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Antibacterial Properties of Soft Solids (Chitosan and Polyacrylic Acid Gel Particles) in Solution and on a Bio-Surface (VITRO-SKIN)†

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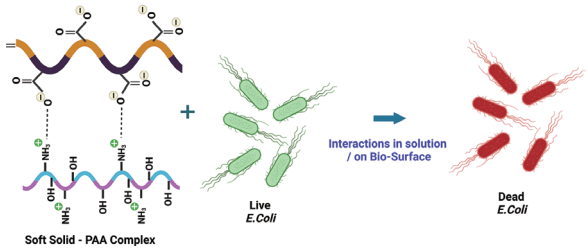
Antimicrobial resistance presents a critical challenge to public health, driving the exploration of innovative strategies against microbial threats. Soft solids, notably polyelectrolyte gel complexes, offer promising antimicrobial alternatives with tailored physiochemical properties and biocompatibility. Primarily, soft solids incorporating chitosan and polyacrylic acid (PAA) complexes have gained importance for their antimicrobial efficacy, stemming from electrostatic interactions between oppositely charged components. This paper evaluates non-covalent interactions within chitosan and polyacrylic acid complexes to reduce *Escherichia coli* (*E. coli*) contaminants. Chitosan, derived from chitin, is valued for its biodegradability and low toxicity, and is currently used in drug delivery and wound healing systems. Conversely, PAA is an anionic polymer with carboxylic groups, widely used in pH-sensitive hydrogel-based drug delivery systems. In the present study, the antimicrobial effectiveness of chitosan and polyacrylic acid complexes was examined both in solution and on the bio-surface. Distinct patterns of antimicrobial activity were observed at the surface when applied individually and in combination. A synergistic antimicrobial effect of the chitosan and polyacrylic acid complex (gel particles), resulted in a remarkable reduction in viable cells both in solution and on the surface. This understanding enhances the potential use of soft solids in addressing the challenge of deactivating antimicrobial resistance pathogens.



Keywords: chitosan, polyacrylic acid, gel particles, *E. coli*, VITRO-SKIN

1. Introduction

Contaminated environmental surfaces function as reservoirs for the transmission of many healthcare-associated pathogens. All these pathogens have been demonstrated to persist in the environment for hours to months and can pose a significant hazard due to touch-based transmission (Chemaly et al., 2014). The current surface disinfection treatments are inefficient for decontamination as some of the nosocomial pathogens may survive and give rise to substantial problems in terms of public health (Dancer, 2014). As per the 2023 report from the World Health Organization, antimicrobial resistance (AMR) poses a major threat to global public health and development. In 2019, bacterial AMR alone caused 1.27 million deaths worldwide (WHO, 2023). Polyelectrolyte complexes have remained one of the most exciting subjects of scientific research, in recent decades, due to desirable physicochemical and biological properties (non-toxicity, biocompatibility, softness, hydrophilicity, biodegradability) having high drug encapsulation efficiency with quick response to stimuli (light, pH, temperature, antigens, ionic strength, etc.). These complexes found potential applications in pharmacy, the food industry, wastewater treatment, pulp, and paper production (Meka et al., 2017). Polyelectrolyte complexes are formed as ionically crosslinked networks between polyelectrolytes with opposite charges in solution without any chemical covalent cross-linker. The significant interactions between two polyelectrolyte polymers may include reversible electrostatic and dipole-dipole associations and hydrogen and hydrophobic bonds (Luo and Wang, 2014). Polyelectrolyte complexes consist of a neutralized polyelectrolyte core surrounded by excess polyelectrolyte, stabilizing the colloids against aggregation (Dautzenberg and Karibyants, 1999).

Chitosan is a derivative product obtained by the deacetylation of chitin, which is biodegradable and possesses low toxicity, and can be used in drug delivery, bioadhesion, wound healing, etc. (Singla and Chawla, 2010). Polyacrylic acid is an anionic polymer having anionic/acidic (–COOH) pendant groups on the polymer chains and is widely used for biomedical applications, especially for pH-sensitive hydrogel-based drug delivery systems (Rizwan et al., 2017). This study aimed to investigate (I) the antimicrobial efficacy of chitosan and polyacrylic acid separately and as a mixture in solution and at a bio-substrate (VITRO-SKIN) surface and (II) the effect of the ratio of chitosan and

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polyacrylic acid within the mixture against *Escherichia coli* (*E. coli*).

2. Materials and methods

2.1 Materials

Polyacrylic acid (PAA) (purity of 99.0 %, molecular weight 450 kDa) was obtained from Polysciences Inc. (Warrington, PA, USA). Medium molecular weight chitosan (molecular weight 190–310 kDa) with a degree of deacetylation 75 %–85 % was purchased from Sigma-Aldrich®. Trypticase soy broth (TSB), trypticase soy agar (TSA), ampicillin sodium salt, and neutralizer broth (D/E broth) were obtained from Thermo Fisher Scientific Inc. Artificial skin substrate (VITRO-SKIN®) was purchased from IMS Inc. (Portland, ME, USA).

2.2 Bacterial strain

E. coli (ATCC 25922GFP) was obtained from ATCC. The culture was cultured by growing *E. coli* in trypticase soy broth (TSB) supplemented with 100 µg/ml ampicillin at 37 °C and harvested in the log phase at an OD600 of 0.3–0.4 by centrifugation at 3200× *g* (RCF: relative centrifugal force) for 15 min. The cell concentration was adjusted to 10^9 CFU/ml (OD600 0.5) and further diluted with sterilized water to obtain a bacterial concentration of 10^6 CFU/ml.

2.3 In vitro antimicrobial activity assay

Viability assays and microbial removal from the substrate were conducted as described previously (Nandakumar, 2018). Viability assay was performed in solution without the artificial skin substrate. For this purpose, a bacterial suspension of 10^6 CFU/ml was directly added to the suspensions of the desired polyelectrolytes/polyelectrolyte mixtures, vortexed for 30 s at 1500 rpm (37 × *g*) and neutralized with D/E broth. The cell viability in the suspension was determined via the agar plate count method after incubating at 37 °C overnight before counting colony-forming units (CFU). Centrifugation speed (1500 rpm (37 × *g*)) chosen for this experiment was verified not to affect concentrations of free bacteria in the suspension. Results were expressed in terms of the total number of bacteria recovered (CFU) on the logarithmic scale.

Bacterial removal test protocol was as follows: (i) spreading the polyelectrolytes over 1 cm^2 area of prehydrated skin substrate and air-drying at room temperature for 30 min, (ii) polyelectrolytes coated skin substrate was exposed to the bacterial suspension of 10^6 CFU/ml followed by further drying for 20 min, (iii) skin substrates were neutralized using D/E broth and vortexed at 3000 rpm (150 × *g*) for 30 s continuously to recover the remaining bacteria bound to the substrate. For solution studies, the interaction time for the polyelectrolytes and *E. coli* was 30 s, and the solution was vortexed at 1500 rpm (37 × *g*) continuously. Enumeration of bacteria using the agar plate count method was achieved as described above.

Removal efficacy following treatment was expressed as $\log_{10}$ bacterial removal using **Eqn. (1)**:

$$\log_{10}\text{ bacterial removal} = \log_{10}(\text{Initial bacterial inoculum on skin}) - \log_{10}(\text{bacteria remaining on skin}) \quad (1)$$

2.4 Synthesis of chitosan–polyacrylic acid polyelectrolyte complexes (soft solid)

The polyelectrolyte complex was prepared as follows: chitosan was dissolved in a 1.0 % w/v lactic acid solution for 12 h under mechanical stirring in order to form a 1.0 % w/v chitosan solution. The polyelectrolyte complexes were prepared by free mixing the specific ratios of polyacrylic acid and chitosan and the volume was adjusted to 5 mL. The polyelectrolyte complex was cast onto a petri dish plate, followed by keeping the plates at 60 °C on a hot plate overnight. The polyelectrolyte film formed was removed and sterilized in UV for 20 min in a laminar bio-hood and stored at room temperature for further testing.

2.5 Measurement of swelling behavior

The swelling behavior of the polyelectrolyte complex was estimated using mass balance at room temperature. The dry polyelectrolyte films were weighed and immersed in 25 ml of distilled water in a petri dish at room temperature. After 24 h, the swollen films were removed from the water, wiped off, and weighed. The swelling ratio was calculated using **Eqn. (2)**:

$$\text{DSW}\ (\%) = [(W_s - W_d)/W_d] \times 100 \quad (2)$$

where W_s and W_d denote the weight of the swollen and dry samples, respectively.

2.6 Measurement of the hydrophobicity of cell surface

The hydrophobicity of the bacterial cell surface was determined by microbial adherence to solvents (MATS) according to Bellon-Fontaine et al. (1996). Briefly, 2.4 ml of bacterial cells (washed thrice) suspended in 100 mM KNO_3 were vortexed at 1500 rpm (0.3 × *g*) for 90 s with 0.4 ml of chloroform and hexadecane and left undisturbed for 20 min. The extent of bacterial partitioning and adhesion to the solvents enabled comparisons between the electron donor properties and the hydrophobic nature of bacterial cell surfaces and was quantified using the following **Eqn. (3)**:

$$\text{Hydrophobicity}\ (\%) = (1 - A/A_0) \times 100 \quad (3)$$

where A_0 is the optical density of the aqueous suspension at 600 nm before mixing and A is the optical density of the aqueous suspension after mixing with the solvent pair.

2.7 Substrate preparation and characterization

VITRO-SKIN® is a commercially available artificial skin substrate coated with collagen, gelatin, and silica particles to mimic the physicochemical properties of natural human skin, including pH, topography, and ionic strength. As per the manufacturer's manual, the substrate was prepared by hydrating a 1.0 cm × 1.0 cm patch overnight using a glycerol: water (15:85) binary mixture in a humidity chamber. The zeta potential of the substrate was measured using Paar Physica Electro Kinetic Analyzer at 10 mM KCl and pH 6.7. In addition, contact angle measurements were conducted using the sessile drop method within 60 s of deposition and were used to estimate the critical surface energy of the artificial skin substrate using the Owens–Wendt model (Owens and Wendt, 1969).

2.8 Characterization of bacteria

The *E. coli* strain employed in this study (ATCC 25922GFP) was characterized by surface energy using a light scattering technique described elsewhere (Zhang et al., 2015). Briefly, *E. coli* cells at set concentration were suspended in ethanol: water binary mixture of varying surface tensions, and vortexed for 30 s at 1500 rpm (37 × *g*) before leaving them undisturbed for 20 min. The samples were then centrifuged at 43 *g* (RCF) for 45 s and measured for optical density at 600 nm. Suspension with the highest optical density (OD 600 nm) was determined as closest to the surface energy values of the bacterial cell. Zeta potential measurements of *E. coli* were conducted using Brookhaven ZetaPlus at 10 mM KCl and pH 8.0.

3. Results and discussion

3.1 Characterization of the bacteria and substrate

This study used the MATS assay to investigate the surface hydrophobicity, hydrophilicity, and Lewis acid-base properties of the *E. coli* strain. The chosen bacteria showed strong interactions with a weak acidic polar solvent (chloroform: 12.5 ± 2.5 % adhesion) compared with interactions with a nonpolar solvent with a similar Lifshitz-van der Waals component (hexadecane: 2.8 ± 0.5 %). The low percentage of bacteria adhering to a nonpolar solvent such as hexadecane indicated that the strain evaluated had low hydrophobicity with base-like (electron donor) behavior. The quantitatively important existence of chemical groups such as $–COO^-$ and $–HSO_3^-$ on the surface of microorganisms could explain their strong electron donor character (Pelletier et al., 1997). Comparable results were obtained from the measured surface energy values of 47.5 ± 1.5 mJ/m^2, which are consistent with the findings of Oh et al. (2018) (57.2 ± 1.5 mJ/m^2). Additionally, a zeta potential of –25.33 ± 1.46 mV (pH 4.0, 10 mM KCl) and an IEP at pH 2.0 were observed, as reported by Ammam (2012).

VITRO-SKIN substrate was characterized for surface energy from contact angle measurements and was estimated to be 40.2 ± 2.3 mJ/m^2 using the Owens–Wendt model (Nandakumar, 2018). Streaming potential measurements of the artificial skin substrate indicated a zeta potential of +22.5 ± 0.7 at 10 mM KCl and pH 6.7, in agreement with the reported zeta potential of +23 mV for human skin at pH 6 (Morykwas et al., 1987).

3.2 Intermolecular interactions in polyelectrolytes in solution

The interaction between chitosan and polyacrylic acid at pH 4, and thus the subsequent formation of the soft solid in water, is schematically illustrated in **Fig. 1**. At low pH values, e.g., pH 4 (typically below the pKa of around pH 6.5), chitosan carries a net positive charge due to the protonation of its amino groups. This positive charge enables chitosan to readily interact with negatively charged species in its surroundings, such as proteins or anionic polymer molecules. The electrostatic attraction between the positively charged chitosan and the oppositely charged species can form complexes or aggregates (Yilmaz Atay et al., 2019). In contrast, polyacrylic acid (PAA) contains carboxylic acid (–COOH) groups, which can undergo ionization and form charged species based on the pH of the solution. As the pH increases and approaches the pKa value of PAA, typically around 4–5, the carboxylic acid groups begin to deprotonate.

The polymer is partially ionized at this pH range, with a balance between protonated (–COOH) and deprotonated ($–COO^-$) carboxylic acid groups. This state allows for interactions with water molecules, leading to increased

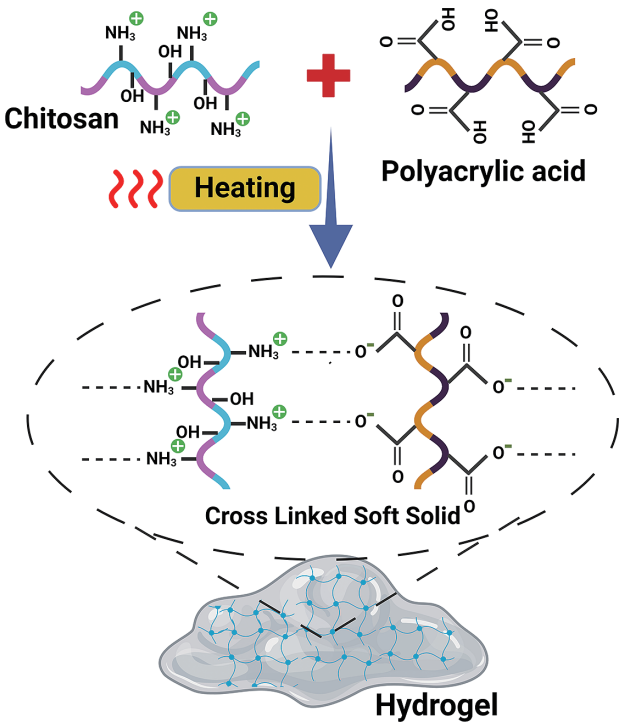

Fig. 1 Formation of soft solid particles.

hydrophilicity and solubility of PAA resulting in maximum swelling behavior and high solubility in water.

At the experimental pH values (pH 4.0 ± 0.2), beyond the pKa, PAA is in its deprotonated form, with the carboxylic acid groups carrying a negative charge ($–COO^-$). The negatively charged electrostatic repulsion among PAA molecules leads to the expansion of the polymer chain and an increase in its hydrodynamic volume. In addition, hydrogen bonds can form between the hydroxyl and amino groups of chitosan and the carboxyl groups of polyacrylic acid. These interactions can contribute to the stability of the chitosan–polyacrylic acid complex. The coulomb repulsion between chain segments, on the one hand, and a non-monotonic change in the hydrogen bonding between chain segments between the negatively charged segments can prevent the polymer chains from collapsing or aggregating, maintaining their solubility in water (Katiyar and Jha, 2017).

The interactions between the charged functional groups present in both polymers primarily determine the pH-dependent behavior of the soft solids resulting from complexation between chitosan and PAA. It is important to note that the specific interactions between chitosan and polyacrylic acid at the experimental pH can be influenced by experimental conditions, such as polymer concentration, mixing ratio, interaction time, and reaction temperature. Complexation can occur due to hydrogen bonding and ionic interactions between oppositely charged functional groups. The resultant complex soft solids can undergo changes in structure, swelling, and other properties due to the balance between electrostatic attraction and repulsion between the partially charged functional groups (De la Torre et al., 2003). Chavasit et al. (1988) observed that the maximum complex formation between chitosan and polyacrylic aid occurred at different mole ratios within 3 to 6 pH range, due to the degree of ionization of the functional groups. At pH 4, the degree of ionization of chitosan and polyacrylic acid was approximately 0.95 and 0.2, respectively. The following complex formation mechanism occurs at pH 4:

$$NH_3^+ + HOOC \rightarrow NH_3^+ {}^-OOC + H^+$$

Swelling characteristics of the soft solid can be used to assess the antimicrobial efficacy by cell attachment and subsequent cell membrane disruption. It is a vital characteristic of the polyelectrolyte complex to determine hydrophilicity. The water absorption in the polyelectrolyte complex is due to interactions between water and hydrophilic groups, such as hydroxyl groups (OH), and carboxylic groups ($–COO^-$), which produce electrostatic and hydrogen-bonding interactions. It thus indicates the successful formation of crosslinked networks (Hatakeyama H. and Hatakeyama T., 1998). Since the formation of polyelectrolyte complex is based on electrostatic interactions, it suggests that the greater the number of protonated cationic groups, the higher the antimicrobial activity. Chitosan forms polyelectrolyte complex with higher swelling ability in aqueous media, where the C = O and N–H groups of chitosan in the complex could be protonated, consequently resulting in a higher net positive charge, thus leading to better antibacterial activity (Mohamed and Fahmy, 2012).

The swelling experimental results indicate an increase in water uptake after 24 h in the tested polyelectrolyte complexes. An inverse correlation was noticed between the water uptake and the degree of polyelectrolyte complex formation, as the water uptake changes from 300 % to 800 %. Under the tested conditions, the polyelectrolyte complex containing 100 µg/ml of chitosan has the highest water uptake (781 %). It is noteworthy that a comparable equilibrium swelling ratio of 850 % was documented at pH 5 in a study conducted by Jozaghkar et al. (2022). This equilibrium swelling ratio was achieved at a specific ratio of chitosan to polyacrylic acid, namely 0.01:1. This finding underscores the importance of pH and the ratio of chitosan to polyacrylic acid in influencing the swelling behavior of the soft solids. This performance may be due to the large pore size of the polyelectrolyte complex formed due to the imbalance of the concentration of cations and anions present in the polyelectrolyte complex (Tsao et al., 2010). As the chitosan concentration increases in the polyelectrolyte complex, the polymer chains may be connected into higher polymer density networks through more ionic crosslinks resulting in less water uptake.

Maximum log reduction (one million to one thousand) of *E. coli* cells was accomplished in the presence of a polyelectrolyte complex containing >100 µg/ml of chitosan 30 s of interaction. Complete degradation of the polyelectrolyte complex was observed after (~10 h) when the chitosan concentration was less than 100 ug/ml in the complex, contributing to 0.5–1.0 log reduction of *E. coli* cells. This response can be attributed to a natural release of polymer chains from this hydrated network because of its few ionic and high porosity crosslinks, as demonstrated for other polymers (Chellat et al., 2000).

3.3 Antimicrobial efficacy of chitosan, polyacrylic acid, and their complexes (soft solid) in solution

The antimicrobial efficacy of chitosan, polyacrylic acid, and their complex (soft solids) at different ratios of polyelectrolytes in the solution is shown in **Fig. 2**. There is no significant reduction of *E. coli* up to 1000 µg/ml of chitosan; however, 1 log and 6 log reduction (complete kill) are observed at 1000 and 10,000 µg/ml concentrations of chitosan, respectively. Jeon et al. (2014) showed a concentration-dependent bactericidal activity of 76 nm spherical chitosan against *E. coli*. Of all concentrations examined by them, 2000 µg/ml of chitosan showed the

most antimicrobial activity resulting in complete inhibition of *E. coli* during 6 h of incubation. All *E. coli* were killed in 120 min in the presence of 5000 ppm of chitosan in solution from initial 1E+05 (100,000) cells. Liu et al. (2004) reported a similar result using a bacterial strain similar to the one used in the present study. Under the present test conditions, the enhanced antimicrobial activity of chitosan is primarily due to the protonation of amino groups in the chitosan molecule. The most commonly hypothesized antibacterial effect of chitosan is to interact with the negatively charged bacterial cell wall, thereby altering membrane permeability. Several studies have shown that chitosan molecules in a solution can bind to DNA in the cell nucleus, where they can inhibit mRNA synthesis, thereby preventing its replication, leading to the cell death (Ardean et al., 2021). Another suggested mechanism is that chitosan serves as a chelating agent, binding to essential metal ions and thus limiting microbial growth (Yilmaz Atay, 2019). Kong et al. (2010) reported that the antimicrobial mechanism of chitosan at acidic pH (pH < 6) can be attributed to the electrostatic interactions between a positively charged amino group in chitosan and negatively charged bacterial surface molecules such as lipopolysaccharides and outer membrane proteins, resulting in the alteration of cell membrane permeability. The change in membrane permeability leads to the leakage of intracellular substances, eventually resulting in cell death.

On the contrary, there is no loss in viability of *E. coli* cells in the presence of polyacrylic acid. Polyacrylic acid is a negatively charged polymer, and its interaction with *E. coli* cells is repulsive.

The antimicrobial efficacy of chitosan and polyelectrolyte mixtures (soft solids) of varying ratios of chitosan and polyacrylic acid in solutions is plotted in **Fig. 2**. The tested polyelectrolyte complexes with the ratio of 1000 µg/ml chitosan and 9000 µg/ml polyacrylic acid, resulted in ~ 5E+03 (5,000) viable cells (out of 1 million initial cell count) after the 30 s exposure. According to Hu et al. (2002), the soft solid's zeta potential increases as the chitosan to polyacrylic acid ratio increases. When the content of chitosan (aminoglycoside units) exceeds that of polyacrylic acid, some of the excess chitosan is adsorbed onto the surface of the chitosan–polyacrylic acid complex (soft solid), increasing the surface charges of the soft solid and resulting in an increase in zeta potential. The antimicrobial mechanism of this complex is speculated to be the complex acting like a molecular ionic sponge, attracting the anionic microbial membrane into the three-dimensional porous structure of the polyelectrolyte complex, leading to membrane disruption and microbial death (Tsao et al., 2010).

3.4 Antimicrobial efficacy of chitosan and polyacrylic acid mixtures on the VITRO-SKIN surface

The removal of *E. coli* from VITRO-SKIN in the presence of different concentrations of chitosan and polyacrylic acid is shown in **Fig. 3**. The viable *E. coli* cells are reduced on the VITRO-SKIN as a function of the increase in the chitosan concentration alone. In contrast, no change was observed when exposed to polyacrylic acid alone. From the streaming potential studies, the surface charge of the VITRO-SKIN is found to be +22 mV due to the presence of collagen (Brohem et al., 2011; Li et al., 2009). Due to electrostatic repulsion between the amino group of chitosan and the VITRO-SKIN, the functional group may be oriented away from the VITRO-SKIN (i.e., exposed to the air). Elemental analysis of VITRO-SKIN reveals the

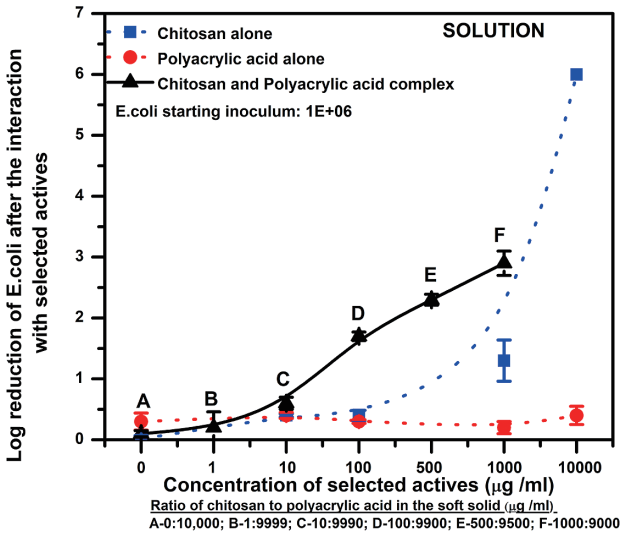

Fig. 2 Reduction of *E. coli* cells in the presence of chitosan, polyacrylic acid, and soft solids in solution. The raw data are publicly available at J-STAGE Data (https://doi.org/10.50931/data.kona.25965100).

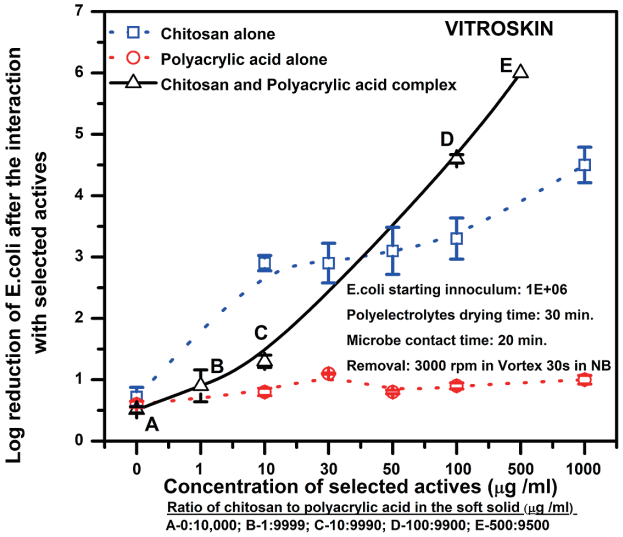

Fig. 3 Reduction of *E. coli* cells in the presence of chitosan, polyacrylic acid, and the soft solid at the bio-surface. The raw data are publicly available at J-STAGE Data (https://doi.org/10.50931/data.kona.25970965).

presence of 1.7 mg of nitrogen per cm^2 (data not shown) that originates from the collagen in the VITRO-SKIN. The zeta potential measurement indicates that the IEP of collagen is about 9.3, which carries a net positive charge (Li et al., 2009).

As described earlier, chitosan includes amino groups that are protonated at lower pH levels, such as pH 4, producing a positively charged polymer. Electrostatic repulsion between the amine functional groups in chitosan and collagen can occur when it comes into touch with a positively charged surface (VITRO-SKIN). On the other hand, chitosan has hydroxyl groups in addition to amino groups. These hydroxyl groups can form hydrogen bonds with positively charged surface functional groups. These interactions can result in chitosan adsorption on the VITRO-SKIN surface, contributing to the overall stability of the chitosan–VITRO-SKIN surface complex. It is reported that the functional group –COOH and NH_2 in collagen may interact through hydrogen bonds with –OH and $–NH_2$ groups from chitosan, as chitosan possesses large numbers of –OH groups, and thus alters the collagen triple helix structure (Sionkowska et al., 2004). This conformational change facilitates an adequate interaction of amino groups in chitosan with the approaching microbe, thus increasing the permeability of the cell membrane. *E. coli* cell outer membrane acts as a permeability barrier and inhibits the transport of macromolecules and hydrophobic compounds entering or leaving bacteria cell membranes. The fluorescence intensity studies reported an increase in the *E. coli* cell membrane permeability observed after interaction with chitosan in 10 minutes (Tang et al., 2010). This behavior is ascertained from the removal studies that log reduction of *E. coli* on the VITRO-SKIN increases proportionally to the tested chitosan concentration.

On the contrary, no significant cell removal from VITRO-SKIN was observed in the presence of polyacrylic acid. Polyacrylic acid is a polyelectrolyte with negatively charged carboxylate groups, especially at lower pH values where carboxyl groups remain protonated. A positively charged VITRO-SKIN surface can attract and facilitate the adsorption of PAA through electrostatic interactions and hydrogen bonding. The adsorbed PAA layer may create a charged or steric barrier that hinders the approach and attachment of *E. coli* cells to the surface. PAA's ability to absorb water and swell may contribute to the creation of a hydrated layer that hampers bacterial adhesion. The electrostatic repulsion between negatively charged PAA and the negatively charged bacterial surface may prevent direct contact and adhesion. The interactions between collagen and polyacrylic acid result in the formation of polyelectrolyte complex through electrostatic interactions involving the NH_3^+ group of the collagen in the VITRO-SKIN and the $–COO^–$ groups of polyacrylic acid (Barbani et al., 1999). Besides, due to their similar surface charges, electrostatic repulsion is anticipated between polyacrylic acid and the approaching *E. coli* cells. Also, collagen has no antimicrobial activity, hence no reduction in cell number on the VITRO-SKIN is observed in the presence of polyacrylic acid.

The complete reduction of *E. coli* cells (total kill) is observed in the presence of a polyelectrolyte mixture (500 μg/ml of chitosan and 9500 μg/ml of polyacrylic acid). In the polyelectrolyte complex, swelling and porosity of the mixture are governed by the percentage of the ionic components in the complex. The pKa values for polyacrylic acid and chitosan have been reported to be 4.0 and 6.5 at pH 4, with ionization levels of 0.2 and 0.95, respectively (Choi and Rubner, 2005). In the present experimental conditions (pH 4.0), more carboxyl groups than amino groups are undissociated. As the chitosan concentration increases from 1 μg/ml to 500 μg/ml, a more stable porous structure in the mixture is anticipated. Under the tested conditions, the available dissociated carboxyl groups in polyacrylic acid interact via non-covalent forces with the groups present in the chitosan. Our research findings suggest that in order to achieve the antimicrobial effect of the soft solids, it is necessary to have direct contact with bacterial suspension. It is worth noting that the reduction in pH alone cannot account for this effect (Gratzl et al., 2015).

3.5 Antimicrobial mechanism of soft solid on a positively charged bio-surface

When the chitosan–PAA complex interacts with a positively charged bio-surface (VITRO-SKIN), the interactions are influenced by the charge distribution on the complex and the bio-surface, as shown in **Fig. 4**.

The electrostatic repulsion between the positive charges of chitosan and the positive charges on the bio-surface can facilitate the repulsion of chitosan molecules away from the bio-surface. The positively charged chitosan molecules in the soft solid align and orient themselves away from the VITRO-SKIN and towards the negatively charged bacterial cells, promoting electrostatic interactions and adhesion. However, the carboxylic acid groups in PAA can undergo ionization on the positively charged bio-surface, leading to the formation of negatively charged carboxylate groups ($–COO^–$) on PAA. The negatively charged carboxylate groups can lead to electrostatic interactions with the positively charged bio-surface, enabling the adhesion of the complex on the surface. The adsorbed chitosan layer can physically encapsulate the *E. coli* cells due to its three-dimensional structure, leading to physical entrapment and hindered mobility of *E. coli* cells. Chitosan contains hydrophobic groups (such as acetyl groups in the case of partially deacetylated chitosan) along with its positively charged amino groups. Furthermore, the hydrophobic regions of chitosan can interact with hydrophobic regions on the bacterial cell surface, including lipids and other

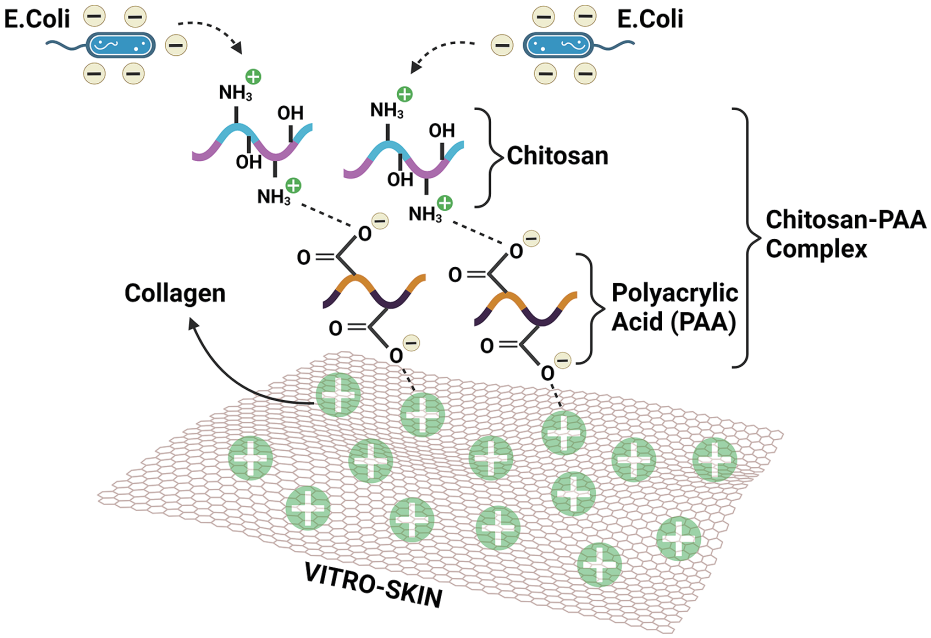

Fig. 4 Interaction of soft solids with *E. coli* at VITRO-SKIN.

hydrophobic molecules present in the cell membrane. Chitosan's hydroxyl and amino groups can also form hydrogen bonds with various functional groups on the bacterial cell surface, such as hydroxyl, carbonyl, and amine groups. The combination of electrostatic interactions, hydrophobic interactions, and hydrogen bonding creates a multifaceted interaction between chitosan and bacterial cells. Chitosan's interaction with the *E. coli* cell membrane could potentially lead to leakage of intracellular components. A previous study has confirmed the leakage of proteins and other intracellular constituents caused by chitosan (Kong et al., 2008). In Gram-negative bacteria, high negative charges given by lipopolysaccharide (LPS) can be neutralized by positive charges from chitosan, resulting in disruption of the bacterial outer membrane, enabling chitosan to penetrate the cell membrane resulting in cell death (Feng et al., 2021). The 20-minute interaction time allows for prolonged exposure of the bacteria to the antimicrobial properties of the hydrogel. Additionally, an acidic environment due to the carboxylic acid groups in PAA can hinder bacterial growth and reproduction, further inhibiting the viability of *E. coli*. Topuzoğullari (2020) observed that positively charged poly electrolyte complexes, generated at greater [quaternized 4-vinylpyridine]/[PAA] ratios, resulted in higher antibacterial activity of these compounds is dependent on these free quaternized 4-vinylpyridine groups. The free quaternized 4VP groups that do not interact with PAA due to insufficient acrylic acid groups and the polyelectrolyte complex characteristics become similar to free polycation, which causes a similar antibacterial activity. In the present study, a similar trend was observed as more protonated chitosan was observed over PAA due to the higher degree of ionization of chitosan over PAA at pH 4.0.

3.6 Antimicrobial mechanism of soft solid in water

In water, the chitosan–PAA complex can exhibit antimicrobial properties against gram-negative bacteria, as shown schematically in **Fig. 5**. The electrostatic attraction between the positively charged chitosan and the negatively charged bacteria promotes the adsorption of the complex onto the bacterial cell membrane. This interaction can disrupt the integrity of the cell membrane, leading to leakage of cellular contents and eventually bacterial death.

Additionally, the carboxylic acid groups (–COOH) in PAA can release hydrogen ions (H^+) in the solution, leading to a decrease in the pH locally around the complex. The acidic environment generated by the release of H^+ ions can further disrupt the bacterial cell membrane, destabilizing the bacterial cells and inhibiting their growth. Both the soft solid and the bacteria are in constant motion in water due to induced shear force. The diffusion of the complex and the bacteria increases the chances of their encounter and subsequent collision. The number of chitosan–PAA complex molecules available in the solution also influences the interaction with *E. coli*. The greater the number of complex molecules, the higher the probability of interactions occurring with bacterial cells. The chitosan content in the gel is critical to its antibacterial efficacy against gram-positive and gram-negative bacteria in the solution. The creation of hydrogen and covalent connections among the functional groups of the chitosan chains is enhanced as the concentration increases, minimizing dispersion, and causing the structure to acquire a densely overlapping coiled conformation. The extensive intra- and intermolecular bonding at higher molecular densities is related to the random-coil structure of chitosan in solution, which is often recognized

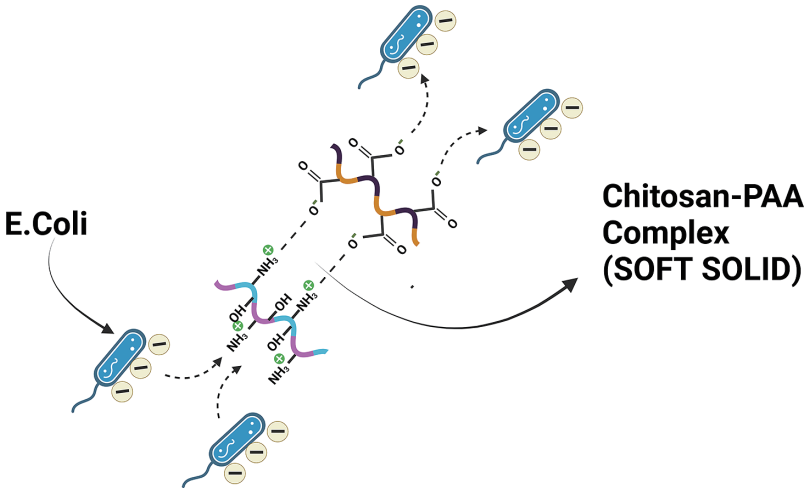

Fig. 5 Interaction of soft solid with *E. coli* in the solution.

in the literature. This, in turn, places geographical constraints on functional groups. As a result, fewer charged sites are accessible for interaction, limiting binding to bacterial cell walls (Goy et al., 2016). The soft solid allows for a larger surface area available for contact with bacteria, increasing the likelihood of collision between the soft solid complex and *E. coli* cells. Increased availability of complex molecules enhances the chance of multiple binding events and can lead to stronger adhesion and antimicrobial effects.

4. Summary and conclusions

This present study showed the antimicrobial efficacy of soft solid containing chitosan and polyacrylic acid emerges as an additional methodology for combating microbial challenges, both in solution and at bio substrate surfaces. In solution, the remarkable efficacy of chitosan at a concentration of 10,000 μg/ml in achieving a 6-log reduction (complete eradication) of microbial populations highlights its unparalleled potential as a potent antimicrobial agent. This level of microbial reduction surpasses that of many commercial antimicrobial agents, which typically achieve a 99.9 % (3 log) reduction in microbial populations. Simultaneously, the introduction of polyacrylic acid reveals its inherent compatibility, as it exhibits no detrimental effect on *E. coli* viability. The strategic blending of chitosan and polyacrylic acid at a ratio of 1000 μg/ml and 9000 μg/ml, respectively, provides a balanced compromise, resulting in a controlled reduction to approximately 5E+03 (5,000) viable cells—a noteworthy accomplishment from an initial population of one million. On the surface, a decrease in viable *E. coli* cells with increasing chitosan concentration on the VITRO-SKIN indicates that chitosan alone has an antibacterial action.

Conversely, the unaltered state in the presence of polyacrylic acid underscores its selective approach. However, the true marvel lies in the combination of chitosan and polyacrylic acid, as demonstrated by the total reduction of *E. coli* cells in the presence of a polyelectrolyte mixture containing 500 μg/ml of chitosan and 9500 μg/ml of polyacrylic acid. The interplay of intramolecular forces between chitosan and polyacrylic acid, encompassing electrostatic attractions, hydrogen bonding, and potentially hydrophobic interactions, appears to result in their synergistic efficacy. This study underlines the synergistic potential of these soft solids and emphasizes the importance of careful formulation to achieve the best antibacterial results. By exploiting the precise interplay of intramolecular forces within soft solids, it is possible to engineer advanced wound dressings, implant coatings, and biomedical textiles that inherently possess antimicrobial properties. These materials could prevent the initial adhesion of pathogens, minimize infection risk, and mitigate the formation of virulent biofilms, which are critical challenges in modern healthcare settings.

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Data Availability Statement

Data on log reduction measurements of soft solids (chitosan and polyacrylic acid gel particles) in solution are

available publicly in J-STAGE Data (https://doi.org/10.50931/data.kona.25965100).

Data on log reduction measurements of soft solids on a bio-surface (VITRO-SKIN) are available publicly in J-STAGE Data (https://doi.org/10.50931/data.kona.25970965).

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