release contrast

agents in magnetic resonance imaging [138]. Microscopy was employed to evaluate the biocompatibility of magnetic nanogels, which consisted of PDEAEMA-based nanogels encapsulated with Fe<sub>3</sub>O<sub>4</sub> nanoparticles on red blood cells and platelets. In autologous plasma, the presence of biomolecules such as proteins, carbohydrates and lipids adhered to the surface of these magnetic nanogels, which prevented interaction with red blood cells and preserved their morphology while red blood cells were lysed after 15 min contact with these magnetic nanogels in physiological saline solution. In both situations, no interaction with platelets was observed [139]. Table 1 provides a short overview of common *in vitro* biocompatibility tests taken for various nanogel-based systems.

Biocompatibility can be influenced by physico-chemical properties of the nanogels. For example, the temperature at which MTT and LDH assays are performed for some temperature sensitive nanogels can influence the outcome of these tests, which in particular is relevant in cases of temperature triggered drug release. The cell viability of both Human breast cancer cell line (MCF-7) and Jurkat cells were approximately 100% when incubated with poly N,N'-diethylacrylamide (DEEAm) at concentration range of 0.1–2.5 mg/mL and poly (N-isopropylacrylamide) (NipAAm) at concentrations up to 1 mg/mL for 24 h when MTT and LDH assays were performed at 37 °C. However at 22 °C, which is below the critical temperature, the permeability of the cell membranes of both MCF-7 and Jurkat cells for LDH increased at concentration up to and above 1 mg/mL for polyDEEAm and polyNIPAAm respectively due to their improved solubility and interaction with the cell membrane [140].

#### 2.4. *In vitro* biocompatibility tests

Biocompatibility testing is part of the main conformity assessment to demonstrate the safety of the product for use in medical devices. In this respect the ISO-standard used in the biological evaluation of medical device, ISO 10993, is guiding the conformity assessment, e.g. with respect to cytotoxicity testing as in ISO 10993:5. According to this standard, cytotoxicity tests can be assessed by morphological means or measurements of cell damage, cell growth, and specific aspects of

cellular metabolism. Rapid and inexpensive calorimetric assays such as the (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT), 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium (WST-1) and lactate dehydrogenase (LDH) assays are routinely performed to evaluate the cyto-tolerance of newly synthesized nanogels based on cellular metabolism [121]. The MTT assay measures the viability of cells by determining the activity of mitochondrial NAD(P)H-dependent cellular oxidoreductase enzyme which is capable of reducing MMT to an insoluble purple formazan. Similarly, the WST-1 assay predicts relative cell proliferation through the reduction of the tetrazolium WST-1 salt to formazan by mitochondrial dehydrogenases while the LDH assay quantitatively measures the leakage of LDH from the cytoplasm as a result of cell membrane damage [121].

Apart from these standard methods, several methods have been employed to evaluate the apoptotic, necrotic, and genotoxic effects of nano-sized gels. A double staining with Propidium iodide and Hoechst applied to a cellular suspension in the presence of various concentrations of the gel has been utilized to assess apoptotic and necrotic effects. These are sometimes paired with methods like the comet assay [122–124] and the annexin-v staining [125] to detect single strand breaks in DNA and the translocation of phosphatidylserine on the outer leaflet of the cell membrane respectively. In addition to this, the generation of reactive oxygen species is measured with the 20,70-dichlorofluorescein diacetate assay [124] and microscopic [126] assessment are utilized to estimate the cytotoxicity of nanogels [127,128].

In order to prevent the inhibition of oxygen transport and encourage proper blood clotting, nanogels, which may directly or indirectly come into contact with blood, should not cause the lysis or coagulation of red blood cells and platelets respectively. Hemocompatibility is usually evaluated with hemolysis and blood clotting tests because a biomaterial may influence the mechanisms of coagulation and thrombosis [129, 130].

**Table 1**  
Summary of *in vitro* biocompatibility tests.

Nano/microgel description	Intended application	Test model	Biocompatibility test	Ref
Silica nanoparticles coated with N-propyl acrylamidophenyl boronic acid-co-dextran-maleic acid nanogels	Insulin delivery	A549 cells	MTT assay	[55]
Rosmarinic acid crosslinked with trimethylolpropane triglycidyl ether	Drug conjugation and delivery	Cos-1 cells	Hemolysis and blood clotting test Toluidine blue staining	[127]
Au-NR and N-isopropylacrylamide-co-acrylic acid nanogel composite	Drug delivery Photothermal therapy	MCF-7 cells	MTT assay LDH assay Cell hemolysis test on blood agar	[133]
PEI microgels functionalized with 1,3-propane sultone	Drug delivery Gene delivery Tissue engineering	Vero 79 cells	WST-1 assay	[135]
Gum Arabic-gelatin nanogels	Drug delivery Gene delivery	MCF-7 cells	Hemolysis test MTT	[136]
80 mol% 2-hydroxypropylamin groups with BENZA-20 or HEXA-20 or CHOLA-20 or DODA-20	Drug delivery	THP-1 cells	WST-1 assay	[137]
Poly (N-(2-mercaptoethyl) acrylamide)	Antioxidant Antibacterial	L929 fibroblasts DLD-1 cells	WST-1 assay Hemolysis test Blood clotting index Hoechst and Propidium iodide staining	[130]
Gum Arabic nanogels modified with diethylenediamine and taurine	Antibacterial	L929 fibroblasts	WST-1 assay Hemolysis test Blood clotting index Hoechst and Propidium iodide staining	[129]
Oligo (ethylene) mono methyl ether methacrylate	Photo initiator in water borne photocuring	HeLa cells	MTT	[134]
PDEAEMA-based nanogels labelled with Fe <sub>3</sub> O <sub>4</sub> magnetic nanoparticles	Clinical diagnostics and therapy	Red blood cells	Microscopic assessment	[139]

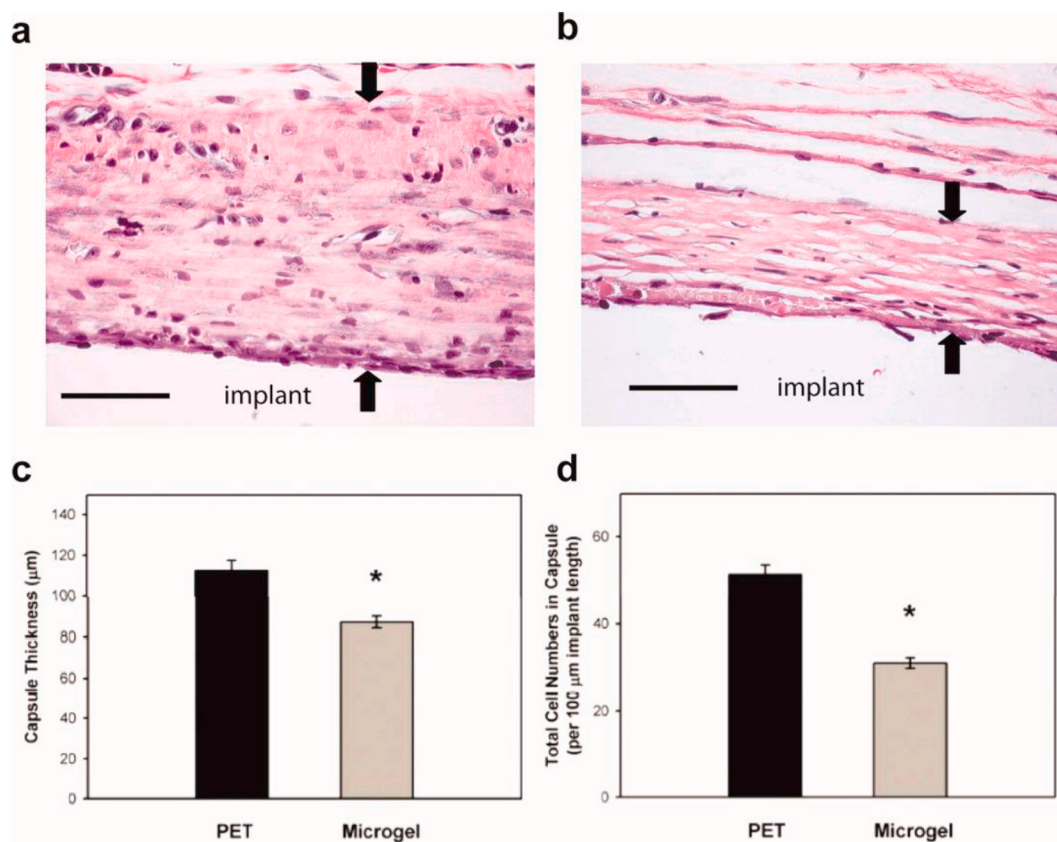
## 2.5. Biotolerability

As far as we know, no standard procedures are available to test the biotolerability of nanogels, nor for the FBR against biomaterials in general. *In vivo* studies, however, often gives a clear picture of the wide repertoire of interactions that occur once nanogels are introduced into a living host [141,142]. The provisional matrix, which is formed by the non-specific adhesion of serum proteins, attracts fibroblasts and other cells involved in acute inflammation. Therefore preventing the adhesion of proteins to the surface of the biomaterial may limit the extent of the FBR [143]. Coating poly (ethylene terephthalate) (PET) with films of pNIPAm cross-linked with the non-fouling poly (ethylene glycol), reduced cellular adhesion both *in vitro* and *in vivo* in comparison with uncoated PET substrates. Histological assessment of the implants after intraperitoneal implantation in mice at 48 h showed a thinner and denser collagen capsule on coated PET in comparison to uncoated PET as shown in Fig. 2, indicating a shorter chronic inflammation and a faster resolution of the immune response on the coating [144].

Since nanogels are hydrogels at nanometer scales, zwitterionic nanogels may promote a non-fibrotic incorporation of biomaterials in the host tissue. The zwitterionic polycarboxybetaine methacrylate (pCBMA) is the only hydrogel known to resist the formation of a fibrotic capsule after 3 months of subcutaneous implantation in mice [145]. However, a disadvantage of zwitterionic hydrogels is their low mechanical strength. To improve the mechanical strength of zwitterionic hydrogels while mitigating the foreign body response, triazole groups were introduced in pCB to form triazole zwitterionic hydrogels (TR-ZW) [146]. This resulted in a 250% percent tensile strain, which is higher

than the 65% tensile strain of pCB. *In vitro* assessment showed similar cell adhesion to the TR-ZW when compared to pCB and the biocompatible poly (2-hydroxyethyl methacrylate) (PHEMA). The expression of interleukin 10 was also increased, which is a characteristic of the pro healing macrophage phenotype. Histological evaluation after subcutaneous implantation of the TR-ZW in mice showed that the introduction of hydrophobic triazole groups increased the adsorption of plasma (3–10 ng/cm<sup>2</sup>) on the TR-ZW hydrogels in comparison to pCB (0.3 ng/cm<sup>2</sup>). However, this did not interfere with the attenuation of a fibrotic capsule and increased blood vessel formation when compared to PHEMA [146]. Temperature sensitive PNIPAM nanogels elicited mild inflammation, which kept declining in severity from one week up to 3 months after injection into the right kidney of rabbits at an embolization rate of 0.10 mL/s. After 3 months, no signs of neovascularization, foreign body granuloma, extravasation, mural hemorrhage and vascular spasms were found. However, fibrous tissues were found in embolized vessels together with the PNIPAM nanogels, which contributed to the persistence of the embolic effect 1 and 2 months after the operation [147].

Biocompatibility and biotolerability are dynamic and multifactorial properties that depend on the concentration and physico-chemical properties of the biomaterial, location, the types of interaction between material and host tissue. The intended functionality is essential since the given definition of biotolerability is not applicable to nanogels used as embolic agents. The research presented above proves the complexity involved in defining the biocompatibility or biotolerability of nanogels. Changes in the biomaterial or in the host environment can potentially cause a once biocompatible biomaterial to become toxic or



**Fig. 2.** Microgel coatings reduce chronic inflammation associated with materials implanted subcutaneously in the rat dorsum for 4 wk. Explants were evaluated for fibrous encapsulation by staining collagen (pink), elastin (black), and nuclei (black). Representative images for unmodified PET (a) and microgel-coated PET (b) disks, and the original implant location is designated. Black arrows indicate capsule measurements. Microgel coatings reduced fibrous capsule thickness by 22% compared to unmodified PET controls as quantified in (c), \* $p < 0.04$ . The density of capsule-associated cells was also significantly reduced in microgel-coated samples (\* $p < 5.6 \times 10^{-3}$ ) compared to unmodified PET controls as quantified in (d). Data is represented as the average value  $\pm$  standard error of the mean using  $n = 4-7$  samples per treatment group. Scale bar is 50  $\mu\text{m}$ . (Reproduced with permission from ref 144. (Copyright 2010 Wiley).

non-compatible [142]. This makes a single definition of biocompatibility unrealistic. Rather, biocompatibility and biotolerability could be redefined for classes of nanogels that have similar biomedical applications. In addition to this, the biocompatibility of nanogels should not be based only on MTT and LDH assays as sometimes found in the literature. The MTT assay can be influenced by factors such as pH and the presence of superoxide, polyphenols, and pyruvate analogs. Superoxide is capable of reducing MTT to Formazan, resulting in an inaccurate estimation of cell viability [148]. Moreover, MTT formazan formed needle-like crystals at nanomolar concentrations that could induce apoptosis of SH-SY5Y cells which resulted in increased plasma permeability and leakage of cell content [149]. Therefore, these assays should always be combined with tests that evaluate apoptotic, necrotic and hemolytic effects of newly synthesized nanogels.

In contrast to drugs, the preclinical and clinical testing of biomaterials is not well defined [150]. Therefore, standard tests can help avoid inaccurate claims of biocompatibility. In addition, choosing the appropriate standard toxicity tests provide some understanding about the physiological, chemical and biochemical processes that become operative in cells or tissues upon contact with nanogels and this is key in defining their biocompatibility and biotolerability [151]. This knowledge may in turn facilitate the development of nanogels with defined properties that may result in successful clinical application [152].

### 3. Nanogel-based antimicrobial systems

Over the past few decades, multidrug-resistant bacteria have become a serious health concern due to the abuse and misuse of antibiotics [153, 154]. A high therapeutic dosage of antibiotics is required to treat the resistant bacterial infection, therewith further contributing to the increased emergence of drug resistance. Therefore, it is imperative to develop novel antimicrobial agents and new treatment strategies [155, 156]. The recent advancements in the development of antimicrobial nanogels offered a promising approach to tackle multidrug-resistant bacteria [157–160]. This section will first provide the applications of nanogels used as delivery tools for conventional biocidal agents; then, the focus will be on the recent development of nanogels with intrinsic antimicrobial actions.

#### 3.1. Nanogels as antimicrobial delivery vehicles

To reduce the applied dosage of biocidal agents and enhance the bioavailability at the same time, the conventional biocidal agents can be conjugated or encapsulated into nanogels and enriched in the infected zone, and released to kill bacteria. The widely used biocidal agents in literature include but are not limited to antibiotics [161–167], bioactive antimicrobial agents [168–172], silver and gold nanoparticles [173–182], and antimicrobial peptides [183–187].

##### 3.1.1. Antimicrobial agent-loaded nanogels

In addition to developing new antibiotics, reduction of the administered dose is also important to cope with the rise of drug-resistant bacteria. Small molecular active antibiotics are easily cleared and quickly inactivated, while the remaining active antibiotics have difficulty penetrating the tissue barrier and killing bacteria in the infected area. To enhance the bioavailability of antibiotics, conventional antibiotics can be encapsulated into nanogels. Encapsulation protects the drugs from degradation and may improve the penetration by tuning specific (bio)chemical interactions with the cell wall, and subsequently (triggered) release of the antibiotics to the infected zone to improve the therapeutic concentrations [162,163].

Among all the commercially available antibiotics, vancomycin is considered a last resort medicine for the treatment of sepsis and lower respiratory tract infections caused by Gram-positive bacteria. Vancomycin is a glycopeptide antimicrobial as alternative penicillin to treat penicillinase-producing strains of *Staphylococcus aureus*, and it is also

widely used for the treatment of serious infections involving methicillin-resistant *S. aureus* (MRSA) [188,189]. However, the incidence of infections with vancomycin-resistant bacteria strains have greatly increased [190,191]. Recently, several studies have used nanogels to deliver vancomycin to the bacterial infection site [161,166,192,193]. However, the hydrophilic nature of vancomycin may cause undesired leakage or non-specific release when loaded into nanogels. To overcome the problem, a bacterial-lipase sensitive polymeric triple-layered nanogel (TLN) was synthesized by a convenient arm-first procedure using an amphiphilic diblock copolymer mPEG-PCL (monomethoxy poly(ethylene glycol)-*b*-poly( $\epsilon$ -caprolactone)) to initiate the ring-opening polymerization of the difunctional monomer DEGDP (3-oxapentane-1,5-diyl bis(ethylene phosphate)) [161]. In this approach, the bacterial lipase-sensitive hydrophobic PCL interlayer formed a hydrophobic and compact molecular fence surrounding the cross-linked polyphosphoester core to prevent vancomycin release from the drug reservoir before reaching the infection sites. The approach eliminates potential adverse side effects due to non-specific drug leakage. After reaching the bacterial infection sites, the TLN sensed the lipase-secreting bacteria and the PCL fence degraded to release the loaded vancomycin. To further achieve the selective eradication of intracellular bacteria, a macrophage targeted antibiotic delivery nanogel (MNG-V) was developed by the same group using a mannosylated PEG-arms shell and a vancomycin-loaded polyphosphoester cross-linked core, as shown in Fig. 3 [192]. The conjugated mannose moieties could target the mannose receptors expressed on macrophages and facilitated the uptake of nanogels to accumulate the drug at the bacterial infection site *in vivo* through macrophage transport [194]. Subsequently, after bacteria are phagocytosed by macrophages, the rapid triggered vancomycin release was achieved in the presence of the bacterial enzymes (e.g., phosphatase or phospholipase) by degrading the polyphosphoester core of the nanogel. The released vancomycin further killed the bacteria, when the absolute concentration of vancomycin was increased to 10  $\mu$ g/mL, the counting the colony forming units (CFU) of surviving intracellular bacteria, treated by MNG-V, was about 50 times lower than in cells treated with free vancomycin, which also significantly improves the therapeutic efficacy of vancomycin in the infected zebrafish embryo model. However, the increased macrophage uptake of nanogels may also speed up the immune clearance to reduce the therapeutic efficacy [195].

To avoid the immune clearance by macrophages, Li et al. reported red blood cell (RBC) membrane coated vancomycin-loaded supramolecular gelatin nanoparticles (SGNPs) for adaptive and “on-demand” antibiotic delivery [193]. As shown in Fig. 4, the RBC membranes also act as detoxifiers to absorb the exotoxins produced by bacteria to relieve bacteria-induced inflammation. Meanwhile, the overexpressed bacterial gelatinases in the infection microenvironment could effectively hydrolyze SGNPs into small fragments, and triggered the release of loaded vancomycin to kill pathogenic bacteria locally. In this way, the bacterial infection was treated by the biomimetic antibiotic delivery system with a minimum dose of antibiotics. Another group combined the two unique strategies, toxin neutralization property of RBC membranes and the ‘on-demand’ drug release nanogel, to achieve the specific intracellular release of vancomycin to treat the MRSA infection [166]. Different from the prior wrapping of RBC membranes onto pre-formed nanogels is that they first prepared the cell membrane-derived vesicles and then polymerize the hydrogel cores inside the vesicles by a redox-responsive cross-linker containing a disulfide bond. Therefore, in an extracellular environment, the RBC-nanogels effectively neutralized MRSA-associated toxins, which in turn promoted further bacterial uptake by macrophages. The intracellular reducing environment facilitated an accelerated drug release profile, which resulted in more effective bacterial inhibition.

Alternatives to synthetic antibiotics, a growing number of antibacterial studies using natural antimicrobial compounds, have emerged to solve the severe issues mainly raised from the overuse of antibiotics [196–198]. However, the intrinsic problems of natural compounds, such



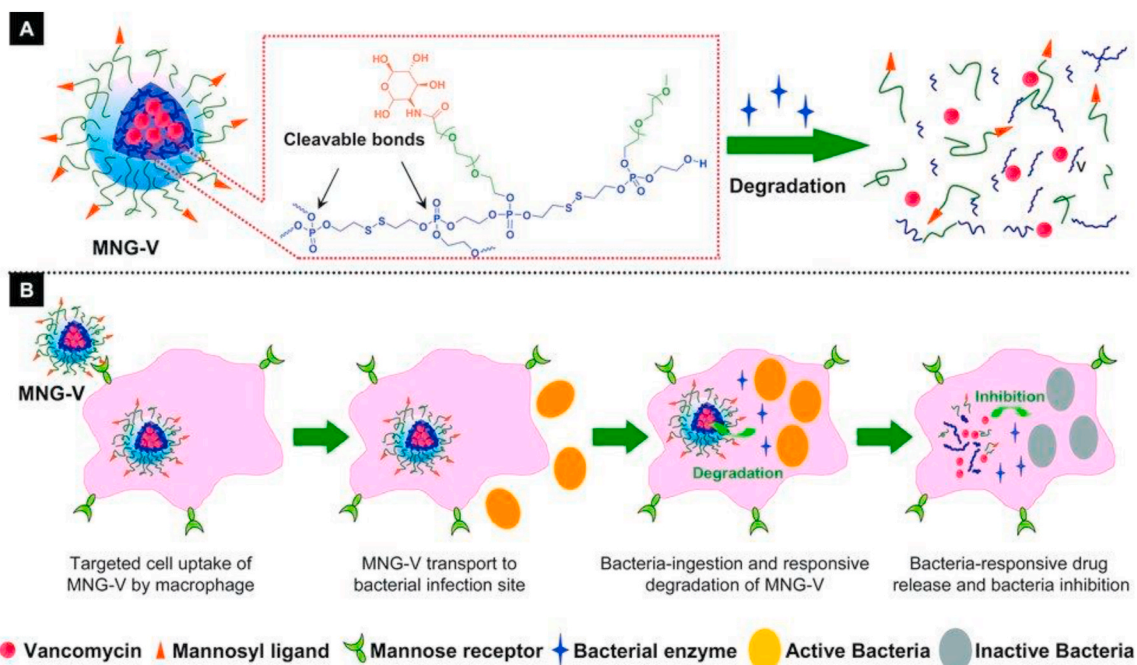


Fig. 3. (A) Schematic illustration of a vancomycin-loaded mannosylated nanogels (MNG-V) and the bacteria-responsive drug release; (B) Schematic illustration of targeted uptake of MNG-V, transport, degradation, drug release and bacteria inhibition. (Reproduced with permission from ref 192. (Copyright 2012 Wiley)).

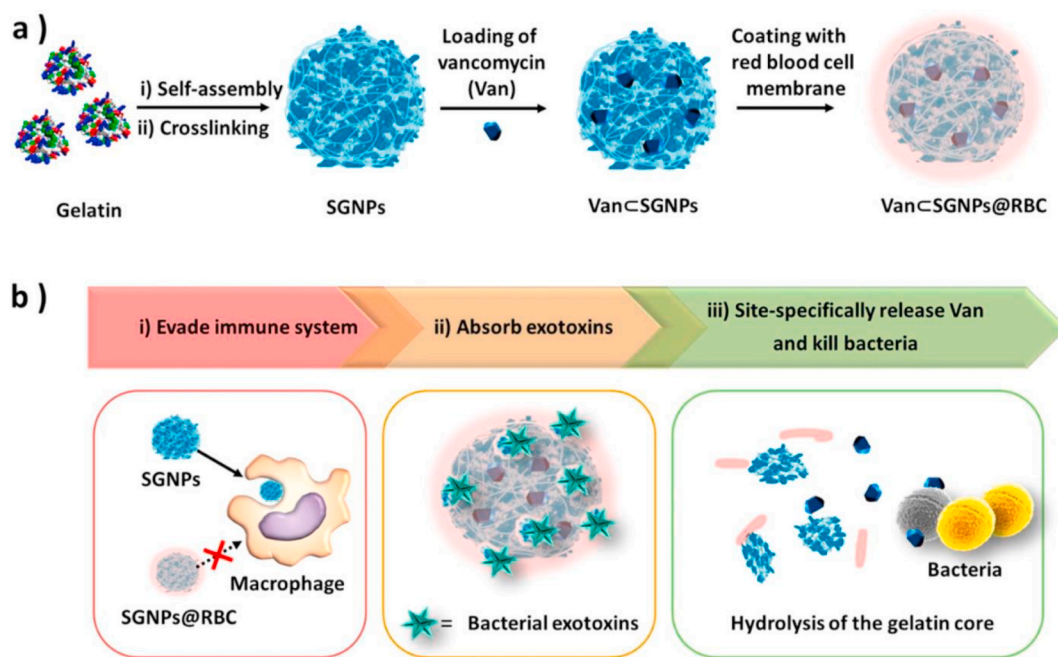


Fig. 4. (a) Preparation of vancomycin encapsulated supramolecular gelatin nanoparticles with RBC membrane coating layer (VanCSGNPs@RBC). (b) Schematic representation of adaptive and multifunctional VanCSGNPs@RBC in the treatment of a bacterial infection. (Reproduced with permission from ref 193. (Copyright 2014 American Chemical Society)).

as low activity, low availability, and instability, hinder their applications. Recent advances have been made by introducing nanogels to transport and protect these natural compounds for enhanced effectiveness [199–201]. For instance, essential oils consist of multiple phenylpropanoids, which are natural antifungal agents but with intrinsic hydrophobicity and rapid evaporation. These oils have been encapsulated into chitosan nanogels to improve antimicrobial activity and stability and are applied in the food industry [202–204]. Curcumin, a natural yellow phenolic compound present in turmeric, has numerous

pharmacological properties such as anticancer, anti-inflammatory, antimicrobial, anti-malarial, and antioxidant, as studied in both pre-clinical and clinical studies [205–207]. However, the rapid degradation of curcumin at physiological pH and poor aqueous solubility limits their application. To tackle the problem, Li and coworkers fabricated a strawberry-like novel temperature-sensitive nanogel using (p (NIPAM), temperature-sensitive polymer to encapsulate poly (3,4-ethylene dioxthiophene) nanoparticles (PEDOT, photothermal agents) and curcumin through reformative precipitation polymerization [201]. The release of

curcumin was achieved by the shrinkage of p (NIPAM) networks triggered by a light-induced temperature increase of PEDOT. This temperature-sensitive nanogel system showed excellent antioxidant and antibacterial performance.

Other than the conventional antibiotics and natural antimicrobial compounds, some chemical antiseptic and disinfection moieties, such as chlorhexidine and Triclosan, have received great attention in oral and skin health applications, for instance, periodontal therapy and wound healing [208,209]. The mechanisms of action of chlorhexidine are bactericidal as well as bacteriostatic, causing membrane disruption [210]. Triclosan combats bacteria by non-specific interaction with the cell membrane but also blocks the lipid synthesis explicitly to inhibit the bacterial growth [211]. However, both of them are small molecules that have poor bioavailability; especially, Triclosan has poor solubility in aqueous solutions, and is under scrutiny because of its environmental and potential hazardous effects [212], which makes it important to provide systems that greatly diminish the used concentration without losing the antimicrobial effects. Therefore, controlled delivery of them to the target sites can help to reduce the toxicity and enhance the biological effectiveness. Some investigations have used nanogels as a carrier to be applied in both aqueous dispersion and surface coatings, which will be addressed in the coating section [213–216]. Recently, in our group, a p (NIPAM-co-DMAPMA) based nanogel quaternized with 1-bromodecane was designed to induce intraparticle micellization and thereby create a hydrophobic domain inside the nanogel to host hydrophobic drug, e.g., Triclosan [217]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results showed that the synergy of physical destruction of the microbial cell membrane by quaternary ammonium compounds and active nano-injection of Triclosan dramatically enhanced the antimicrobial efficiency 1018 fold as compared to free Triclosan against *S. aureus*.

Notably, bacteria generally have negatively charged cell membranes consisting of lipid layers and peptidoglycan [218]. Therefore, nanogel carriers with a positive surface charge could enhance the interaction between the nanogel and the surface of the bacteria. Paunov and co-workers have conducted a series of experiments on the enhancement of the antimicrobial effect of antibiotics or cationic antimicrobial agents using nanogel carriers with cationic surface functionality [219–222]. In one of these studies, the antibacterial agent berberine was loaded into polyacrylic acid-based nanogels, the surface of which was subsequently coated with a layer of cationic polyelectrolyte, poly (diallyl dimethylammonium chloride) (PDAC) [221]. The PDAC-coated nanogels encapsulated with berberine exhibited enhanced antimicrobial efficacy against both *Escherichia coli* (*E. coli*) and *Chlamydomonas reinhardtii*, compared to the free berberine. In another work, the biocompatible cationic polyelectrolyte polyethyleneimine (PEI) was used to functionalize the polyacrylic acid-based nanogel. The nanogel was loaded with two small cationic antimicrobial molecules, tetracycline hydrochloride and lincomycin hydrochloride, which can potentially overcome antibiotic resistance [220]. The functionalized antibiotic-loaded cationic nanogel carriers enhanced the antimicrobial activity by specific electrostatic adhesion to the microbial cell wall, and due to accumulation, a higher local antibiotic concentration was achieved.

### 3.1.2. Silver-loaded nanogels

Metal nanoparticles exhibit unique chemical, physical, and biological properties that are significantly different from bulk metals or isolated molecules. For example, silver nanoparticles (AgNPs) display antimicrobial and anti-inflammatory properties, thus have been exploited as antimicrobial agents [223,224]. The proposed mechanism of action of AgNPs vary: the destruction of the bacterial cell wall by their favorable surface properties; generation of free radicals that can alter the membrane permeability; inactivation of indispensable enzymes by thiol group modification due to the release of silver ions [225,226]. To achieve the controlled or sustained release of AgNPs locally while limiting the cellular uptake and possible cytotoxicity of AgNPs,

encapsulation of AgNPs inside nanogels, so-called hybrid nanogels or nanocomposites have been developed [227–231]. However, most of the hybrid nanogels were prepared with prefabricated AgNPs, followed by the addition of AgNPs into the polymer network [232,233]. Alternatively, efforts are heading towards studying greener and easier methods for AgNPs containing antimicrobial hybrid nanogels [177,234–237]. Ferrer and coworkers developed the hybrid nanogels (~160 nm) consisting of a lysozyme rich core and a dextran rich shell containing AgNPs (~5 nm), which were synthesized “*in situ*” in the nanogel solution without requiring additional reducing agents [238]. Lysozyme was found to enhance nucleation and stabilization of AgNPs while limiting their growth. Furthermore, with varying lysozyme content, they were able to tune the size of both AgNPs and nanogels and the loading capacity; larger nanogels with greater loading of smaller AgNPs were obtained following increased lysozyme concentration [236]. Similarly, Khan et al. also reported an environmentally greener reducing agent, glucose, to form the AgNPs nanogel “*in situ*”, but with a microwave-assisted heating technique [237]. Also, AgNPs nanogel have been synthesized and stabilized simultaneously “*in situ*” with the help of gamma irradiation, the mean size of AgNPs ranged from 10 to 50 nm [177]. Analogously, Choi et al. developed a one-step process to synthesize nanogels containing AgNPs involving electron beam irradiation of silver nitrate and poly (acrylic acid). The size of the prepared nanogels decreased with increasing irradiation doses, while the antibacterial effects increased due to a larger surface area for bacterial interaction of smaller AgNPs [234].

### 3.1.3. Antimicrobial peptide-loaded nanogels

As an attractive alternative to commercial antibiotics, antimicrobial peptides (AMPs) have gained large interest over the last decades and some found their way into clinical practice [239]. AMPs are natural compounds driven from innate immune systems and can kill bacteria by non-specific membrane disruption, which is less likely to induce bacterial resistance [240]. With their both hydrophobic and hydrophilic moieties, AMPs affect bacteria in several ways: AMPs destabilize the membrane by pore formation, causing the leakage of intracellular components, and the carpet mechanisms where the coverage of AMPs on the outer and inner membrane is uneven, which induces the imbalanced surface tension resulting in cell membrane collapse [241,242]. However, some undesirable characteristics, such as high toxicity, easy degradation, and low bioavailability by binding to serum proteins, mucins, and other anionic components, have impaired the clinical translation of AMPs [240,241]. Therefore, recently increased attention has been placed on nanogels as carriers for the delivery of AMPs to improve the antimicrobial performance [243–250]. The Malmsten group investigated cationic AMPs incorporated into anionic poly (ethyl acrylate-co-methacrylic acid) (MAA) nanogels in different ways that influenced the encapsulation efficiency and release of AMPs, and the membrane interactions and antimicrobial effects of those nanogels. To investigate the mechanisms of membrane interactions of free and nanogel-loaded AMPs, the dimyristoylphosphatidylcholine/dimyristoylphosphatidylglycerol (DMPC/DMPG) (75/25) was chosen as model cellular membrane to obtain solid-supported bilayers with an anionic charge to mimic the bacterial membranes. They found that the peptide incorporation increased with increasing nanogel charge density and peptide amphiphilicity. The nanogel displayed a negative zeta potential even at high peptide loading and therefore did not bind to the bacteria-mimicking membranes. Instead, membrane disruption and antimicrobial effects against *E. coli* relied largely on peptide release, nanogel charge density, primarily influencing the peptide loading and release rate [245]. They also investigated how poly (ethylene glycol) conjugation (PEGylation) of AMPs affected their loading and release behavior from MAA nanogels. They found that the PEG content influenced the nanogel loading and release rather than the PEG conjugation site. PEGylation appeared to shift peptide localization towards the corona instead of in the core of the nanogel [249]. Building on the

studies of AMPs-loaded nanogels synthesized by emulsion polymerization, recently the same group prepared AMPs-loaded nanogels using continuous microfluidic particle generation based on 3D-printed micromixers by complexation of polymyxin B with  $\text{Ca}^{2+}$  cross-linked alginate, as shown in Fig. 5 [248]. As such, Polymyxin B was localized in the particle core and caused particle growth with increasing peptide loading up to > 80%, and the peptide release was strongly accelerated at physiological ionic strength. To offer better stability during the production of AMPs-loaded nanogels, Parilti and coworkers used supercritical carbon dioxide as a green alternative for the conventional solvents to synthesize AMP-loaded poly (2-hydroxyethyl methacrylate) (poly (HEMA)) nanogel via one-pot free-radical dispersion polymerization as a powder [243]. The antibacterial tests revealed the sustained release of the AMP from this poly (HEMA) nanogel that was swollen in water.

### 3.2. Nanogels as active antimicrobial agents

Although antimicrobials-containing nanogels significantly show antibacterial efficacy and potentially slow down the emergence of multidrug-resistant bacteria, the development of new materials with intrinsic antimicrobial activities is still in urgent demand. Metal-based nanoparticles can be directly used but, as mentioned before, usually with high toxicity. Nanogels provide a versatile platform to generate novel intrinsically antimicrobial structures due to their large surface area to mass ratio allowing multivalent interactions with bacteria, their chemical reactivity, and highly compatible surface for facile functionalization, and their high colloidal stability and excellent biocompatibility.

Whereas in the previous section nanogels were applied as carriers for antimicrobials and the susceptibility of microorganisms to these antimicrobials are in most cases expected to be unchanged (or in some cases enhanced), the susceptibility of microorganisms towards nanogels with intrinsic antimicrobial action need to be assessed separately. Antimicrobial susceptibility tests have been designed to evaluate the time or concentration dependent antimicrobial potential of newly synthesized antimicrobial agent by determining the minimum inhibitory concentration (MIC) against microorganisms, indicating the antimicrobial concentration that inhibits visible growth of a planktonic bacterial suspension after incubation [251,252]. Because the MIC can be influenced by factors such as the inoculum preparation method and concentration, type of growth medium and incubation time, these tests have been standardized by the Clinical and Laboratory Standards Institute,

International Standard Organization (ISO) and European committee of antimicrobial susceptibility testing (EUCAST), making clinical evaluation possible, however these guidelines do not guarantee clinical relevance [251].

Recently, the developments of nanogels with intrinsic antimicrobial moieties, such as quaternary ammonium compounds (QACs) [253–256], guanidine [257], AMPs and their synthetic mimics [258], have been subject of investigation but is still underdeveloped as compared to the previously mentioned systems. Positively charged moieties cause physical damage to the bacterial cell membrane and are one of the major mechanisms of antimicrobial activity of functional nanogels. This strong charge interaction leads to the generation of pores and alteration in membrane permeability and, eventually, bacterial cell death [259]. Among the different cationic compounds that have been used in nanogel functionalization, QACs are the most explored ones. Echeverría and coworkers synthesized a tertiary amine group-containing nanogel by copolymerization of NIPAM and dimethylaminoethyl methacrylate (DMAEMA), and further quaternized with methyl iodide and butyl iodide [256]. The MIC results showed that compared with pNIPAM nanogel (MIC > 10 mg/mL) and unquaternized nanogels (MIC = 5 mg/mL), the methyl quaternized nanogels with highest incorporation of DMAEMA up to 25 wt% provide some antimicrobial ability, although the MIC was still high (MIC = 2.5 mg/mL).

In addition to quaternized nanogels, nanogels with cationic guanidine groups on the surface inhibits bacterial growth by attacking them through electrostatic attraction on the bacteria cell surface [260]. Han et al. prepared a guanidine nanogel by copolymerization of styrene, polycaprolactone-hydroxyethyl methacrylate, and polyhexamethylene guanidine hydrochloride methacrylate. The resultant nanogels exhibit a strong ability to kill *S. aureus* and *E. coli* by destroying the cell membrane and causing the cell lysis. Besides, the nanogels had a positive effect on preventing wound infection as a result of the antibacterial activity [257].

As addressed above, AMPs show broad and effective antimicrobial activity against bacteria, viruses, and fungi, but their high manufacturing cost, susceptibility to proteolysis, and poorly understood pharmacokinetics due to the complexity of their native structures hindered their applicability as drugs. Therefore, there has been increasing research interest towards preparing synthetic AMPs with the amino acids, lysine, arginine, and histidine as cationic components and leucine, valine, and phenylalanine as their hydrophobic analogs [261]. Except being delivered by nanogels, a series of polypeptide nanogels (PNGs) have been fabricated by coordination-assisted self-assembly of a mannose-conjugated AMP, poly (arginine-*r*-valine)-mannose, as a ligand and  $\text{Zn}^{2+}$  ion as a metal ion source to minimize the toxicity of the pristine polypeptide without compromising the antimicrobial activity, as shown in Fig. 6 [258]. The PNGs showed the potential bactericidal effect as revealed from their MIC results (2–16  $\mu\text{g}/\text{mL}$ ) against *S. aureus* and *E. coli*, which were significantly lower than the traditionally used antibiotic, vancomycin (>128  $\mu\text{g}/\text{mL}$ ; *E. coli*). Notably, the PNGs exhibited higher cell viability (above 80%) against mammalian cells with a negligible hemolytic effect compared to the pristine polypeptide (viability below 35%). Determination of the MIC in the above studies is necessary to evaluate the susceptibility of microbes to these nanogels with antimicrobial functionality.

### 4. Antimicrobial nanogel surface coatings for biomedical applications

Bacterial attachment and subsequent biofilm formation on implant surfaces is a severe problem from both an economical and healthcare perspective, since bacterial biofilms show a high resistance towards antibiotics and biomaterial associated infections are notoriously persistent and difficult to eradicate [262,263]. In recent decades, considerable focus has been placed on the creation of surface coatings to prevent initial bacterial adhesion and biofilm formation as an

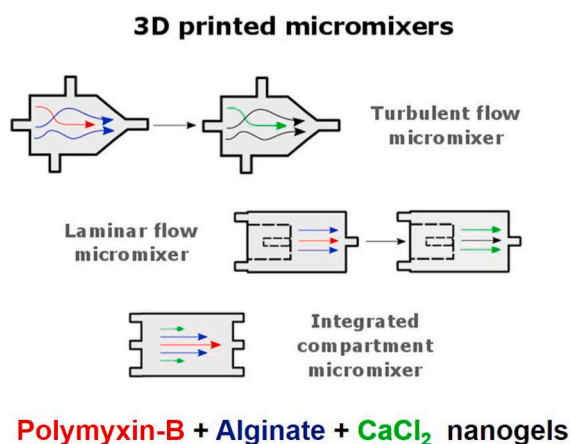


Fig. 5. Preparation of  $\text{Ca}^{2+}$ -cross-linked alginate nanogels loaded with the AMP polymyxin B in a continuous process using 3D-printed micromixers with three different geometric designs: turbulent flow micromixers, laminar flow micromixers, or integrated compartment micromixers. (Reproduced with permission from ref 248. (Copyright 2019 Elsevier)).



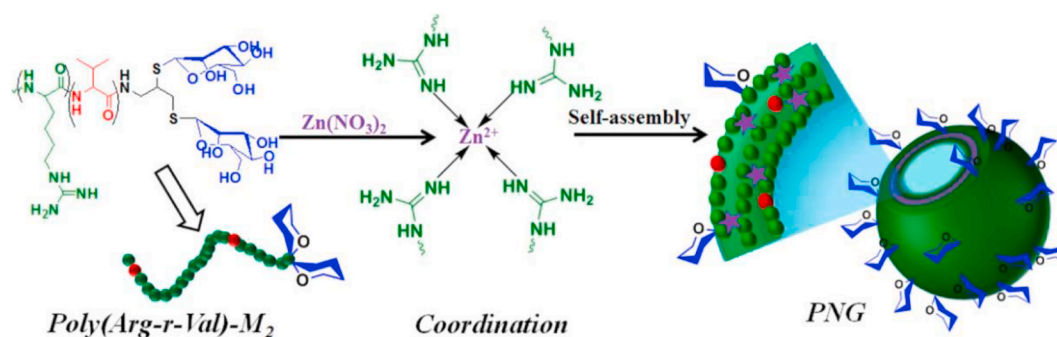


Fig. 6. Schematic presentation of the fabrication of PNG by coordination-assisted self-assembly of a mannose-conjugated antimicrobial polypeptide, poly (arginine-r-valine)-mannose with Zn<sup>2+</sup> ions. (Reproduced with permission from ref 258. (Copyright 2019 American Chemical Society)).

alternative platform since antibiotic resistance is becoming a serious threat. These antimicrobial surface coatings are mainly based on three strategies: an antifouling strategy that can significantly reduce the amount of initial bacterial attachment, an antibacterial (bactericidal) strategy, based on killing bacteria before or after coming in contact with the surface, and a third strategy based on triggered bacterial detachment [264]. Some methods combine strategies to achieve multi-functional surfaces [265].

Based on their physical adsorption capabilities, colloidal particles are known to have excellent ability to form well-organized films regardless of the substrate material [266–269]. Many attempts have been made to perform surface coatings with hard colloidal particles such as polystyrene nanoparticles. However, these coatings were found to be unstable under physiological conditions since the contact point between the particle and surface is limited [270]. From this point of view, nanogels, as being soft deformable colloidal particles, can stick to the surface firmly and create remarkably homogenous coatings [271,272]. Nanogel-based surface coatings can be easily prepared by physical adsorption without requiring any harsh chemical procedures such as grafting or covalent binding [268]. Besides, the characteristics of the nanogel-coated surface can be simply modified by just modulating the chemical functionalities of the adsorbed nanogel particles.

Nanogels have great potential as antifouling and/or antimicrobial coatings on the surface of medical implants. Their advantages, such as high water content, desirable chemical and physical structures, good mechanical properties, and excellent biocompatibility, make them useful candidates as antimicrobial surface coatings [273,274].

#### 4.1. Nanogel-based antifouling coatings

Antifouling surfaces are designed to reduce the initial bacteria attachment by repelling the bacteria and consequently preventing biofilm formation [275]. Apart from zwitterion-based surfaces and polymer brush coatings, these surface coatings are usually created using hydrogels, which can form a hydration layer in an aqueous environment. Hydrogels have been used in the field of biomaterials due to their affinity to water and the formation of a hydration layer, which works as a physical barrier resisting protein adsorption and bacterial adhesion [145,276]. Antifouling hydrogel surfaces can possess strong hydration properties also under physiological conditions such as in serum or salt solutions [277–279]. As mentioned before, nanogels are possessing similar behaviors as hydrogels with a nano-sized feature. Many applications of nanogels inspired by hydrogels, such as antifouling surfaces, have been developed in the biomedical field, based on the excellent tunable-chemical properties and film-forming capabilities of nanogels. Poly (N-isopropylmetacrylamide) (p (NIPMAM)) nanogel coatings have been previously studied to prevent adhesion of cells and proteins on the surface [27,268,280]. More recently, our group focused on these nanogel surfaces as an antifouling coating system that repels bacteria and subsequently prevents biofilm formation and a possible infection

[29]. In this work, internal cross-linking density and the size of the nanogels and thickness of the coating was investigated to find the highest reduction of initial bacterial adhesion. As shown in Fig. 7, all nanogel coatings provided a homogeneous monolayer, with a surface coverage of over 90%. The results presented that the thickest and softest (lowest cross-linking density) nanogel coating exhibited more than 98% reduction in the number of initial bacterial adhesion. These findings clearly suggested that a promising antifouling surface coating was successfully developed.

Similarly, Mergel et al. investigated the effect of electrochemical switching of poly (N-isopropylacrylamide-co-vinylferrocene) p (NIPAM-co-VFc) nanogel coatings prepared with nanogels that have a ferrocene (Fc)-enriched (collapsed/hard) core and a NIPAM-rich shell on the antifouling behavior [281]. The stiffness on the surface was altered by switching the oxidation states of the nanogels with electrochemical stimuli. Quantitative analysis proved that upon oxidation, nanogels coated on the glass surface turn into a significantly softer, highly swollen layer. Furthermore, the bacterial adhesion was examined under flow conditions on coated surfaces on the oxidized and non-oxidized state of the nanogels and also on bare glass. Although the difference in stiffness did not affect the fouling behavior the nanogel coated surfaces showed a remarkable antifouling performance compared to bare glass. These results indicated that the alteration in stiffness of the nanogels is not sufficient to provide a difference in bacteria attachment.

Very recently, addressing the potential use of surface-bound nanogels as coatings for improving the non-fouling properties of the biomaterials, Huang et al. introduced zwitterionic nanogel coated surfaces [282]. Poly (sulfobetaine methacrylate) (PSBMA)/poly (ether sulfone) (PES) nanogels were blended within the PES polymer matrix and further cast on glass surfaces. The biofilm formation of *E. coli* and *S. aureus* on the surfaces both in static and flow conditions was investigated with confocal laser scanning microscopy (CLSM). The CLSM results highlighted that the reduced biomass and biofilm thickness were proving the decreased bacterial attachment on the surface for both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria due to the presence of the hydration layers provided by nanogels. On the other hand, the developed zwitterionic system displayed strong anti-abrasion properties and chemical durability, even in harsh chemical environments.

Addressing the temperature-responsive characteristics of nanogels, Saha and co-workers investigated a synthetic strategy to obtain zwitterionic nanogels that exhibit tunable dual-VPTT [283]. These nanogels were covalently bound on activated SiO<sub>2</sub> quartz sensors, and the anti-fouling activity was tested by quartz crystal microbalance-dissipation (QCM-D) experiments under a flow of protein solution. The nanogel-coated surface showed excellent antifouling characteristics resulting in remarkably reduced protein adsorption. Thus, these dual-stimuli responsive nanogel coated systems can be used as a coating material to prevent protein adsorption, which may suggest reduced bacterial adhesion as well.

Similarly, addressing the potential use of nanogels as coatings for



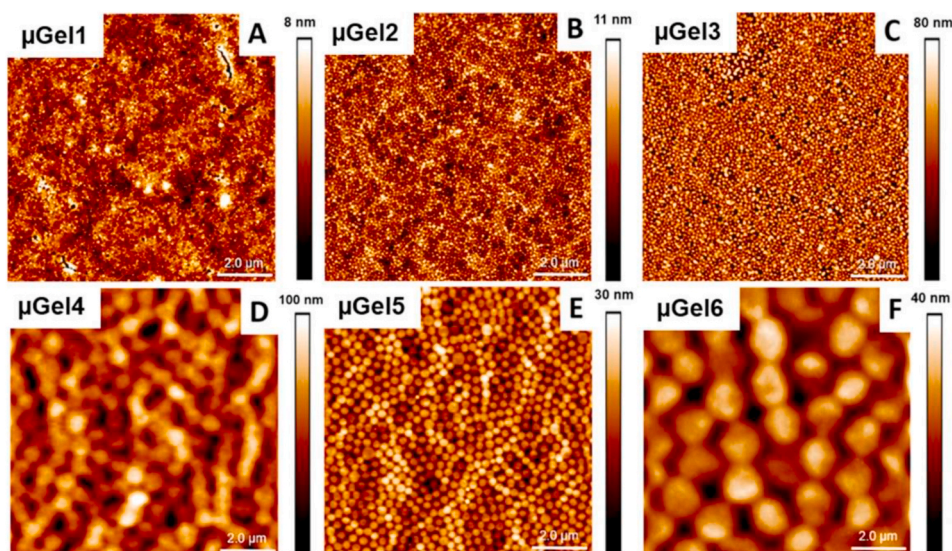


Fig. 7. Atomic force microscopy images of the p (NIPAM) nanogel coated glass surfaces with different internal stiffness/cross-linking density and hydrodynamic radii  $R_h$  at 23 °C in the dry state. (A) nGel1,  $R_h = 114$  nm, (B) nGel2,  $R_h = 109$  nm, (C) nGel3,  $R_h = 101$  nm, (D) nGel4,  $R_h = 787$  nm, (E) nGel5,  $R_h = 301$  nm, (F) nGel6,  $R_h = 650$  nm at 30 °C. (Reproduced with permission from ref 29. (Copyright 2019 American Chemical Society)).

biomaterials, Brosel-Oliu et al. profited from the nanogel antifouling properties for biosensors to measure the bacterial response to antibiotics, avoiding time consuming culturing procedures [284]. To this end, a p (NIPAM) nanogel coating was employed to prevent the attachment of *E. coli* on top of insulating barriers of the biosensor, in order to optimize the sensors sensitivity towards bacterial adhesion on the electrodes of the device. Successful nanogel deposition and immobilization of bacteria was found by confocal microscopy analysis. This approach exhibited the bacterial response to ampicillin when measured by electrochemical impedance spectroscopy, which promotes novel applications in biosensing related to toxicity validations.

In an attempt to further explore the performance of the antifouling behavior of surface-bound nanogels, Liu et al. fabricated pH-responsive membranes with poly (4-vinyl pyridine) (P4VP) nanogels [285]. P4VP nanogels were self-assembled on the membrane surface and in the inner channels, through the phase-inversion procedure of membrane casting solution, as shown in Fig. 8. The nanogels on the surface of the membrane promoted the antifouling properties and self-cleaning performance, which were examined by water flux experiments. Herein the fouling experiments firstly performed by measuring pure water flux before and after the pollution by pure milk filtration. In the inner channels of the membrane, nanogels can respond to pH changes. Upon this environmental trigger, the channels alter their diameter due to the protonation/deprotonation ability of pyridine groups, providing an excellent pH-responsive water gating capability to membranes. Therefore, such nanogels can be potentially used in smart separation applications, in addition to their antifouling properties.

Furthermore, Ji et al. explored the use of heparin-mimicking nanogels to prevent bacteria and protein accumulation on membrane surfaces and also to improve the hemocompatibility of these membranes

[286]. Polyethersulfone (PES) membranes were fabricated by a simple physical blending of heparin-mimicking nanogels, namely poly (acrylic acid-co-N-vinyl-2-pyrrolidone) (P (AA-VP)) and poly (2-acrylamido-2-methylpropanesulfonic acid-co-acrylamide) (P (AMPS-AM)), obtained through conventional free-radical copolymerization. Results revealed that nanogel modified membranes inhibited not only the bacterial adhesion but also the protein adsorption on the surface due to the hydrophilic characteristics provided by the nanogels. In addition to the antifouling behavior of the heparin mimicking nanogel an increase of the adhesion of the L929 cells and an enhanced cytocompatibility was shown on the surface of nanogel-modified membranes, as was also found earlier [287]. Thus, nanogel-blended PES membranes have great potential use for various biomedical applications such as blood purification membranes. Another antifouling and antibacterial membrane approach was investigated by, Li et al. (Fig. 9) [288]. Briefly, electrospun polycarbonate urethane substrate membranes were coated with a nanogel layer by cross-linking of eugenol-modified chitosan and the zwitterionic copolymer poly (sulfobetaine methacrylate-co-2-aminoethyl methacrylate) (PSA). The prepared membranes not only presented a significant bacteria-repelling performance towards *E. coli* and *S. aureus* but also a lower non-specific protein adsorption on the surface. Furthermore, antibacterial results showed that the nanogel-coated membranes could inhibit not only initial bacteria attachment but also kill the adhered bacteria. Moreover, prepared membranes showed no serious cytotoxicity against L929 fibroblasts, with more than 80% of relative cell viability which; thus, it is considered to have a potential use for biomedical implant applications. According to ISO 10993-5 procedure, percentages of cell viability above 80% are considered as non-cytotoxic; within 80%–60% weak; 60%–40% moderate and below 40% strong cytotoxic, respectively [289].

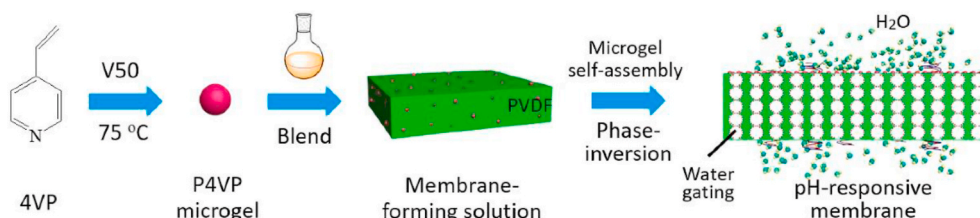
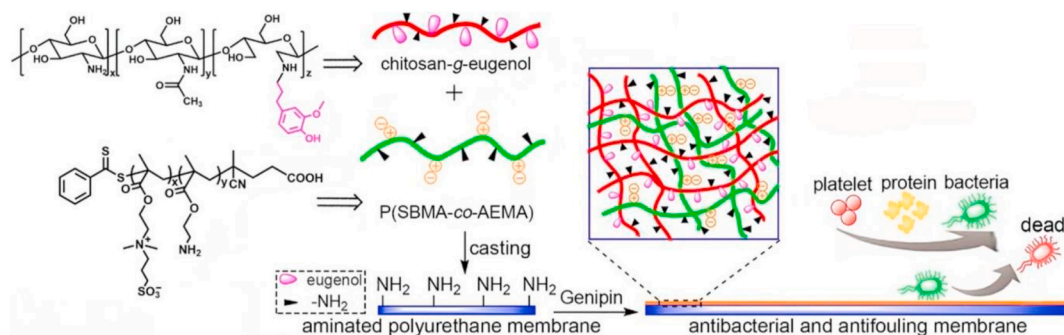


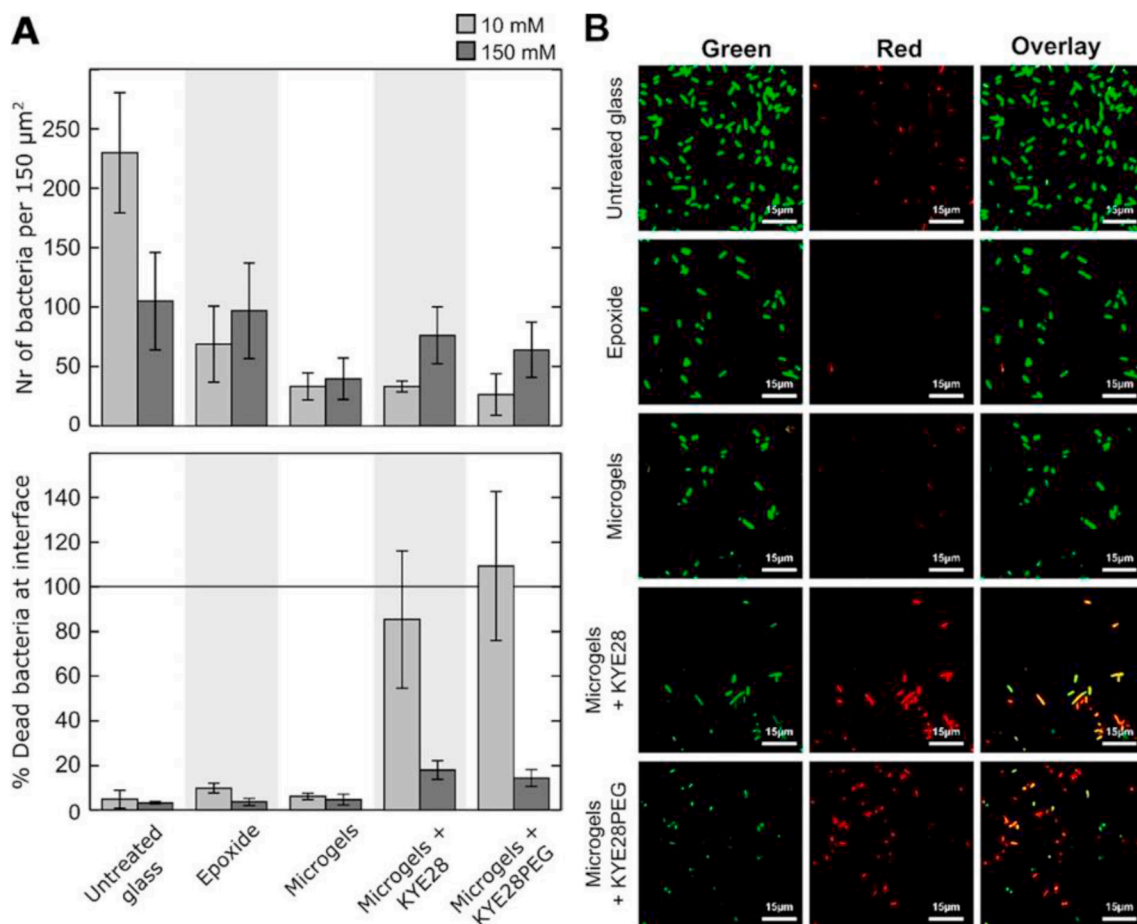
Fig. 8. Fabrication diagram of the pH-responsive membrane prepared with nanogels. (Reproduced with permission from ref 285. (Copyright 2019 Elsevier)).



**Fig. 9.** Integrated antibacterial and antifouling membranes via cross-linking eugenol-modified chitosan and a zwitterionic copolymer on the electrospun polyurethane surface. (Reproduced with permission from ref 288. (Copyright 2018 Elsevier)).

Investigating similar designs of both antifouling and antimicrobial surface coatings, Nyström et al. studied the peptide-loaded poly (ethyl acrylate-co-methacrylic acid) nanogels [290]. Therefore, nanogel films were prepared by covalent immobilization of nanogel on silica substrates with or without the incorporation of peptides. To assess the antifouling properties, nanogel-coated surfaces were immersed in an *E. coli* bacterial suspension under static conditions. The analysis showed that all coatings displayed a strong anti-adhesive effect on *E. coli* bacteria, as shown in Fig. 10. Moreover, peptide-loaded nanogel coatings exhibited efficient bacteria-killing properties at the same time.

Although the primary emphasis has been placed on biomaterials or biomedical devices, nanogel-coated surfaces offer opportunities also in other industries, e.g., for marine equipment to prevent biofouling and its costly consequences. Chen and co-workers developed a self-healing underwater-oil-repellent and biofouling-resistant coating prepared with nanogel spheres modified with hydrophilic polymeric chains, that were then prepared by self-assembly on a glass substrate [291]. The results show that this coating is highly stable under harsh acidic conditions and prevents biofouling meanwhile also displaying a self-healing ability when mechanically damaged. Albeit these coatings are created to



**Fig. 10.** (A) Bacterial adhesion of *E. coli* to surface-modified glass slides after 4 h of incubation in Tris buffer with or without additional 150 mM NaCl (top) and viability of the adhered *E. coli* quantified using BacLight LIVE/DEAD staining (bottom). Data were normalized against *E. coli* killed in 70% isopropanol. (B) Representative CLSM images of adhered *E. coli* on surface-modified glass slides in 10 mM Tris buffer. Images are presented as z-projections of stacks of six images or more, with increased brightness and contrast added after data analyses for improved visualization. (Reproduced with permission from ref 290. (Copyright 2018 American Chemical Society)).

avoid biofouling in marine equipment, it can also be used as a new platform within the biomedical field. Even though, nowadays, most of the coatings for marine equipment are made by environmentally friendly techniques and materials, the biocompatibility and cytotoxicity regulations must be carefully fulfilled when antifouling coatings are used in the body [292].

#### 4.2. Nanogel-based antibacterial coatings

Antibacterial surfaces are identified as surfaces that can kill bacteria. Antibacterial coatings can be categorized into two groups: Contact-killing antibacterial coatings and release-based antibacterial coatings. Contact killing occurs when the bacteria touch the surface and are killed by molecules attached to the surface possessing antimicrobial activity or by the surface pattern itself [293–295]. Release-based antimicrobial coatings are pre-loaded with drugs that are released to kill the bacteria in the near vicinity [296].

As already discussed above, the formation of nanogel-based coatings for antifouling applications has attracted some interest. Apart from these strategies to control the biomaterials associated infections, the research on antibacterial coatings facilitated by nanogels has developed in recent years, as will be described in this section briefly. Nanogels can be combined with conventional antibacterial agents to enhance the killing-mechanism based on a release approach as well as can be functionalized with long tethering molecules to activate the contact killing mechanism.

In our very recent study, we prepared antibacterial surfaces with poly (N-isopropylacrylamide-co-N-[3 (dimethylamino)propyl]methacrylamide) (P(NIPAM-co-DMAPMA)-based nanogels [297]. Through the tertiary amine in the DMAPMA comonomer, nanogels are quaternized which introduced the antibacterial activity due to the bacterial membrane binding and the intercalating ability of the aliphatic QAC. Subsequently, the quaternized nanogels enabled the formation of intraparticle hydrophobic domains allowing for Triclosan incorporation as a very common antimicrobial agent (Fig. 11). The coating with Triclosan-loaded nanogels provided excellent bacteria-killing properties with a killing efficacy of up to 99.99% of adhering bacteria on the surface while still possessing an antifouling activity. This study effectively demonstrated that the first time, the possibility to achieve Triclosan encapsulation into the nanogel for a powerful antibacterial effect.

In one of the recent studies on antibacterial nanogel coated surfaces, Zhao et al. reported the bactericidal activity of coatings formed by QAC-based p (NIPAM) nanogels synthesized with 1-vinyl imidazole and 1,6-dibromohexane [298]. Monolayer nanogel films were prepared on different substrates such as silicon, gold, polystyrene and polydimethylsiloxane. Next, the antibacterial efficiency, due to the QAC moieties in the nanogel coating was examined using *E. Coli* MG1655 bacteria; the bacteria viability was investigated by fluorescent staining and also by colony counting method on agar plates. The nanogel-coated

surfaces exhibited almost 100% efficacy in bacteria-killing. This bactericidal effect of this QAC-based p (NIPAM) nanogel coating was established by showing that after inoculating  $10^4$  *E. Coli* MG1655 bacteria in 1 mL of buffer and exposure of the suspension to the coated surface resulted in only one viable colony on the utilized agar plate. Moreover, they proved that these robust coatings could be performed on many different substrates with a desired cytocompatibility.

In a similar line, another research is introduced by Xue et al. also using QAC-based p (NIPAM) nanogels [299]. Herein, firstly the thin films (quaternized p (NIPAM) nanogel, QPM film) were prepared with quaternized nanogels on silicon wafers by drip coating. Then a glycopolymer (P(SS-co-MAG)), which is containing both sulfonate groups and sugar units, was attached via a chemical modification to the QPM films. *E. coli* bacteria were subsequently used as a model microbe to examine the bactericidal properties of nanogel films. Both nanogel films QPM and QPM modified with P(SS-co-MAG) showed excellent antibacterial activity compared to the bare silicon wafer surface. The bactericidal efficacy of the nanogel coating was solely based on a Live/Dead staining assay after a 2 h incubation in  $10^7$ /mL *E. coli* culture without a corresponding viable cell count to show whether the level of bacterial killing answers the 99% killing required by ISO20743 and JIS Z 2801. However, no significant differences were observed between P(SS-co-MAG) modified and QPM surface; thus, it can be concluded that the killing efficiency was not affected by modification with P(SS-co-MAG). Notably, after the P(SS-co-MAG) modification, the films showed an improved cytocompatibility.

According to ISO20743 and the Japanese industry standard JIS Z 2801, dealing with coated materials, the efficacy of antimicrobial agents is determined by the difference in the logarithmic value of viable cell counts after incubating an inoculum with the antimicrobial compound and its corresponding inert control. This difference is only microbiologically relevant when the reduction in viable cell count is more than two log values or more than 99% when linearly expressed [300]. Furthermore, the initial inoculum concentration must correspond to the microbial challenge associated with the intended application of the antimicrobial agent since microbial density can influence the MIC [300, 301].

Furthermore, Chattopadhyay et al. explored the use of physically cross-linked nanogels (PCNGs) to form protective coatings on mica and highly oriented pyrolytic graphite surfaces [302]. The nanogels used in this study were synthesized in water as a solvent with C-10 alkyl chains modified poly (ethylene imine) (PEI) and azetidinium groups, thus yielding amphiphilic characteristics. PCNGs were possessing a high colloidal stability due to the hydrophobic interactions of long alkyl chains and the ionic repulsion of the azetidinium groups, thus spontaneously self-assembled. Consequently, prepared PCNGs demonstrated excellent antimicrobial activity against a varied range of bacteria. As shown in this work, the nanogels are stable in water up to 120 days.

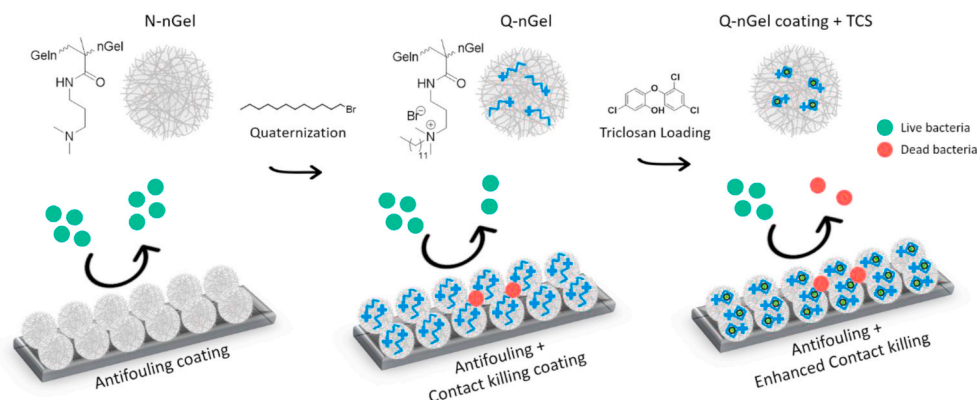


Fig. 11. Schematic representation of antifouling and antimicrobial nanogel coatings (Reproduced with permission from ref 297. (Copyright 2019 Elsevier)).



Considering the stability results of these nanogels, their coatings can be a potential candidate for water-based biomedical applications. To this end, an optimized and successful coating method needs to be performed and the stability tests of the coatings needs to be completed.

Sproul et al. prepared thin nanogel films by using platelet-like particles (PLP), which were synthesized with NIPAM-co-acrylic acid with antimicrobial gold through non-covalent and covalent methods to develop nanogold composites (NGCs) [303]. Antimicrobial assay results suggested that all fabricated thin films by NGC PLPs possessed an effective activity for inhibiting bacterial attachment and growth. Here, viable cell counts were performed at time 0, 3, 6, 12, and 24 h using a testing solution which was made by a 1:10,000 dilution of  $10^5$ /mL CFU\* 0.2mL/sample. Meanwhile, the antimicrobial effects of these particles on inhibiting bacterial growth in suspension were not significant due to the available gold particle surface area which facilitates the bacteria killing; therefore, in the future, applications of NCG PLP's should be applied as a coating on the surface to prevent infections.

Addressing mechanisms confirming antimicrobial effects of nanogel-bound antimicrobial peptides (AMPs), Nyström et al. examined the incorporation of AMPs into a nanogel by electrostatic interactions [290]. Bactericidal properties by contact-killing and release of incorporated peptides of the peptide-loaded nanogels were investigated. The antimicrobial efficiency was dependent on the release rate of the bactericidal peptides from the nanogel network, and it was speculated that the ionic strength in the solution controlled their release. At low ionic strength, peptides remained attached to the nanogels by electrostatic interactions; therefore, the killing mechanism is mostly due to contact killing and partly by the release of the peptides. At high ionic strength, however, the antimicrobial effect was mainly based on peptide release. Such nanogel coatings containing peptides exhibited enhanced antimicrobial and anti-inflammatory properties to control the inflammatory response to biomaterials.

In a similar line of research Nyström et al. investigated the formation of peptide-loaded poly (ethyl acrylate-co-methacrylic acid) (MAA) nanogel multilayers [304]. These nanogels were synthesized with biotin, and the nanogel multilayers were formed by alternating biotin containing nanogels and avidin with layer-by-layer deposition method since avidin can strongly bind to biotin. Next, the loading and release of the antimicrobial peptide into the formed nanogel multilayers were examined at physiological conditions with pH 7.4 and ionic strength 150 mM. At this high ionic strength, the peptide release was observed to be slow because the peptide diffusion through the multilayer from the outside was kinetically hindered. As a result the antimicrobial effects on *E. coli* bacteria of peptide-loaded multilayers were decreased when compared to a peptide-loaded nanogel monolayer. Furthermore, it was proven that the increased loading of the antimicrobial peptide resulted in an improved antimicrobial activity on the surface, both for planktonic and

adhered bacteria. In conclusion, cross-linked nanogel multilayers show great potential as a useful antimicrobial surface coating. However, more attention needs to be given to peptide release phenomena.

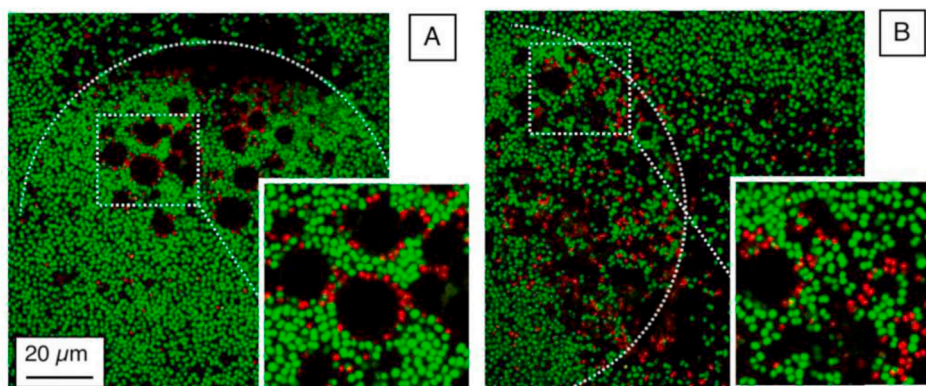
Additionally, Liang et al. examined the coated surfaces prepared by poly (acrylic acid)-based anionic nanogels loaded with the antimicrobial (lipo)peptides [305]. They showed that the contact with macrophages and osteoblasts was not able to trigger antimicrobial release; it was only possible with bacteria. Very interestingly, the bacterial contact was found to trigger the release of these AMPs, consequently resulting in local bacterial killing as shown in Fig. 12. Here, a microprobe experiments is performed in order to understand the contact transfer between bacteria and nanogels loaded with antimicrobial peptides, as L5 and Sub5. The bacteria in contact with the peptide loaded nanogels were killed as can be seen in Fig. 12. The loaded antimicrobials continued being stably complexed within the nanogels for extended periods, even for weeks. Thus, this coating can be assessed as a promising antimicrobial triggered bacteria-release system.

Some examples of nanogels and their applications as coatings can be found in Table 2.

## 5. Conclusions and outlook

Biomedical applications of nanogels have made quick progress in the last years. These developments have allowed an expansion in the field of drug delivery and antimicrobial approaches, by controlling characteristics of nanogels such as size, stability, surface functionality for biodegradability. A current challenge is the development of strategies for the nanogel surface coatings to have both antifouling and antimicrobial features, both *in vitro* and *in vivo*. To address this matter, combining different approaches as exemplified by e.g. the Triclosan loading of the quaternized nanogels earlier presented in this article, is an important example of possible concepts that can give rise to advances in creating effective nanoparticles to prevent infections, both in suspension and on biomedical implant surfaces.

Taken together, nanogels have demonstrated to be not only versatile drug delivery systems but also, when bound onto the substrate, an effective antimicrobial coating system to combat infections on the biomaterial surface is created. One future goal should be the usage and development of these nanogels as multifunctional particles with several additional attributes incorporated to be used for a broad spectrum of biomedical applications with multiple functions, robust biocompatible features, and programmed responses that can be controlled by stimuli and triggers under physiological conditions. Such integrated nanogel-based systems will provide superior material characteristics, especially in the field of biomedical research, to combat biomaterial-associated infections as well as general pathogenic intrusions within the body. Extensive biocompatibility and biotolerability testing of nanogels is a



**Fig. 12.** Contact transfer kills *S. epidermidis* on AMP loaded microgel-modified surfaces. Confocal images showing live (green) and dead (red) *S. epidermidis* after 30 min of contact with microgels loaded with: (A) Antimicrobial peptide L5 (in 0.07 M ionic strength buffer); and (B) Antimicrobial peptide Sub5 (in 0.28 M ionic strength buffer). The dashed lines outline the outer diameter of the probe tip. (Reproduced with permission from ref 305. (Copyright 2019 Elsevier)).



**Table 2**  
Nanogels and their coating applications.

Coating Applications	Nanogels	Ref.
Reduced protein adhesion	p (NIPAM) nanogels cross-linked with PEG diacrylate	[27]
Prevented bacteria attachment on the surface for biomaterials	p (NIPMAM) nanogel coatings	[29]
Allowed cell adhesion and spreading	p (NIPAM) nanogels	[268]
Antifouling surface towards bacteria on the surface	p (NIPAM-co-VFc) nanogels	[281]
Decreased bacterial attachment and biofilm thickness for biomaterials	PSBMA/PES nanogels	[282]
Reduced protein adsorption SiO <sub>2</sub> quartz sensors	(PVCL-co-PGMA-g-PSB) zwitterionic nanogels	[283]
Antifouling properties for biosensors	p (NIPMAM) nanogels	[284]
Antifouling properties and self-cleaning performance for the membrane	(P4VP) nanogels	[285]
Inhibited the bacterial adhesion and the protein adsorption on the membrane	P (AA-VP) and (P (AMPS-AM)) nanogels	[286, 287]
Antifouling and antibacterial membrane surface	poly (sulfobetaine methacrylate-co-2-aminoethyl methacrylate) nanogels by cross-linking of eugenol-modified chitosan	[288]
Antifouling and antimicrobial surface coatings	Peptide-loaded poly (ethyl acrylate-co-methacrylic acid) nanogels	[290]
Prevent biofouling for marine equipment	NIPAM, methacrylic acid (MAA), and poly (ethylene glycol) diacrylate (PEGDA) based nanogel spheres	[291]
Antibacterial and antifouling coating	Quaternized and Triclosan incorporated p (NIPAM-co-DMAPMA)-based nanogels	[297]
Antibacterial surface on silicon, gold, polystyrene and PDMS	QAC-based p (NIPAM) nanogels	[298]
Antibacterial surface on silicon wafers	p (NIPAM) nanogels modified with P (SS-co-MAG)	[299]
Antibacterial surface on mica and graphite surface	Nanogels with C-10 alkyl chains, modified PEI and azetidinium groups	[302]
Antibacterial thin films	p (NIPAM-co-acrylic acid) nanogels modified with gold	[303]
Antimicrobial and anti-inflammatory properties	Nanogel-bound antimicrobial peptides (AMPs)	[290]
Antimicrobial multi and monolayers	Antimicrobial peptide-loaded poly (ethyl acrylate-co-methacrylic acid) (MAA) nanogels	[304]
Antimicrobial triggered bacteria-release system	Poly (acrylic acid)-based anionic nanogels loaded with the antimicrobial (lipo)peptides	[305]

prerequisite for clinical trials and application. To help reach this goal, ISO-standard toxicity tests are designed to guide researchers in acquiring knowledge that will facilitate a better understanding of the interactions between living tissues and nanogels.

The clinical impact that these nanogel-based systems could have reaches far beyond the antimicrobial applications as depicted in this review. General drug delivery approaches would certainly benefit from these nanogels and would be an excellent addition to the liposomal and polymersomal approaches. Therefore, it is expected that nanogels will also impact diseases such as cancer but also due to the diverse range of possible modifications including metallic structures, metal-ions, small molecular conjugates, peptides, antibodies and other biomacromolecules will provide possibilities for applications in imaging, theranostics, biosensing, and gene-delivery. Although hydrogels themselves are already known and used in clinical applications for long times, nanogels require special attentions with respect to their size as they belong to the class of nanotechnology, which is under particular scrutiny in all fields as it is regarded as difficult to predict long-term outcomes.

## Declaration of competing interest

The authors declare the following conflict of interests: P. van Rijn also is co-founder, scientific advisor, and shareholder of BiomACS BV, a biomedical-oriented screening company.

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