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LICENTIATE THESIS

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New strategies for multifunctional antibacterial materials

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CHALMERS UNIVERSITY OF TECHNOLOGY
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THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

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Cover picture
Caption: **Harmony in science flow**
Illustrator: **Hengzhi Ruan**

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Abstract

Healthcare-associated infections (HAI) is responsible for significant financial and human costs in healthcare systems. Therefore, a substantial amount of research has been devoted to developing biopolymer-based strategies that prevent bacterial attachment and biofilm formation on surfaces. Gelatin hydrogels have been used in the last decades for different biomedical applications due to the excellent biocompatibility, easy processability, bioactivities to mimic the extracellular matrix (ECM). However, their poor mechanical properties and thermal stability limited their potential applications. Herein, a facile and economical approach of introducing dopamine and [2-(methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) via in situ synthesis into gelatin hydrogels with the existence of $ZnSO_4$ was applied to overcome these disadvantages. This fabrication method allows the obtaining of gelatin-based hydrogels with fatigue resistance and mechanical stability from -100 to 80 ℃. Moreover, the hydrogels showed adhesive, self-healing, electrical and excellent antibacterial properties leading to their potential use as wearable monitoring sensors and antibacterial coatings. In particular, the hydrogels showed adhesion to various types of surfaces such as paper, skin, wood, plastic, rubber and steel, as well as 99.99% and 100% of antibacterial efficiency against Gram-positive and Gram-negative bacteria respectively. The results indicate widespread applications of the new hydrogels in many biomedical areas.

Keywords: antibacterial coatings, hydrogel, multifunctional materials.

Content

Introduction

Presumably the vast majority of people think so, humans, as the most creative species, dominate the earth. But this is worthy of careful thinking. Some scientists[1] claim that microorganisms are the "masters" of life on our planet, and the survival and evolution of human beings are only the history of using intelligence to compete and symbiosis with microorganisms including bacteria, viruses, fungi and other tiny creatures that are difficult to observe with the naked eye. Indeed, we humans only have 1% of the total number of microorganisms and microorganisms quietly distributed everywhere on earth play a leading role in the cycle of matter. Furthermore, only about 43% of the human body is made up of human cells, and about 57% is made up of microorganisms. One microorganism called bacteria appeared about 3.7 billion years ago on the planet while human have been on the earth only for 7 million years. No one is an island, and it is actually a complex ecosystem (adaptive system in physics), a huge society where trillions of bacteria live. The human body cannot survive alone without bacteria. Meanwhile, in some ways, human activities also make contributions to the accelerated bacterial evolution, for example, some studies have found that due to the overuse of antibiotics, many so-called "super bacteria" are constantly appearing. The super bacteria are not a specific type of bacteria, but bacteria that are resistant to multiple antibiotics, which means that common antibiotics cannot kill them. These bacteria are also called multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Streptococcus pneumoniae*, *vancomycin enterococcus*, etc. Because such bacteria are insensitive to a variety of antibiotics, they are extremely harmful to the human body. Moreover, according to a report released by the United Nations Environment Program[2], climate change, pollution as well as the increased areas of cities and urbans may increase the drug-resistance of bacteria to antibiotics and produce more "super bacteria". The history reminders us not only to be satisfied with the obvious daily needs, but also to be prepared for danger in times of peace and pay special attention to those "invisible things" that may affect our normal body and life order—in a sense, those things that are invisible and intangible may be more fundamental or important.

However, the biggest misconception about bacteria – something that occurs most often once bacteria are merely mentioned - is that they are easily regarded as bad / harmful. There are however helpful bacteria as well as harmful bacteria. Harmful bacteria are pathogenic bacteria that can make people sick, and some even contribute to mortality, such as *Mycobacterium tuberculosis*, *Bacillus leprae*, etc. There are however also many helpful bacteria, called probiotics. Probiotics are a class of active microorganisms that are beneficial to the host. They are a general term for active beneficial microorganisms that colonize the human intestinal tract and reproductive system and can produce definite health effects, thereby improving the host's micro-ecological balance[3]. For example, *Bifidobacterial* and *Lactobacilli* that live in our intestines not only help in digestion, but also protect our bodies from pathogens and synthesize essential vitamins and nutrients. A species called *Clostridium sporogenes* only grows in an oxygen-deficient environment, such as the center of a tumor. When cancer drugs

are injected into tumors, the bacteria help the drugs hit the tumor cells directly without affecting healthy tissue. Probiotics can even change the neurochemical structure of the brain, helping to treat anxiety or depression-related neurological disorders. In addition, some bacteria also play a major role in the environment. The rod-shaped bacteria *Alcanivorax* increase greatly during oil spills at sea because the oil provides them with a lot of food. Therefore, this type of bacteria can be used to clean up marine oil pollution. *Shewanella* living in deep-sea can generate oxygen-seeking nanowires in a low-oxygen environment. When this appendage is connected to a platinum electrode, it can transmit electric current as well as consume toxic waste. If used in sewage treatment plants, *Shewanella* bacteria can not only treat sewage issues, but also provide electricity to human.

In terms of harmful bacteria, in addition to "super-bacteria," they cause healthcare-associated infections (HAI), among many other negative effects. HAI are infections that are contracted while receiving or providing healthcare, impacting both patients and medical staff. Hospitals, long-term care facilities for the elderly, outpatient settings, and even after discharge, are all places where HAI can occur, particularly for patients who rely on medical devices like implants, catheters, hernia mesh, wound dressings, and vascular grafts. This makes HAI one of the most frequent adverse events in healthcare that endangers patient safety. It is also a significant contributor to rising morbidity, mortality, and financial costs for individuals, families, and healthcare systems around the world. Therefore, a number of antibacterial strategies, methods, and materials have been investigated and developed by extensive research, including this thesis, to deal with the problems brought by harmful bacteria, see also Paper 1.

This licentiate thesis is divided into two parts that correspond to two of the research tracks of strategic importance for our work. In the first research track, we focus on a common class of biopolymers called polysaccharides as a viable platform for developing novel antibacterial coatings. Here, an overview of strategies and fabrication methods for designing polysaccharide-based antibacterial surfaces/coatings is provided, summarizing a comprehensive review authored on the topic, see Paper 1. In the second research track a simple approach to synthesizing biopolymer-based gelatin hybrid hydrogels centered around their remarkable antibacterial properties and potential to be used as coatings. Combining with that strong adhesion, improved mechanical properties, self-healing ability, thermal stability, antifreeze properties, electrical conductivity and fatigue-resistance, the newly developed hydrogel has significant potential for biomedical applications.

With this work, we hope to make contribution to a better understanding of the development of antibacterial materials and we are setting up the building blocks for future technology developments where we will further develop novel antibacterial coatings with tailored multifunctional properties for various applications.

The thesis is structured as follows. Chapters 2 and 3 summarize part of the review Paper 1, while emphasizing specific strategies and methods utilized in Paper 2. Chapters 4-5 focus on the materials and methodology for developing the novel hydrogel in Paper 2, with Chapter 6 summarizing the main results in the respective paper.

Strategies for designing antibacterial surfaces

Driven by reproductive fitness, bacteria have evolved their biofilms to withstand physical forces and to aid their survival in environments within animal or human hosts under various assaults[4]. This includes for example the washing effect of saliva and shear forces produced by blood flow. Their biofilms comprise extracellular polymeric secretions, which are primarily composed of proteins, nucleic acids, and exopolysaccharides (EPS)[5]. Therefore, the primary cause of HAI is bacterial adhesion to the surface of biomedical devices, biomaterials and nearby tissues[6]. Bacterial adhesion is a complex process that typically involves two stages[7], see Fig. 1: (i) a typically non-specific interaction whereby bacterial cells and the surface of the material interact quickly and irreversibly and (ii) adhesion proteins that mediate the interaction with molecules on the material surface are excreted by bacteria. While (i) can be easily destroyed by phagocytosis, a cellular process for ingesting and getting rid of microorganisms, foreign objects, etc. Due to the presence of adhesins on the microbial cell surfaces in (ii), this step is irreversible (or only slowly reversible)[8]. Thereafter, biofilm formation aims to improve the interaction between bacteria and the material surface after the adhesion process.

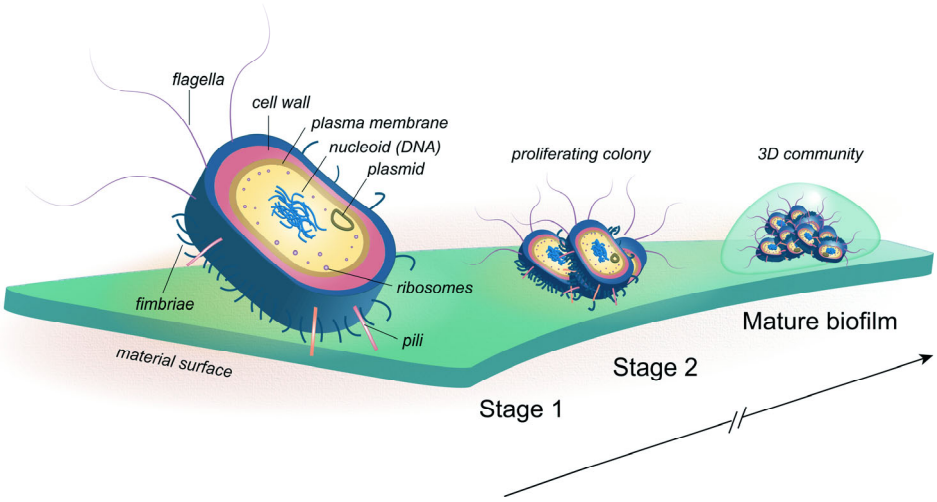

Fig. 1. Illustration of the three stages of bacterial adhesion. In Stage 1, the single free-floating bacterium attaches to the material surface and attempts to anchor itself by adhesion structures such as pili and fimbriae; in Stage 2, during the surface colonization the bacteria proliferate into a colony; in the final stage the colony can produce a biofilm to protect growing bacteria.

Accordingly, the main strategy for creating antibacterial surfaces is to either kill any attached bacteria or prevent bacteria from adhering to the surface of the material. As a result, the following three methods for designing antibacterial coatings have been suggested: release of an antibacterial agent, contact killing, and bacteria repelling[9]. Antibacterial agents that can be released from surfaces can effectively kill both adherent and planktonic bacteria. Contact-killing occurs when bacteria come into contact with an antibacterial surface. And finally, surfaces that repel bacteria can prevent bacterial attachment. For a more comprehensive

overview, the Paper 1 attached to the thesis is available for reference.

Two of these approaches were combined in our research: the novel coating has the ability to both prevent bacteria from adhering and effectively kill bacteria by releasing antibacterial agents.

Fabrication of antibacterial coatings

Antibacterial components must be compatible with scalable, adaptable, and reasonably priced manufacturing processes in order to be converted into commercially viable materials. The key to their applicability will be antibacterial formulations that can be applied as coatings on the surface of a wide range of substrate materials in any shape while also being mechanically and environmentally stable. In general, there are typically six techniques used to fabricate antibacterial coatings (Fig. 2.): dip coating, spin coating, spray coating, 3D printing, electrospinning and layer-by-layer assembly.

The easiest and most widely used industrial technique for creating coatings is dip coating[10, 11]. There are four steps to the dip-coating process. The substrate is submerged into a tank that contains a precursor solution in the first step known as dipping. The substrate is then immersed in the solution after dipping for a predetermined amount of time. The time for which the substrate is held inside the precursor solution is called dwell time, which is the second stage of the process. Once the coating material has completely covered the substrate, it is left in the solution. The third step involves withholding the substrate that has been coated, at a constant speed without judder.

The dip-coating method has the advantage of being simple to install and operate with minimal maintenance, making it cost-effective for large-scale production[12, 13]. The technique is also simple to be used in lab settings, therefore many researchers have used the method to create and test antibacterial coatings. Additionally, the antibacterial properties of the dip coating process have been applied to a number of important areas, including textiles, medical devices, packing materials, implants, membranes, paper coating, and wound healing[14-18].

For a more comprehensive overview, the Paper 1 attached to the thesis is available for reference.

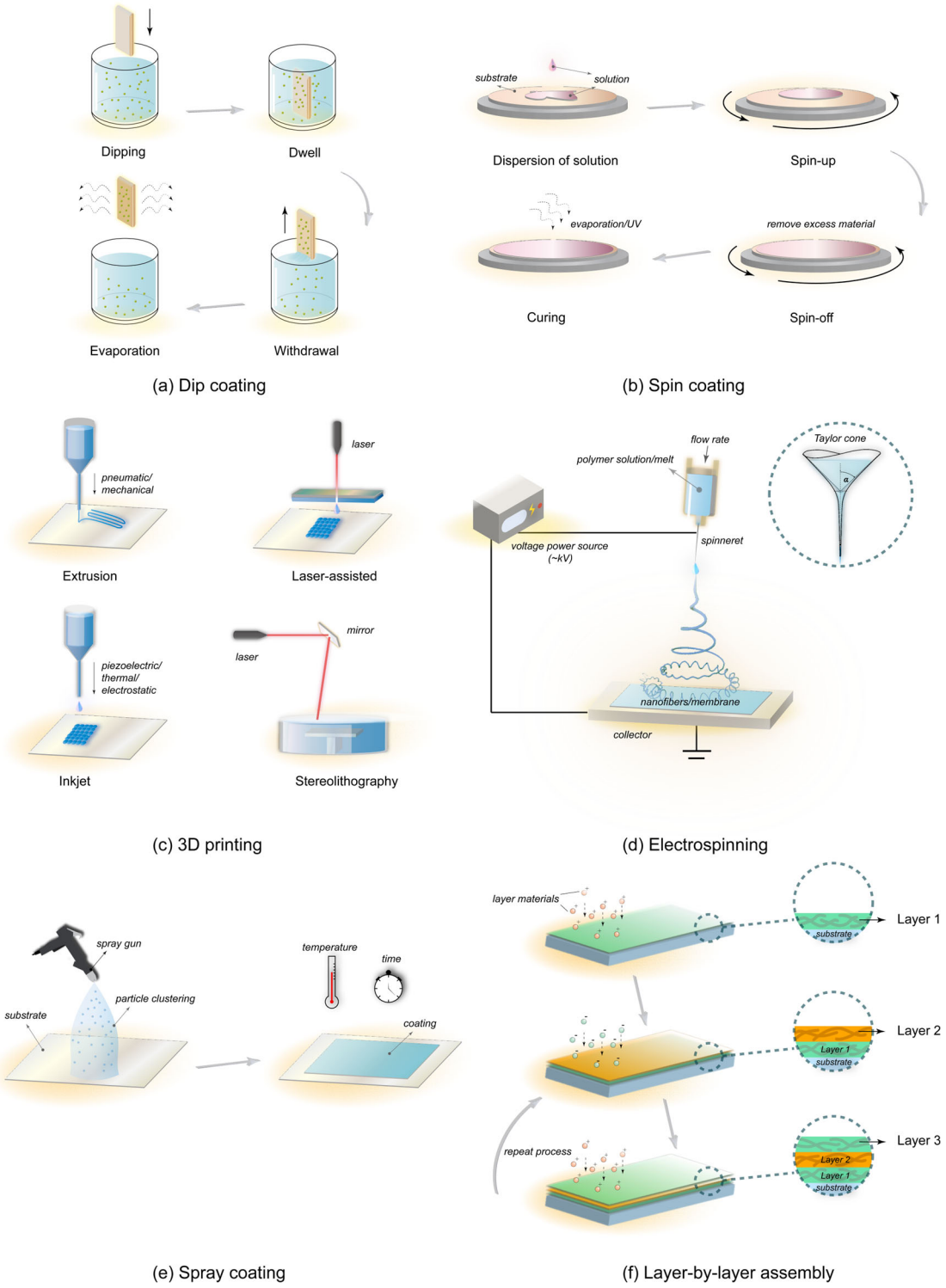

Fig. 2. Schematic overview showing the main methods to fabricate antibacterial coatings (a) dip coating, (b) spin coating, (c) 3D printing, (d) electrospinning, (e) spray coating and (f) layer-by-layer assembly, see Paper 1.

Materials

A special category of materials, hydrogels contain three-dimensional networks that are hydrophilic and self-supporting[19]. Physical and/or chemical crosslinks give the network its structural integrity and produce its various properties. The structure of hydrogels, meanwhile, is comparable to those of natural extracellular matrix, allowing molecules and cells to diffuse through them and adhere to them[20]. In biomedical applications such as drug delivery[21], wound dressing[22], tissue engineering[23], customized sensors[24] and medical device coatings[25], hydrogels have consequently attracted a great deal of attention from research point of view.

Gelatin, a collagen degradation product that exhibits many excellent biological activities including biodegradability, biocompatibility, and non-immunogenicity[26], is one of the frequently used materials to fabricate hydrogels for biomedical applications. Below the sol-gel transition temperature, gelatin can physically crosslink to form a hydrogel. In addition, its rheological properties can be used to modify the viscosity of solutions[27]. These benefits have led to the widespread use of gelatin-based hydrogels in the production of contact lenses, tissue engineering scaffolds, and drug delivery matrices[28]. However, it is still difficult to create gelatin-based hydrogels with the multifunctional properties needed for real-world applications, such as tunable mechanical performance, electrical conductivity, adhesion, stability over a wide temperature range, cytocompatibility, self-healing, antimicrobial properties, etc. Additionally, gelatin's molecular weight distribution and amino acid composition, which can vary significantly depending on processing circumstances, play a significant role in its properties such as gel strength and thermostability[29]. Therefore, improving the multifunctional properties and expanding their functionality are both current topic of interest.

Modern materials and new technologies often take their cues from nature, in the form of biomimetic and bioinspired materials and structures. A typical example is the use of mussel chemistry to create remarkable surface adhesion to various materials[30]. Messersmith *et al.* discovered that the mussel foot proteins containing 3,4-dihydroxyphenylalanine (DOPA) exhibit strong adhesion in seawater[31]. The catechol groups on the side chains of DOPA are thought to be the primary cause of the non-covalent interactions and chemical crosslinking that occur with various substrate surfaces[32]. Other residues in mussel foot proteins, such as charged and hydrophobic moieties, also enhance wet adhesion by regulating hydrophobic or electrostatic interactions[33]. DOPA and its derivatives have been used as fundamental building blocks to create new hydrogels as a result of this property[34]. Additionally, zwitterionic monomers are a different class of building blocks that have both positive and negative charges. They can firmly bind with water molecules through electrostatic interaction, increasing the amount of saturated water and enhancing the electrical conductivity of hydrogels[35]. Specifically, it has been demonstrated that one of zwitterionic monomers, [2-(methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA), prevents the adhesion of bacteria and proteins by producing a dense hydration layer[36, 37]. This

significantly broadens the applications of hydrogels in biomedicine.

In Paper 2, gelatin (gel strength 300, Type A), [2-(methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) and dopamine hydrochloride, zinc sulfate heptahydrate (all purchased from Sigma-Aldrich) were used to prepare the novel hydrogels.

Experimental methods

In Paper 2, we present a simple approach to synthesize a versatile gelatin-based hybrid hydrogel with conductivity, adhesive properties, fatigue-resistance, self-healing ability, antibacterial activities, and mechanical stability from -100 ℃ to 80 ℃. The latter, not only overcomes they challenges associated to gelatin-based hydrogels at physiological temperatures, but could also expand its application in different biomedical applications. In addition to the compositional novelty, several characterization methods such as rheo-tack and rheo-dielectric spectroscopy were for the first time applied to investigate hydrogels. Overall, the combined significant enhancements in multifunctional properties show that the novel hydrogels have a high potential for a number of biomedical applications, such as wearable biosensor to detect the motion of human body and antibacterial coatings.

5.1. Methods to prepare hydrogel materials

By adding short oligomer chains of SBMA, dopamine, and zinc sulphate to hydrogels made of physically crosslinked gelatin long chains, a multi-functional hydrogel material was created. To create GSD/GSDZ hydrogels, dopamine monomers were added to the gelatin solution, where they served as a crosslinking mediator and polymerization initiator[38]. Fig. 8. shows how SBMA-dopamine oligomers can interact with the gelatin network by forming hydrogen bonds as well as ionic bonds with other oligomers and metal ions like zinc.

Table 1: Samples and their composition.

Sample	$ZnSO_4$ in DI water (wt%)	Solutes concentration, wt/v (%)			
		$ZnSO_4$ heptahydrate	Gelatin	Dopamine	SBMA
Gelatin		0.00	17.65	0	0
GZ10		11.76	17.65	0	0
GSD		0.00	25	16.67	25
GSDZ5	5	5.33	25	16.67	25
GSDZ10	10	11.00	25	16.67	25
GSDZ20	20	25.00	25	16.67	25

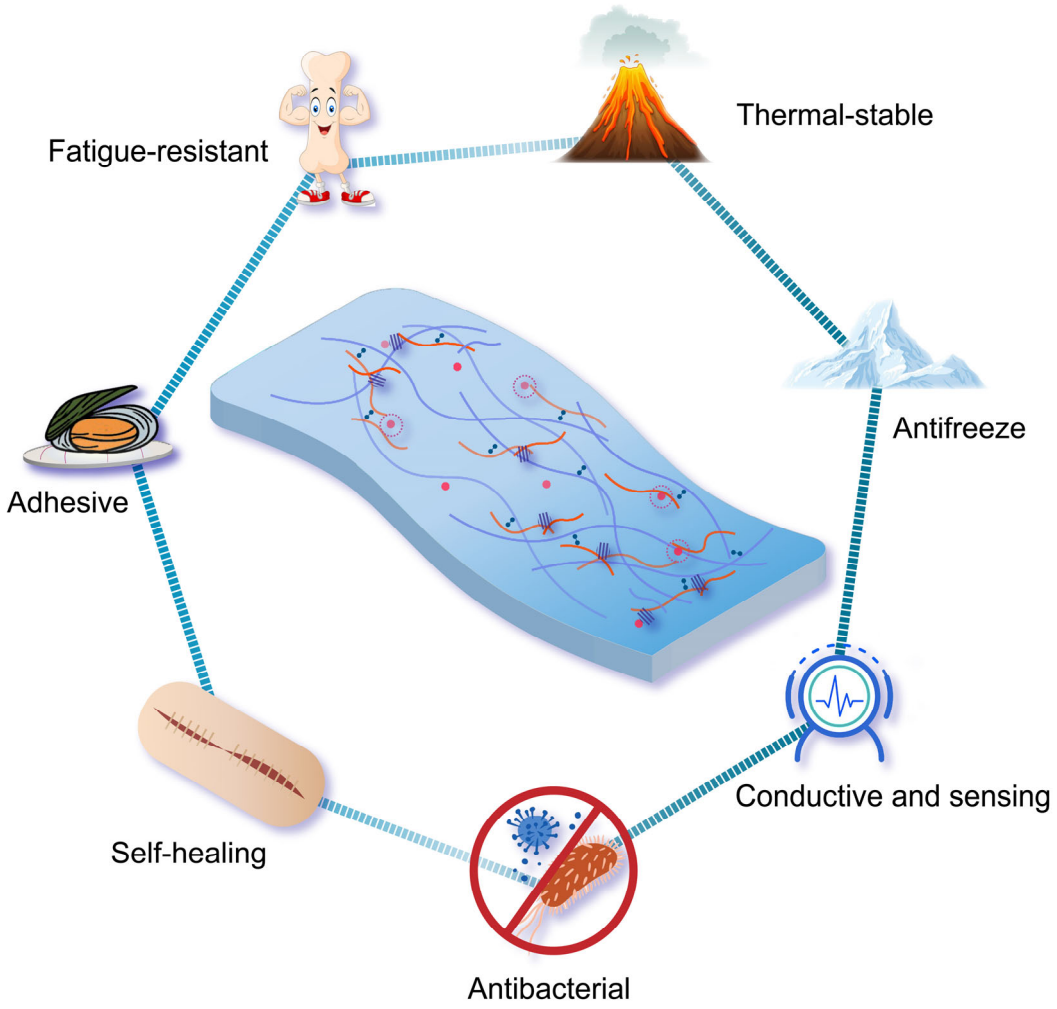

Fig. 3. Schematic diagram of GSDZ hydrogels with multiple performances and applications.

5.2. Methods to investigate the multifunctional properties of hydrogel materials

Polysaccharides or proteins found in nature can be converted into hydrogels, which retain water or liquid in their network structures without dissolving. As research has advanced, more and more findings have demonstrated the importance of mechanical characteristics in biomedical applications for hydrogels, particularly 3D scaffold for cell growth. Today, rheology is the primary technique for evaluating a hydrogel's mechanical characteristics and enables researchers to investigate the viscoelasticity of soft materials. Rheology, on the other hand, can be combined with many different additional analytical methods, making it a potent tool for examining the multifunctional properties of various types of materials.

5.2.1. Rheo-tack

The adhesive properties of hydrogels were initially investigated using a novel Rheo-tack testing method recently developed (Fig. 4.), which allowed for the adjustment of retraction speed, compression force, and sample geometries using standard rotational rheometers to observe the adhesive phenomenon[39].

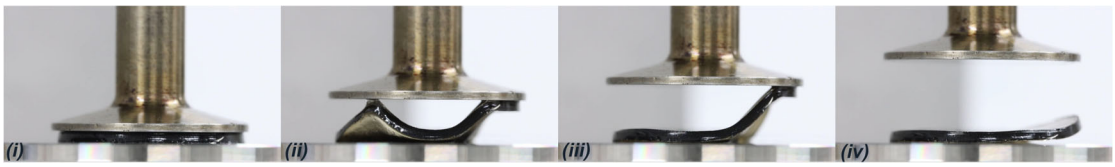

Fig. 4. Photos showing the process of GSDZ hydrogels stretching and detaching during rheo-tack measurements.

5.2.2. Rheo-dielectric spectroscopy setup

A rheo-dielectric setup, which have previously been successfully used to investigate the structure and dynamics of polymeric systems[40], were adopted as a measurement platform to quantitatively analyze the self-healing process as well as the electrical properties of hydrogel materials under compression loads.

5.2.3. Dynamic Mechanical Thermal Analysis (DMTA)

DMTA is a non-destructive method to assess the viscoelastic properties of soft materials and is thus a preferred method to assess their thermal stability in a broad range of temperatures as well determine their critical phase transition temperatures. During DMTA temperature sweep tests, a small-amplitude sinusoidal strain at frequencies ≤1 Hz is applied to the material with the stress response being used to determine the storage (in-phase component) and loss moduli (out-of-phase component), while the temperature is gradually increased or decreased with a pre-defined ramp rate. Thus, DMTA was chosen to investigate the antifreeze properties and thermal stabilities of hydrogel materials. In the meantime, two different sets of measuring geometries were employed in order to accommodate the samples' unique viscoelastic material response. To prevent compliance errors at low temperatures for GSD and GSDZ samples, solid rectangular fixtures (SRF) were used. T counter-act sample contraction, a normal force of -0.5 N was applied. Plate-plate geometries with a normal force of 2 N were chosen for gelatin to prevent sample slips from freezing. We briefly note that sign on the applied forces is in accordance with the convention used by the instrument manufacturer for various measurement fixtures/geometries.

5.3. Methods to evaluate the antibacterial properties of hydrogel materials

We need to first take into account the various approaches for assessing antibacterial properties and what they entail when trying to compare evaluate the antibacterial performance of the coatings before considering any factors influencing the performance of antibacterial coatings. Antibacterial testing currently lacks a clear standard, which makes it challenging to compare the results of various studies and reproduce results. This is further detailed in Paper 1.

5.3.1. Counting colony forming units

The best method for precisely counting viable bacterial cells among various antibacterial

assessments, such as disk diffusion and optical density (OD), is to count colony forming units (CFU). This technique gives the total number of viable bacteria in the sample, making it simpler to quantitatively compare the antimicrobial effectiveness of various tested agents to their control counterparts[41, 42]. Studies demonstrating the antimicrobial effect against suspensions of bacteria, as well as time kill assays based on CFU methodology, could be used to better understand the antimicrobial potential[43, 44].

5.3.2. Scanning electron microscopy

Additionally, scanning electron microscopy (SEM) can be used to show how bacterial cells or biofilm matrix change in morphology after coming into contact with antibacterial coatings, offering mechanistic insights. Bacterial cells can be grown on surfaces for the desired amount of time in order to assess bacterial adhesion and growth on the surface and assess the potential of test agent coated surfaces to prevent the formation of biofilm.

Summary of results

6.1. Mechanical and fatigue-resistant properties

Traditional hydrogels applications are largely restricted by their weak internal structure and poor stretchability. The obtained GSDZ hydrogels, however, were significantly stretchable and had improved mechanical properties. To examine the ductility and toughness of the GSDZ hydrogels, tensile tests were carried out, Fig. 5(a). Through a variety of dynamic interactions, including hydrogen bonding, hydrophobic effects, and electrostatic interactions, the dopamine and SBMA oligomer enabled effective energy dissipation. However, when the zinc sulfate solution concentration was increased to 10%, there was only a very slight reduction in the strain-at-break and stress-at-break compared to GSD hydrogels. According to Fig. 5(b), SBMA oligomers and dopamine could increase the interactions between chains and the flexibility of chains in comparison to gelatin hydrogel samples due to its low Young's modulus and high strain-at-break of GSD and GSDZ hydrogels.

Along with poor mechanical properties, hydrogel materials also have typically poor performance under repeated deformations, i.e., fatigue resistance. Therefore, the stress-strain hysteresis was measured using cyclic stretching in order to examine the fatigue behaviour. Using GSDZ5 hydrogels as an example, Fig. 5(c) displays 10 consecutive cyclic loading-unloading tensile curves with no rest time in-between. The cyclic stress-stretch curves approached a steady state after the first loading cycle, and the hysteresis loops from the second to the tenth loading were essentially very similar. As a result, the orientation of polymer chains began to follow the direction of stretching, which could be the reason for the extra energy loss, given that minimal relaxation could have occurred between the load cycles. This steady state could be the result of the hydrogel networks' dynamic bonds, like hydrogen and ionic bonds, continuously breaking and reforming to form structural morphologies that are optimized to withstand the type of applied load. In this sense, the hydrogels can be considered as being dynamically adaptive.

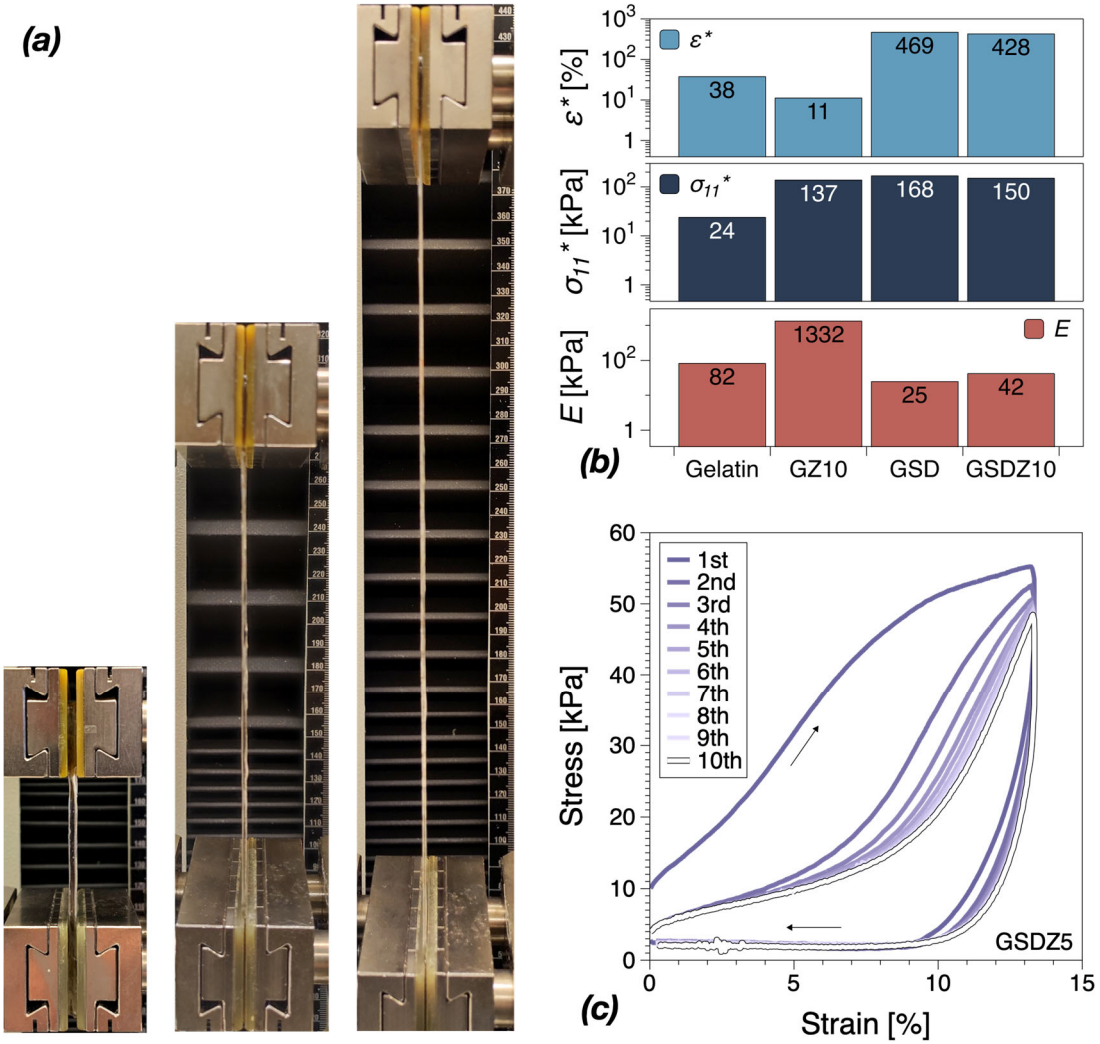

Fig. 5. (a) Images of GSDZ10 hydrogel samples during tensile tests. (b) Summary of ultimate strain, stress and calculated Young's modulus. (c) Consecutive loading–unloading cycles of GSDZ5 hydrogels.

6.2. Adhesion properties

The obtained GSDZ hydrogel could be attached to a variety of surfaces, including paper, skin, wood, plastic, rubber, and steel, thanks to the various supramolecular interactions, Fig. 6(a). Additionally, it was proposed that after absorbing a significant amount of water molecules through ionic solvation, SBMA segments may form a dense hydration layer for protection[45]. As a result, SBMA was developed and put into use along with other elements to extend the useful life of GSDZ hydrogels in common settings. The GSDZ10 hydrogels could still maintain high adhesion force after being contaminated by various drinks, as shown in Fig. 6(b), which suggested promising antifouling properties. Increasing the zinc sulphate content also increased the maximum adhesion force and adhesion energy, Fig. 6(c). When the concentration of $ZnSO_4$ was increased from 5% to 10% at a retraction speed of 0.1 mm/s, the adhesion energy increased from 4.07 mJ to 26.27 mJ and the maximal adhesion force increased from 17.26 N to 31.54 N.

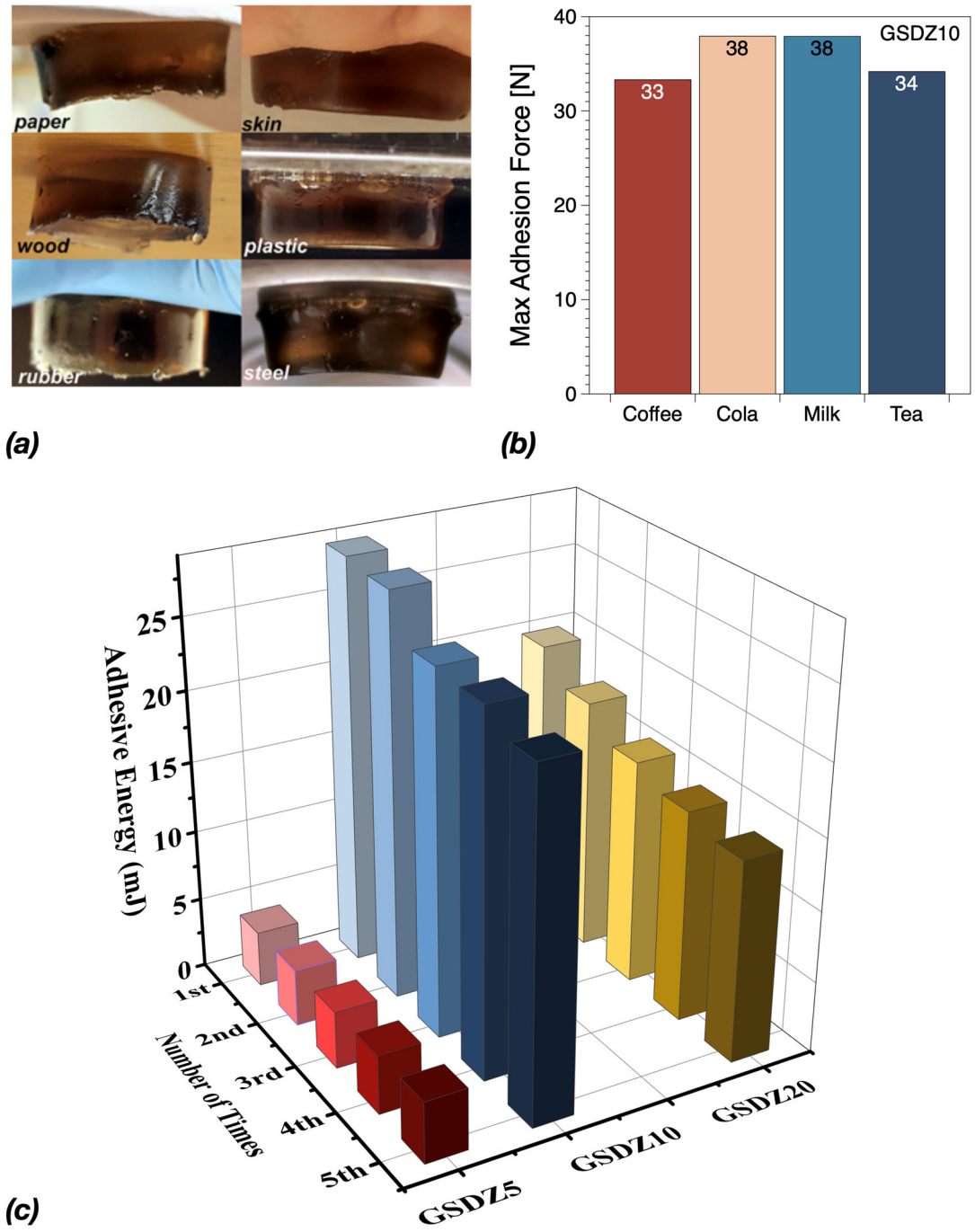

Fig. 6. (a) Photos of GSDZ hydrogels attached to different types of surfaces. (b) Maximum adhesion force of GSDZ10 hydrogels after being dipped into several types of drinks. (c) Evaluation of calculated adhesive energies of GSDZ5, GSD10 and GSDZ20 during cyclical adhesion tests.

6.3. Self-healing properties

The hydrogel network's dynamical physical crosslinking, which is reversible, gave the obtained hydrogel samples excellent self-healing capabilities. All hydrogels were able to repair themselves after the initial injury (cutting) at room temperature without the need for outside stimulation, while heat could further accelerate this process, as shown by microscopy images

of GSDZ5 in Fig. 7(a). The sample's wound had been sealed after three hours of healing, and it was further repaired under heat treatment at 60 °C. The repaired specimen could already be stretched at this point without breaking at the cut. Additionally, after heating and cooling, the chopped hydrogel samples could be reshaped and repaired with the addition of a small amount of water, Fig. 7(b).

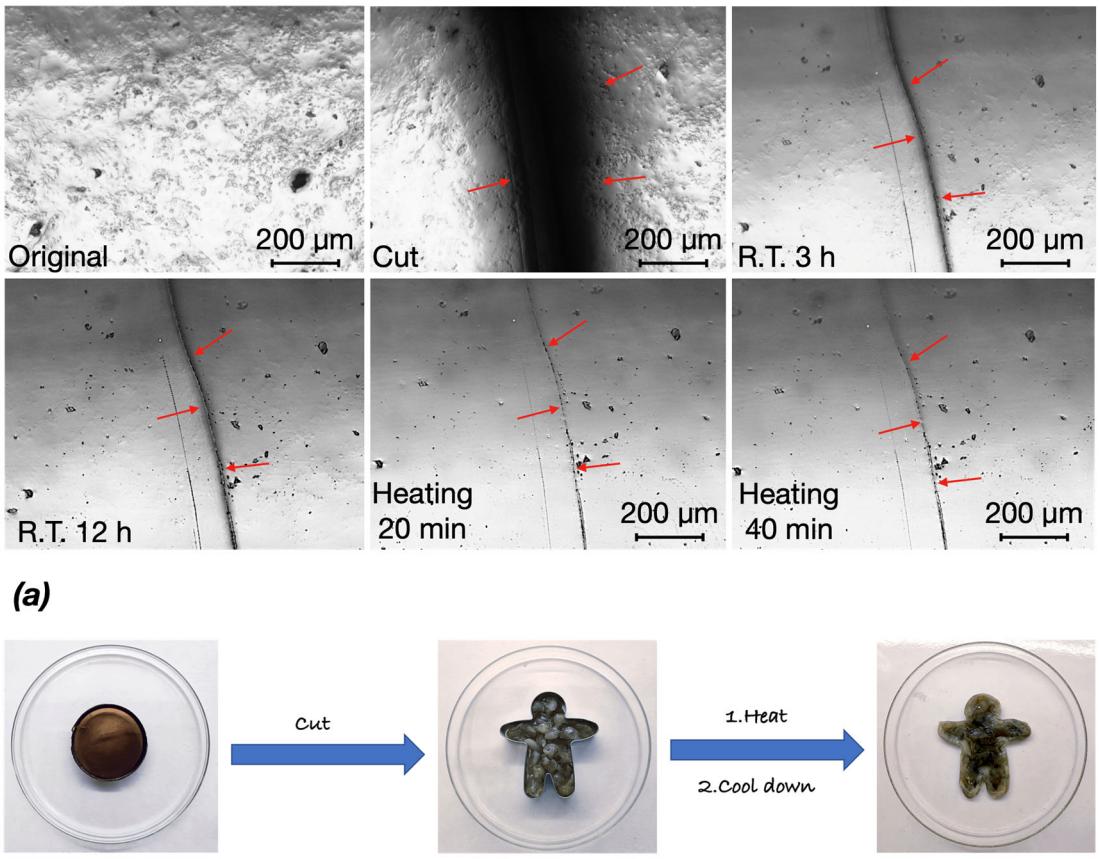

Fig. 7. (a) Optical microscopy images showing the process of self-healing on GSDZ5 hydrogel samples. (b) Photos showing that the GSDZ hydrogels can be remolded. R.T. stands for room temperature.

6.4. Electrical and sensing properties

Fig. 8(a) displays dielectric spectra as expressed by the real part of the complex conductivity obtained throughout the self-healing procedure. Because zinc sulfate contributes to the ionic conductivity, aided by the additional water content from wet cutting, there is a significant increase in the conductivity after cutting. This is accompanied by a distinct intermediate frequency-independent plateau after cutting evidencing the addition ionic contribution to the conductivity. Since zinc sulfate is hygroscopic, the rate at which the sample dries during extended testing may have a significant impact on the dynamics of dielectric processes during self-healing. GSDZ20 hydrogels could be used as flexible wearable devices with good electrical conductivity to achieve real-time monitoring of organ function in humans, as well as individualized diagnosis and treatment. According to Fig. 8(b), the GSDZ20 hydrogel could be applied to human fingers and used as a soft sensor to record finger motions. As the sample

was bent, it became more resistant, and the peaks appeared when the finger was bent 90°.

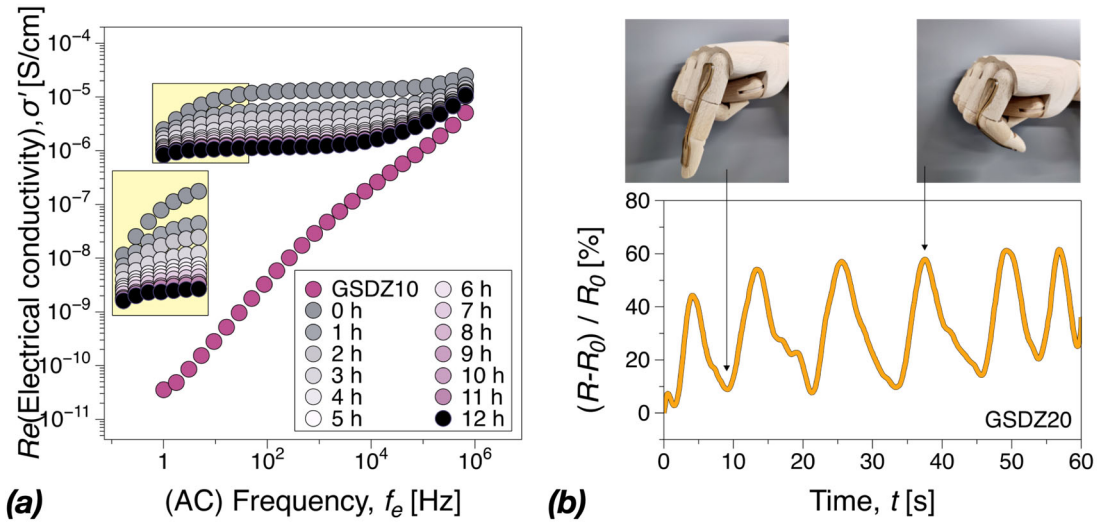

Fig. 8. (a) Self-healing characterized through dielectric spectroscopy on GSDZ10; (b) The resistance variation of the GSDZ20 wearable sensor as a function of time when fingers moved.

6.5. Antifreeze properties and thermal stability

With decreasing temperature, starting from room temperature, the dynamic shear moduli of pristine gelatin sample began to sharply increase around -7 °C, as seen in Fig. 9, indicating the sample was freezing. Meanwhile, GSD hydrogels that can withstand freezing went through a transition to a glassy state as the temperature was gradually decreased, see the inset glass transition temperatures, T_g, determined as the peak in storage modulus, G''. The transition in itself occurs over a much broader temperature range, as highlighted as example in the coloured areas spanning the peak in G''. At low temperatures, GSDZ5, GSDZ10, and GSDZ20 hydrogels exhibited a similar tendency to GSD. With increasing temperature, starting from room temperature, around 37 °C, a reverse transition from the gelatin gel to the gelatin solution occurred[46], causing the G' and G' of the gelatin samples to drop sharply. In contrast, The G' value for the GSD and GSDZ hydrogels was higher than the G'' value throughout the entire measurement process, indicating the integrity of the polymer network and improved thermal stabilities, which may have resulted from an increase in supramolecular interactions in the hydrogels, such as the coordination bonds within the GSDZ hydrogel network[47].

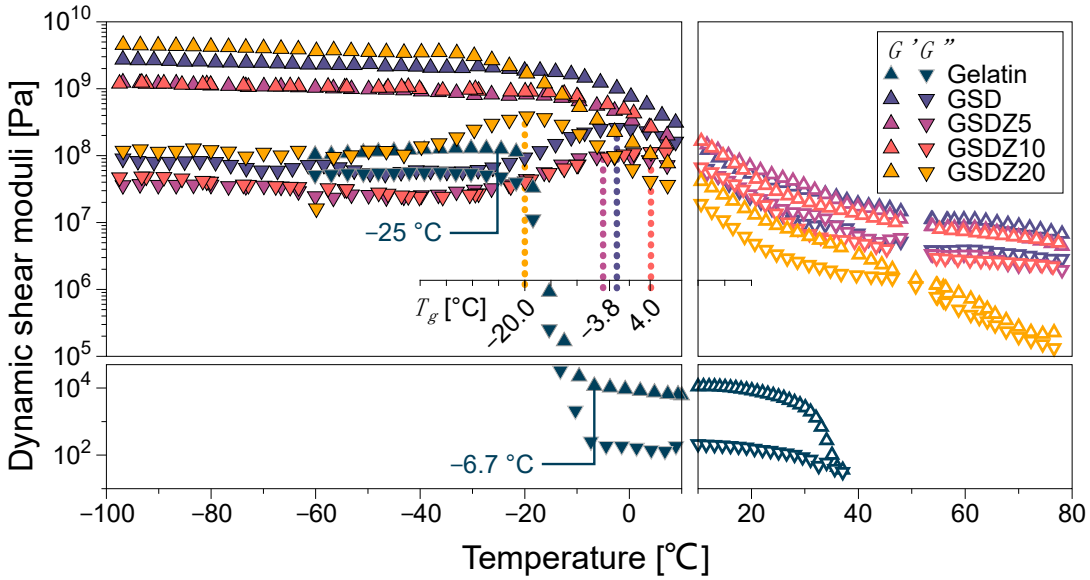

Fig. 9. Shear Dynamic moduli, G', G'', in the (-100,80) ℃ temperature range.

6.7. Antibacterial properties

Two different bacterial strains *Escherichia coli* UTI89 (*E. coli*; Gram negative) and *Staphylococcus aureus* CCUG10778 (*S. aureus*; Gram positive) were used to examine the antimicrobial potential of produced hydrogels. *E. coli* was grown in lysogeny broth (LB broth) and *S. aureus* was grown using tryptic soy broth (TSB broth).

Non-coated and hydrogel-coated discs were subjected to bacterial culture for 24 hours at 37 °C to evaluate the antibacterial effectiveness. Colony forming units (CFU) counting and SEM imaging were used to measure the biofilm-preventive activity. The bare steel disc contained 1.43 × 10^8 CFU/mL and 1.67 × 10^8 CFU/mL of *S. aureus* and *E. coli*, respectively, as shown in Fig. 10(a). While the steel discs without the hydrogel coating had 5.83 × 10^3 CFU/mL and 8.83 × 10^3 CFU/mL, respectively, of *S. aureus* and *E. coli*, showing the hydrogel coatings' potent antibacterial properties. It is evident that as zinc sulphate concentration was increased, the number of bacterial cells that are viable declined. Surprisingly, when the zinc sulphate solution concentration was increased to 10% or more, no *E. coli* colonies were detected. With increasing concentrations of zinc sulphate, the bactericidal activity against *S. aureus* was also enhanced. The antibacterial effectiveness was calculated as a relative percentage of reduction to the control in order to further quantify viability reduction, i.e.:

$$E = \frac{N_0 - N_i}{N_0} \qquad (1)$$

where E is the antibacterial efficiency, N_0 is the numbers of CFU determined for control sample, and N_i is the numbers of CFU determined for stainless steel coated by GSDZ hydrogel.

All GSDZ hydrogel coatings, as shown in Fig. 10(b), demonstrated over 99.99% antibacterial efficiencies against Gram-positive and Gram-negative bacteria, with GSDZ10 and GSDZ20 demonstrating 100% antibacterial efficiencies against *E. coli*. This amounts to outstanding

antibacterial performance.

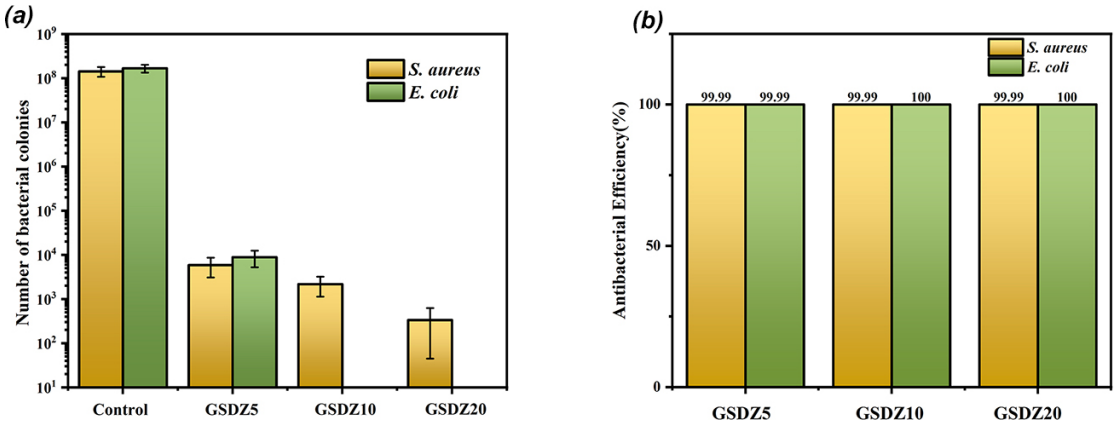

Fig. 10. Comparison of antibacterial activities of control samples, GSDZ5, GSDZ10 and GSDZ20 hydrogel coatings against *S. aureus* and *E. coli* (a) and corresponding antibacterial efficiency (b).

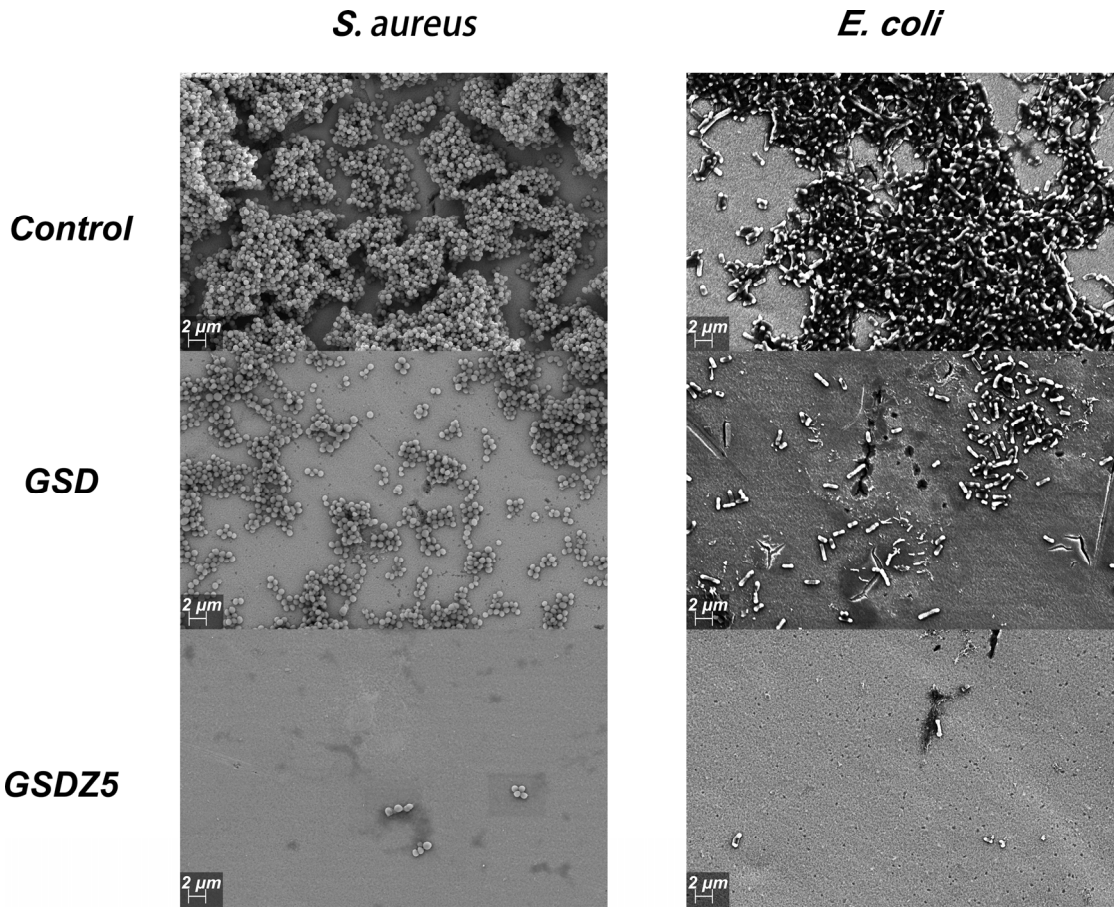

Fig. 11. SEM images of *E. coli* (a) and *S. aureus* (b) grown on the control sample, GSD and GSDZ5 hydrogel coatings.

The SEM images (Fig. 11) demonstrate that the stainless steel lacked antibacterial qualities because, as predicted, bacteria had spread all over the surface. As a result of improved hydrophilicity and the addition of the zwitterionic SBMA[48], the number of bacteria on the

steel disc with GSD hydrogel coatings decreased dramatically, indicating excellent antibiofouling properties against *S. aureus* and *E. coli*. On samples coated with GSD hydrogels, there were fewer microcolonies and fewer bacterial cells per microcolony. The fact that the remaining bacteria still maintained their structural integrity and had smooth membranes suggests that the GSD hydrogel coating may not be bactericidal but rather works to prevent bacterial adhesion to surfaces. When zinc sulphate was added, it was observed that GSDZ hydrogel coatings rendered the bacterial cells inactive by severely harming their membranes. The SEM image showed dead bacteria with leaked cytosol and ruptured bacterial cell membranes, indicating that the GSDZ5 hydrogel coating effectively killed both Gram-positive and Gram-negative bacteria in addition to repelling their adhesion.

Conclusion and future work

Through the in-situ synthesis of oligomers of dopamine and [2-(methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA), we created a versatile gelatin-based hydrogel with outstanding antibacterial properties, strong adhesion, excellent fatigue resistance, thermal stability, antifreeze properties, electrical conductivity, and self-healing. The properties of the hydrogel materials could be further tuned for additional biomedical applications, such as wearable sensors monitoring organ functions and antibacterial coatings on biomedical devices, by varying the content of zinc sulphate. We emphasize that based colony forming units (CFU) counting and SEM imaging, 99.99% and 100% antibacterial efficiency against *S. aureus* and *E. coli* respectively, which is a significant achievement.

Future work on the design of new antibacterial coatings will be continued using at least two strategies: *(a)* hydrogel coatings and *(b)* hierarchical surface patterns.

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Time flies, and I find myself in the middle of my PhD study. In the beginning, my group and I were debating which of chemistry, physics, and biology could have the greatest influence on the advancement of science. Yet if you ask me right now, I'd say they're all significant. I could always stay in my comfort zone and fail to discover new possibilities without the help of my three supervisors and teammates. My co-supervisor, in particular, gave me a book about microorganisms, which introduced me to the world of biology. Also, I went to Roland's class the day before my first teaching assignment since I was unsure of how I should instruct master's and bachelor's level students. I tried to imitate Roland. However, Roland urged me to be authentic and to be myself. I was taken aback since I was used to hearing from my family and friends that I had to act in a specific way because it was the norm. I later understood that it's crucial for me to voice my opinions because, frequently, my opinions differ from others' perceptions and may generate misunderstandings. This appears to be a double-edged sword because it both makes my research more creative and enriches in a complicated way my personal life. So, I really appreciate that my supervisors, teammates, group members and friends continue to support me. You are the treasure that I have found in Europe.

The most important thing I have learned during my PhD so far is that everything has its own meaning to exist and has its own reason to last. Nature and culture are prosperous for the diversities, and I think the world could be better if we allowed greater diversity and inclusiveness in human civilization.

6th March 2023, Rome, Italy,
Hengzhi