HYDROGEL ANTIBACTERIAL COATING FOR SILICONE MEDICAL DEVICES

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Abstract

Effective antibacterial coatings are in demand in medicine, especially for urological medical devices such as catheters and stents. We propose the production method of an antibacterial hydrogel coating on polydimethylsiloxane (PDMS, silicone), a popular surface for medical materials. The coating process consists of the following steps: PDMS surface activation (introduction of hydroxyl groups), silanisation (introduction of amine groups) and application of chitosan/alginate hydrogel with the addition of lysozyme as an antibacterial agent using the layer-by-layer method. We investigated the effect of polyion concentration on the coating mass, swelling ratio and stability. We analysed the adsorption of Micrococcus luteus, Escherichia coli and Proteus rettgeri on a PDMS surface using confocal laser scanning microscopy. The chitosan/alginate hydrogel coating with immobilised lysozyme protected the PDMS surface against adhesion for all three tested bacterial strains.

Keywords: *antibacterial coatings, medical devices, layer-by-layer, alginate, chitosan, lysozyme*

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1. Introduction

Bacterial infections are a significant problem in medicine, including those caused by medical devices that interfere with the patient's body. There is a great need to develop antibacterial coatings for medical devices. It is important especially in urology, where an antibacterial coating should be applied to catheters and stents. These two medical devices can be colonised with bacterial biofilm and cause urinary tract infections. Catheter--associated urinary tract infection (CAUTI) causes approximately 40% of all nosocomial infections [1]. With short-term catheterisation (up to 7 days), infection occurred in 10%-50% of cases, while with long-term catheterisation (more than 28 days), infection was present in all examined cases [2]. The risk of infection increases by 3%-7% with each day of catheterisation [3]. In turn, stent-associated urinary tract infection causes 25%-40% nosocomial infections [4]. Additionally, urea-producing bacteria on the surface of urological stents cause its encrustation, which is also dangerous for the patient [5].

One of the strategies for modifying the surface of medical devices is coating them with a hydrogel with antibacterial properties. Due to their structure, high water content and mechanical properties, hydrogels can mimic human tissue, making them attractive for biomedical applications [6]. One of the techniques for producing hydrogel coatings is the layer-by-layer method. This method consists of alternately immersing the coated material in a solution of polycation and polyanion. The advantage of this technique is controlling the coating thickness by selecting the number of polyion layers. We focused on a hydrogel made of a pair of natural polyions: chitosan and alginate. This hydrogel is widely used: for example, as part of a composite membrane for pervaporation dehydration of alcohol [7], nanolaminates [8] or fruit bar coatings [9] in the food industry, targeted delivery of probiotic bacteria [10], the platform for the formation of stem cell aggregates [11], nanoparticle coating for antifouling protection and folic acid-binding in tissue engineering [12].

One of the most commonly used materials for producing long-term urological catheters and stents is silicone, which is a chemically inert material that is difficult to modify. [13]. In this article, we describe a method of producing a chitosan/alginate antibacterial hydrogel coating on a polydimethylsiloxane (PDMS) surface consisting of the following steps: surface activation (introduction of hydroxyl groups), silanisation (introduction of amine groups) and application of chitosan/alginate hydrogel with the addition of an antibacterial agent using the layer-by-layer method. We decided to use lysozyme as a natural nonantibiotic antibacterial agent.

2. Materials and Methods

2.1. Materials

Chitosan from shrimp shells with low viscosity and a deacetylation degree of ~79.5%, alginic acid sodium salt, (3aminopropyl)-triethoxysilane 97% (APTES), lysozyme hydrochloride from egg white ≥ 30 000 U/mg, fluorescein diacetate 98% (FDA) and acridine orange hydrochloride hydrate ≥ 98% (AO) were provided by Sigma-Aldrich PDMS was prepared from Sylgard® 184 Silicone Elastomer Kit (Dow Chemical Company, USA). Nutrient Broth M002 medium (peptone 5.0 g/l, sodium chloride 5.0 g/l, HM peptone B 1.5 g/l, yeast extract 1.5 g/l; final pH 7.4 ± 0.2) was provided by HiMedia Laboratories (India). *Micrococcus luteus* ATCC 4698, *Escherichia coli* ATCC 8739 and *Proteus rettgeri* PCM 1383 bacterial strains were provided by the Polish Collection of Microorganisms Institute of Immunology and Experimental Therapy (Poland). The other chemicals were of analytical grade and were used without further purification.

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2.2. Preparation of Chitosan/Alginate Hydrogel Coating with Immobilised Lysozyme

2.2.1. Chemical Activation of the PDMS Surface

A mixture of 30% hydrogen peroxide and concentrated sulfuric acid in a 1:1 volume ratio was used to activate the PDMS surface. The freshly prepared activation solution was cooled in a stream of water for 5 min. After washing with ethanol and deionised water, PDMS samples were incubated in the activation solution for 30 min. After activation, the samples were rinsed with deionised water and dried at 60°C for 10 min.

2.2.2. Introduction of Amino Groups on the Surface of Activated PDMS

To introduce the amino groups, the PDMS surface was silanised using APTES. A 2% (v:v) APTES solution in 96% ethanol, which had been previously adjusted to pH 5.0 with 5% aqueous acetic acid, was used. The APTES solution was prepared 10 min before PDMS activation. At this time, hydrolysis of APTES took place with the formation of reactive silanol groups. Then the APTES solution was heated to 60°C, and PDMS samples were placed in it for 30 min. After this time, the samples were washed with 96% ethanol to remove unbound APTES. The silanised samples were dried at 100°C for 30 min to fix the functional groups to the PDMS surface.

2.2.3. Preparation of the Hydrogel Coating With the Layer-by-Layer Method

The hydrogel coating was prepared by alternately dipping the silanised PDMS in the polyanion and polycation solutions. Alginate dissolved in 10 mM acetate buffer at pH 5.0 was used as the polyanion solution. The polycation solution was prepared by dissolving chitosan in 2% (w:v) acetic acid solution in water and then diluted with 10 mM acetate buffer (acetic acid and sodium acetate) at pH 5.0 to the desired concentration. After that, a defined amount of lysozyme was added to the chitosan solution. Polycations (alginate and chitosan) were used at concentrations of 5, 10, 15 or 20 mg/ml. Lysozyme was added to the chitosan solution to obtain a 0.5 or 1.0 mg/ml enzyme concentration.

The silanised PDMS samples were immersed in a phosphate buffer at pH 3.0 to ionise the surface amine groups. The samples were then immersed alternately in alginate or chitosan/lysozyme solutions. The incubation time was 1 min in each of the solutions. Additionally, the samples were washed in phosphate buffer at pH 3.0 (after incubation in alginate solution) or in ammonium buffer at pH 9.0 (after incubation in chitosan/lysozyme solution) to remove unbound polymers. Prepared this way, chitosan and alginate layers were treated as a single bi-layer. The coating process was carried out until 10 bi-layers were obtained. Then, the samples were lyophilised and left for further testing.

2.3. Fourier-Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) analysis

The efficiency of PDMS surface activation and silanisation was determined by FTIR spectroscopy using a Nicolet™ 6700 spectrometer (Thermo Fisher Scientific). The ATR mode was used, and spectra were analysed with the OMNIC 8.3 software (Thermo Fisher Scientific). Measurements were made in the range of 4000-400 cm^1 with a spectral resolution of 4 cm^1 and 32 scans per single measurement. Spectra were recorded for at least four randomly selected areas for the sample. One characteristic spectrum of each modification step was selected for presentation.

2.4. Analysis of the Swelling Ratio and Stability of the Hydrogel Coating

PDMS discs with a diameter of 38 mm were subjected to the activation and silanisation processes, described in sub-chapters 2.2.1 and 2.2.2, respectively, and then weighed on an analytical balance. Stability and swelling studies were carried out for four polymer concentrations: 5, 10, 15 and 20 mg/ml (the same concentrations for alginate and chitosan

in the experiment). The lysozyme concentration in the chitosan solution was 0.5, 1, 1.5 and 2 mg/ml, respectively. Then, the samples were coated with hydrogel according to the procedure described in sub-chapter 2.2.3, freeze-dried, placed in a desiccator for 30 min and weighed. Samples with dry hydrogel were immersed in phosphate-buffered saline (PBS) at pH 7.2 for 30 min. After this time, the samples were gently drained of excess water and weighed immediately. All analyses were performed for three independent samples (n = 3). The swelling ratio (SR) was calculated with Eq. (1):

$$SR = \frac{m_w - m_d}{m_d - m_0}, \quad (1)$$

where m_w is the mass of wet hydrogel, m_d is the mass of dry hydrogel and m_0 is the mass of silanised PDMS disc.

To determine the stability of the hydrogel, the same samples were again immersed in PBS at pH 7.2 for 24 h. After this time, the samples were removed from the PBS, gently rinsed in distilled water and lyophilised. The samples were placed in a desiccator for 30 min and then weighed. The stability after 24 h (S_{24}) was calculated with Eq. (2):

$$S_{24} = \frac{m_{d24} - m_0}{m_d - m_0}, \quad (2)$$

where m_{d24} is the mass of dry hydrogel after 24 h of incubation in PBS.

2.5. Bacterial Biofilm Adsorption

PDMS discs with a diameter of 14 mm were coated with a chitosan/alginate hydrogel with immobilised lysozyme according to the methods described in sub-chapter 2.2. In this experiment, 5 mg/ml of alginate and chitosan solutions were used. Three variants of the hydrogel coating were prepared: without the addition of lysozyme (H0) and with the addition of lysozyme at a concentration of 0.5 mg/ml (H0.5) or 1.0 mg/ml (H1.0). Uncoated PDMS was used as a control. All variants were performed in three independent replications (n = 3). All the samples for microbiological tests were prepared under sterile conditions, and all solutions were previously filtered using 0.2-µm filters.

Test samples were placed in Falcon tubes containing 4.9 ml of sterile Nutrient Broth M002 medium. Then, 100 µl of a suspension of bacteria derived from a 24-h culture in Nutrient Broth M002 was added to each tube. Three bacterial strains were used in the study: *M. luteus*, *E. coli* and *P. rettgeri*. Falcon tubes with inoculated medium and test samples were incubated on a shaker for 48 h at 37°C and 100 rpm. The samples were then subjected to microscopic observation.

2.6. Observation of Bacterial Biofilm Adhesion With Confocal Laser Scanning Microscopy (CLSM)

After 48 h of incubation with the bacterial suspension, samples, prepared as described in sub-chapter 2.5, were washed in sterile PBS to remove cells that had not adhered to the surface. After washing, the samples were stained with fluorescein diacetate (FDA) by immersion in 1 ml of FDA solution at a concentration of 20 µmol/l in PBS for 30 min. After this time, microscopic observation was made. Then, the same samples were stained with AO by immersion in 1 ml of AO solution at a concentration of 1 mg/ml in PBS for 10 min. After this time, the samples were washed with PBS and re-examined under the microscope. Each sample was observed in a minimum of three fields of view.

All samples were analysed using a CLSM (LSM 880, Zeiss, Germany) with the following parameters: excitation beam 488 nm and 405 nm for FDA and AO, respectively; collection ranges of 493-624 nm and 465-695 nm for FDA and AO, respectively.

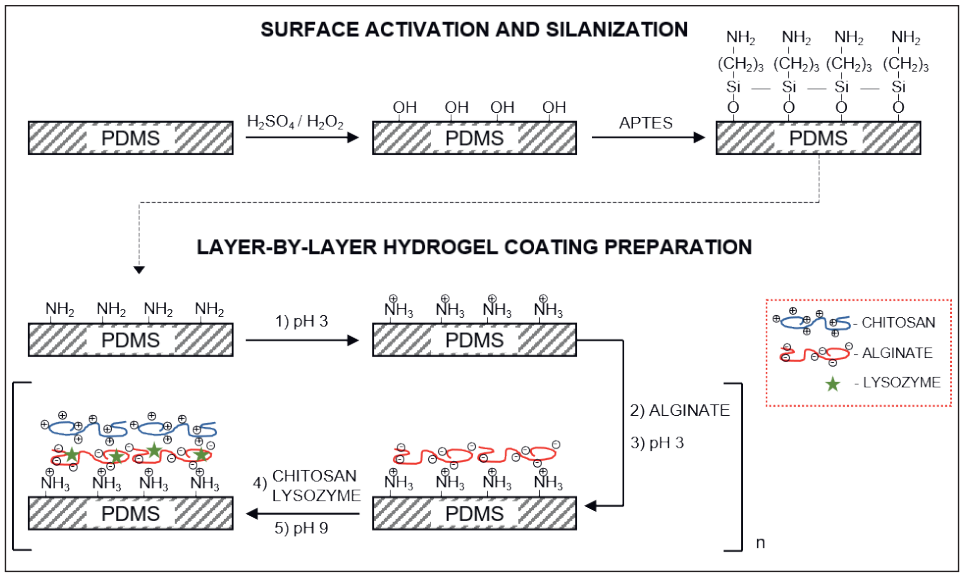

Figure 1. Scheme of the preparation of chitosan/alginate hydrogel with immobilised lysozyme as a coating on a polydimethylsiloxane (PDMS) surface

3. Results and Discussion

3.1. Characterisation of Chitosan/Alginate Hydrogel Coating With Immobilised Lysozyme

The scheme of the multi-step method for the preparation of chitosan/alginate hydrogel with immobilised lysozyme as a coating on PDMS surface is shown in Figure 1.

The first step was to chemically activate the PDMS surface to introduce hydroxyl groups, which are the basis for further modifications. In the second step, surface amino groups were introduced by silanisation using APTES. In this process, the trialkosilane hydrolyses to reactive silanols. These molecules condense with each other and then bind to the hydroxyl groups of the substrate by hydrogen bonds. In the last stage, covalent bonds are formed [14]. The effectiveness of PDMS surface activation and silanisation was confirmed by FTIR-ATR (Figure 2). This analysis confirmed the effectiveness of PDMS surface activation and silanisation. The spectrum of the material after chemical activation shows a characteristic broad band from the hydroxyl groups in the range of 3570-3200 cm^{-1} [15]. The spectrum of the sample after silanisation contains two characteristic absorption bands corresponding to aliphatic primary and secondary amines: a broad band in the range of 3400-3325 cm^{-1} and a band in the range of 1650-1590 cm^{-1} [15].

The final stage of functionalisation of the PDMS surface was applying layers of oppositely charged polymers, chitosan and alginate, which formed a hydrogel coating. Both polymers in solution with pH 5.0 exist in the ionised form. Under the process conditions, lysozyme also has a positive net surface charge, thus forming a complex with negatively charged alginate. The structure of the hydrogel is cross-linked by electrostatic interactions between alginate and chitosan [16]. The hydrogel application process was carried out using the layer-by-layer method by dipping the samples in polycation and polyanion solutions. This process allows obtaining different thicknesses of the hydrogel coating by selecting the number of polymer bi-layers applied. In this study, we always used a coating made of 10 bi-layers. Figure 3 shows the influence of alginate and chitosan concentrations on the

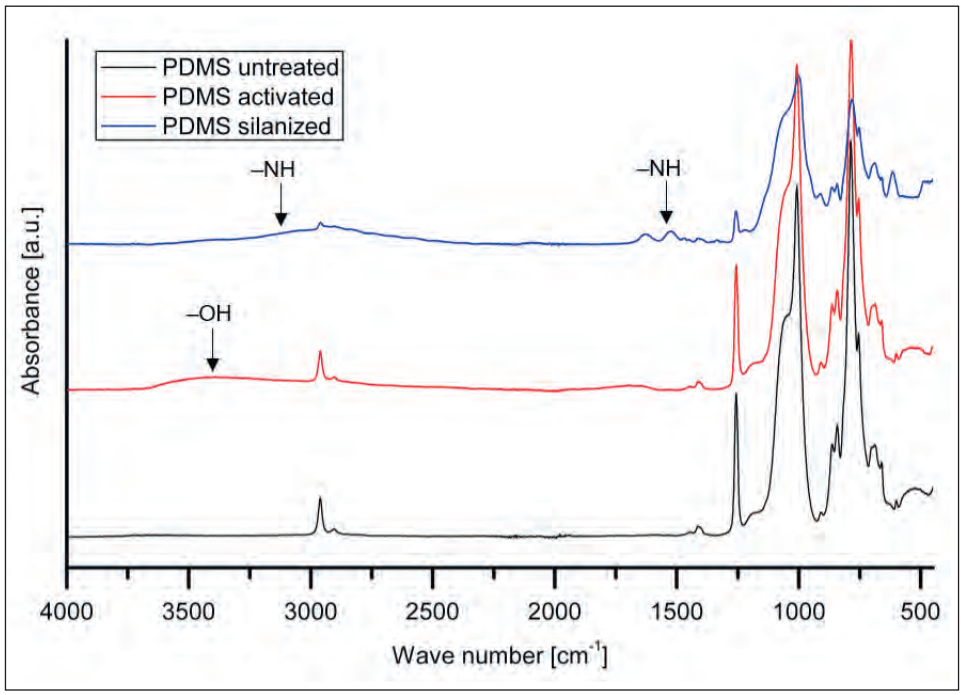

Figure 2. Fourier-transform infrared attenuated total reflectance spectra of a polydimethylsiloxane (PDMS) surface after chemical activation and silanisation

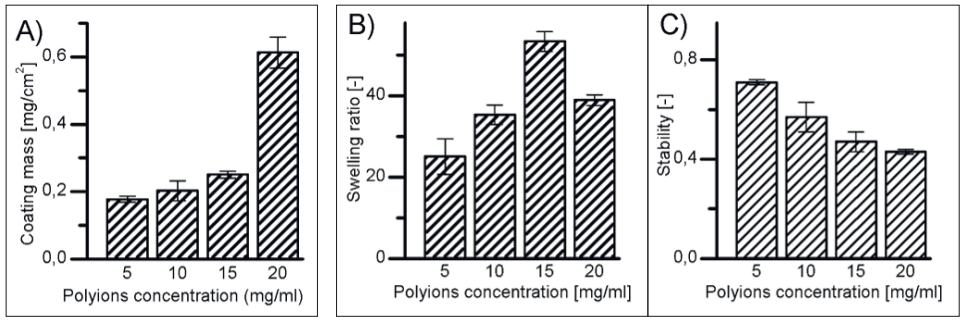

Figure 3. Effect of alginate and chitosan concentration on the properties of the obtained hydrogel cover: (A) the mass of attached hydrogel, (B) the swelling ratio and (C) the stability after 24 h of soaking in phosphate-buffered saline

properties of the obtained coating: the mass of the attached hydrogel, the swelling ratio and stability after 24 h of soaking in PBS. As expected, a higher concentration of polymers resulted in a higher amount of deposited hydrogel (Figure 3A). Moreover, the degree of the swelling ratio initially increased with an increase in concentration, but for 20 mg/ml, there was a decrease in the value of this parameter (Figure 3B), probably because the hydrogel detached from the coating after 30 min of the experiment. The stability of the coating appeared to decrease as the concentration of alginate and chitosan increased (Figure 3C).

Increasing the concentration of polyions from 5 to 15 mg/ml did not cause a significant increase in the mass of the attached hydrogel. The use of 20 mg/ml polyions allowed for a marked increase in the coating mass, but it was characterised by high instability. The highest stability of 0.71 ± 0.01 was obtained at a polyion concentration of 5 mg/ml. For these reasons, we decided to use alginate and chitosan concentrations of 5 mg/ml for the bacterial biofilm adsorption studies.

3.2. Analysis of the Adsorption of Bacterial Biofilm on a PDMS Surface With a Hydrogel Coating

We used two different staining methods to visualise live bacteria as well as bacterial cells (regardless of their physiological state) for biofilm adsorption studies. We used FDA to stain live bacteria cells. This dye penetrates the bacterial cell wall and is metabolised by bacterial esterases to fluorescein, which has a difficult escape from the cell due to its negative molecular charge [17]. A characteristic feature of ester derivatives of fluorescein is the ability to stain living cells selectively. Dead bacterial cells with damaged cell walls are unable to maintain the dye inside the cell [18]. The second dye was AO, which binds to the nucleic acids of cells regardless of their physiological state. It allows for the staining of both living and dead cells [19].

Bacterial adsorption studies were carried out for three bacterial strains: *M. luteus*, *E. coli* and *P. rettgeri*. *M. luteus* was used as a model gram-positive bacterium susceptible to lysozyme activity. *E. coli* is a model gram-negative bacterium that is also commonly found in urinary tract infections. *P. rettgeri* is also common in urinary tract infections and has a high ability to colonise the surface of medical devices [20]. As Figure 4 shows, there were live bacteria on the surface of unmodified PDMS after 48 h of incubation in the bacterial suspension for all three tested bacterial strains. As expected, the highest number of bacteria was *P. rettgeri* (Figure 4C). This observation confirms that medical devices made of PDMS are susceptible to the adsorption of various types of bacterial cells.

Figures 5-7 show a PDMS surface with different chitosan/alginate hydrogel coatings after 48-h incubation in bacterial suspensions. Adsorption studies for *M. luteus* showed that the PDMS surface with a hydrogel coating without the addition of lysozyme had a significant number of viable bacterial cells (Figure 5, H0). The coating with 0.5 mg/ml lysozyme showed single viable cells and a small amount of total bacterial cells (Figure 5, H0.5) and the coating with 1.0 mg/ml of lysozyme did not show any bacterial cells (Figure 5, H1.0). *M. luteus* are very sensitive to lysozyme activity. The bacteria were

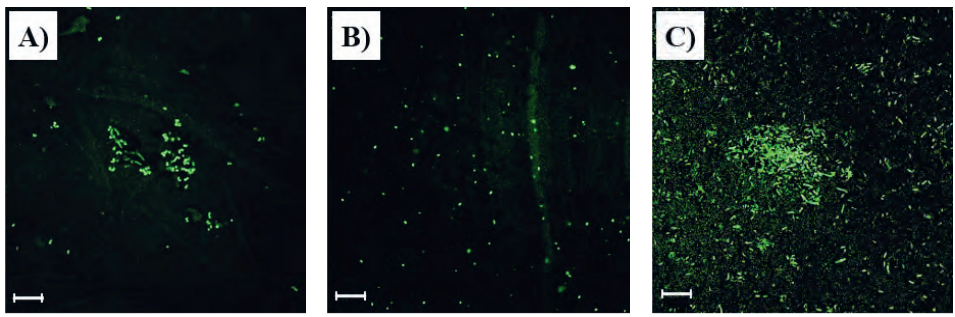

Figure 4. Confocal laser scanning microscope images of living bacterial cells adsorbed on an unmodified polydimethylsiloxane (PDMS) surface after 48 h of incubation in bacterial suspensions: (A) *Micrococcus luteus*, (B) *Escherichia coli* and (C) *Proteus rettgeri*. The staining is with fluorescein diacetate. The scale bar represents 20 μm

probably killed in the suspension by the enzyme released from the coating due to the higher concentration of lysozyme. These data confirm that the lysozyme immobilised in the structure of the hydrogel coating does not lose its activity.

Adsorption studies for *E. coli* and *P. rettgeri* showed similar results as *M. luteus*. There were many live bacteria on the PDMS surface with a hydrogel coating without the addition of lysozyme (Figure 6, H0, and Figure 7, H0). Coatings with 0.5 mg/ml lysozyme showed a significant reduction in the number of viable bacteria and many total bacterial cells (Figure 6, H0.5, and Figure 7, H0.5). Coatings with 1.0 mg/ml lysozyme showed only single viable cells with many total bacterial cells (Figure 6, H1.0, and Figure 7, H1.0). Almost all bacteria that got into the hydrogel coating with a higher concentration of lysozyme were killed. Hence, using this coating on medical devices made of PDMS could be effective in preventing urinary tract infections.

It is worth noting that *M. luteus* had the lowest adhesion to the surface of unmodified PDMS but was present in the highest number in the hydrogel coating without lysozyme. *P. rettgeri* had the highest adhesion to the PDMS surface and the lowest ability to colonise the hydrogel coating without any antibacterial agent. The hydrogel coating strongly hydrophilises the surface of a very hydrophobic polymer, a phenomenon that could cause that the cells of adherent bacteria to lose their ability to attach to the material.

4. Conclusions

The subject of this work was the synthesis, characterisation and microbiological studies of chitosan/alginate hydrogel coating with immobilised lysozyme for medical devices made of PDMS. The proposed methodology of PDMS surface activation, silanisation and coating preparation with layer-by-layer polyion deposition yielded a stable hydrogel on the material surface. Increasing the concentration of chitosan and alginate increased the amount of deposited hydrogel coating but reduced its stability. The highest stability after 24-h incubation in PBS (0.71 ± 0.01) was obtained for the lowest tested concentration of polyions, which was 5 mg/ml. The unmodified PDMS surface was slightly susceptible to the adhesion of *M. luteus* and *E. coli*. However, many *P. rettgeri* bacterial cells adhered to this surface and began to form biofilm after 48 h of incubation. The presence of the hydrogel coating causes a significant decrease in the number of *P. rettgeri* on the material surface. The strongly hydrophilic nature of the hydrogel probably causes difficulties in the adhesion of bacterial cells. However, the chitosan/alginate hydrogel coating does not provide bactericidal properties as its own. The addition of 1.0 mg/ml lysozyme to the chitosan solution killed almost all bacteria inside the coating structure for all tested bacterial strains: *M. luteus*, *E. coli* and *P. rettgeri*. Chitosan/alginate hydrogel coating with immobilised lysozyme could be considered effective in preventing adsorption of bacterial biofilm. The presented coating could be used for urological catheters or stents made of PDMS to provide protection against microorganisms causing infections and, due to low friction, may limit the formation of urinary tract microdamage that occurs during patient catheterisation or stenting procedures.

5. Acknowledgements

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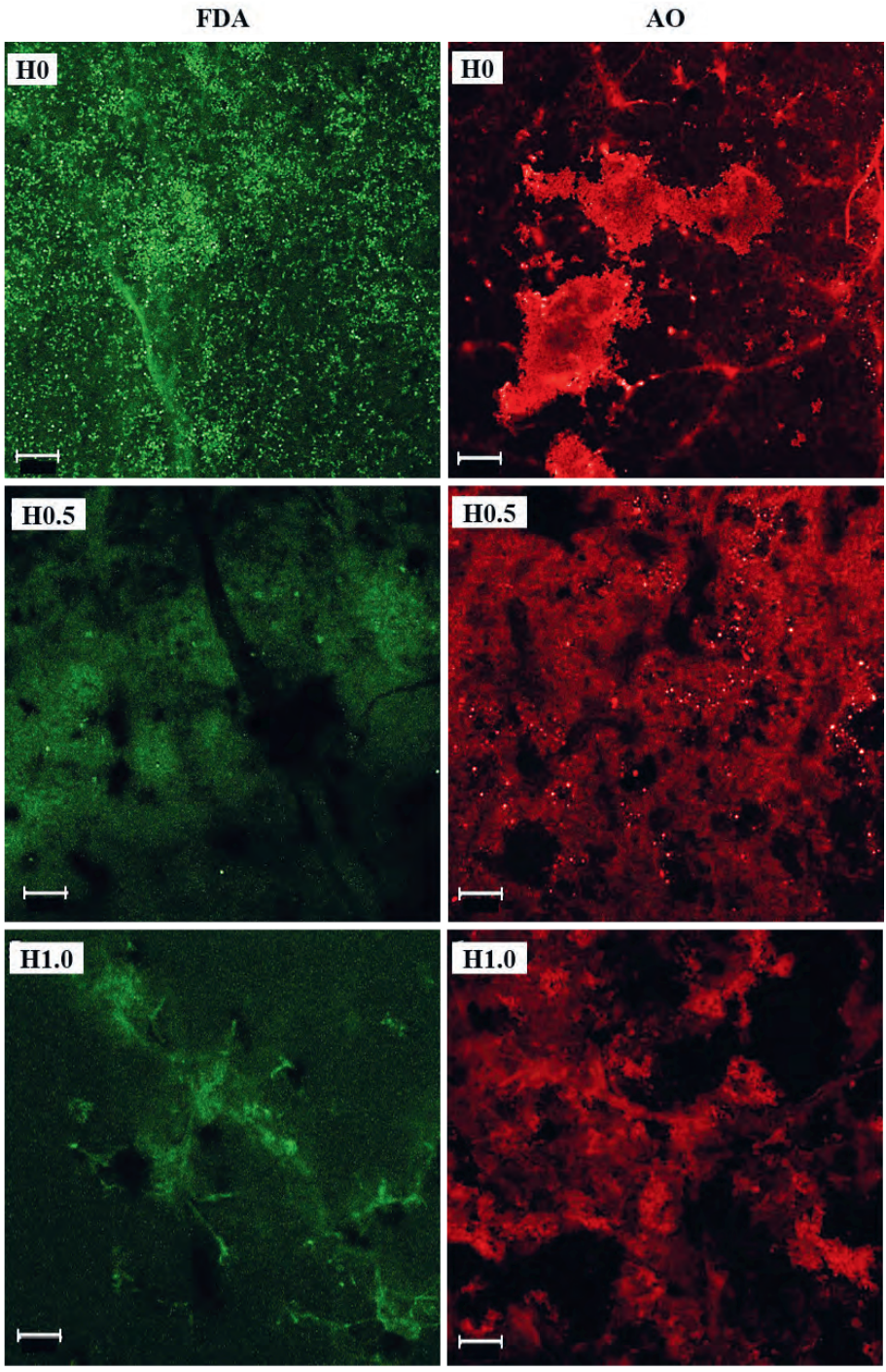

Figure 5. Confocal laser scanning microscope images of living (green) and all (red) *Micrococcus luteus* adsorbed on the polydimethylsiloxane (PDMS) surface with chitosan/alginate hydrogel coating without lysozyme (H0), with 0.5 mg/ml lysozyme (H0.5) or with 1.0 mg/ml lysozyme (H1.0) after 48 h of incubation in bacterial suspensions. Staining is with fluorescein diacetate (FDA) and acridine orange (AO). The scale bar represents 20 μm

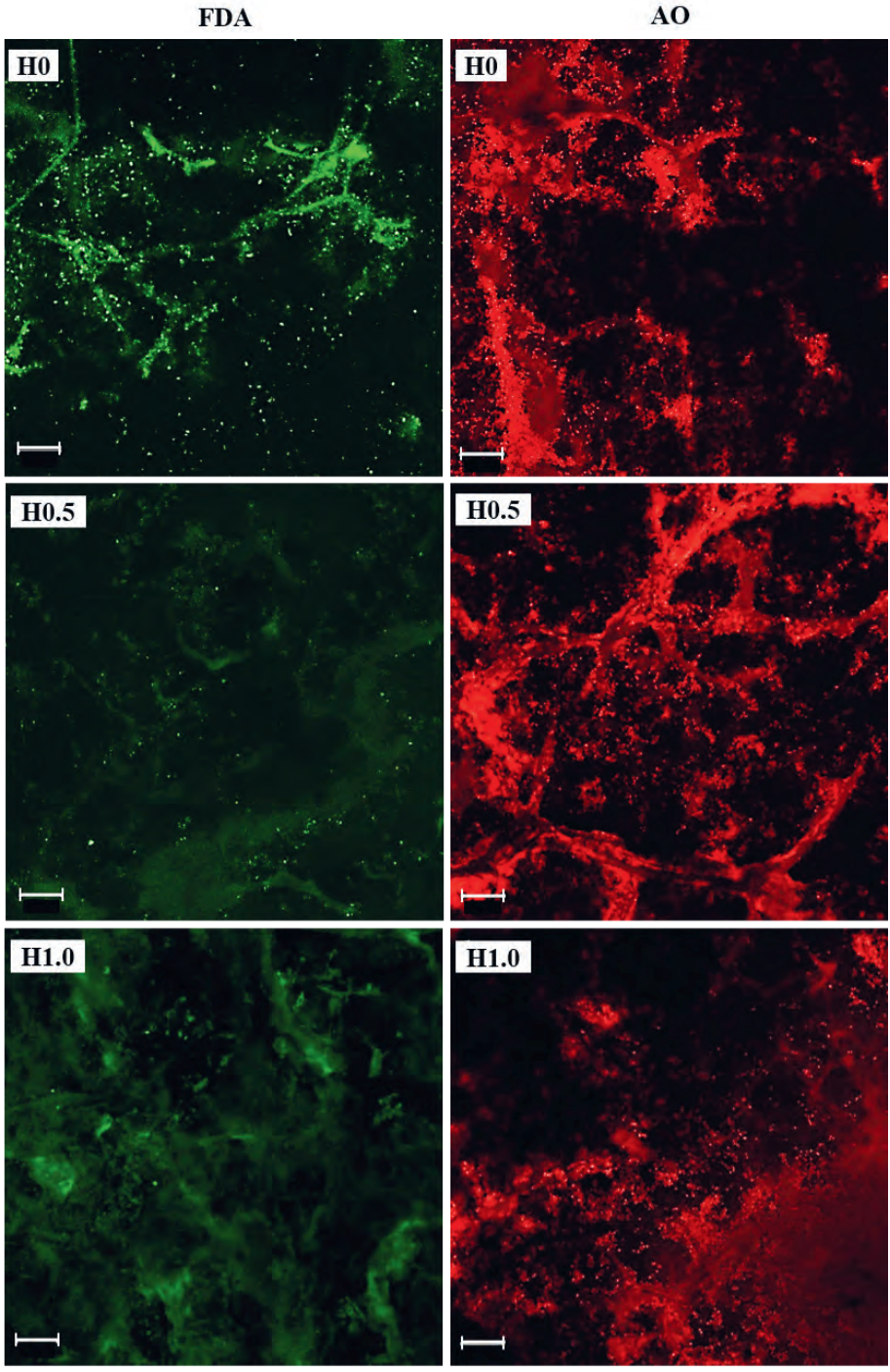

Figure 6. Confocal laser scanning microscope images of living (green) and all (red) *Escherichia coli* adsorbed on the polydimethylsiloxane (PDMS) surface with chitosan/alginate hydrogel coating without lysozyme (H0), with 0.5 mg/ml of lysozyme (H0.5) or with 1.0 mg/ml of lysozyme (H1.0) after 48 h of incubation in bacterial suspensions. Staining is with fluorescein diacetate (FDA) and acridine orange (AO). The scale bar represents 20 μm

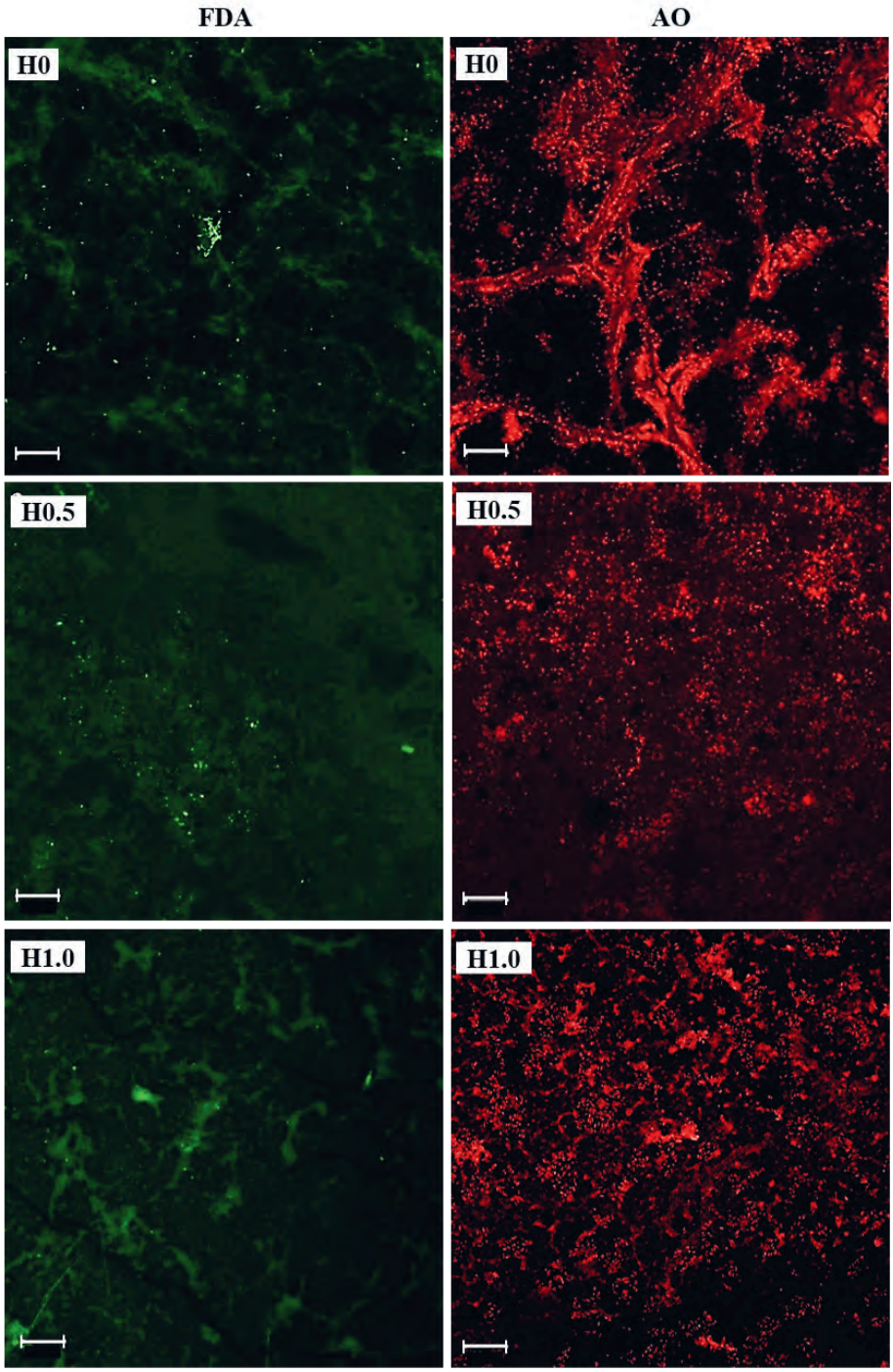

Figure 7. Confocal laser scanning microscope images of living (green) and all (red) *Proteus rettgeri* adsorbed on the polydimethylsiloxane (PDMS) surface with chitosan/alginate hydrogel coating without lysozyme (H0), with 0.5 mg/ml of lysozyme at a concentration (H0.5) or with 1.0 mg/ml of lysozyme (H1.0) after 48 h of incubation in bacterial suspensions. Staining is with fluorescein diacetate (FDA) and acridine orange (AO). The scale bar represents 20 μm

https://doi.org/10.15259/PCACD.26.012

6. References

[1] S.M. Jacobsen, D.J. Stickler, H.L.T. Mobley, and M.E. Shirtliff, "Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis," Clin. Microbiol. Rev., 21, 1, 26–59, 2008. **DOI**: 10.1128/CMR.00019-07.

[2] R.M. Donlan, "Biofilms and Device-Associated Infections," Emerg. Infect. Dis., 7, 2, 277–281, 2001. **DOI**: 10.3201/eid0702.010226.

[3] L.E. Nicolle, "Catheter associated urinary tract infections", Antimicrob Resist Infect Control., 3-23, 2014. **DOI**: 10.1186/2047-2994-3-23.

[4] H. Rebl, J. Renner, W. Kram et al., "Prevention of Encrustation on Ureteral Stents: Which Surface Parameters Provide Guidance for the Development of Novel Stent Materials?" Polymers, 12, 558, 2020. **DOI**:10.3390/polym12030558.

[5] A. Mosayyebi, C. Manes, D. Carugo, B.K. Somani, "Advances in Ureteral Stent Design and Materials", Curr. Urol. Rep., 19:35, 2018. DOI: 10.1007/s11934-018-0779-y.

[6] J. Liu, S. Qu, Z. Suo. and W. Yang1, "Functional hydrogel coatings", Natl Sci Rev, 8, nwaa254, 2021. **DOI**: 10.1093/nsr/nwaa254.

[7] W. Zhang, C. Pan, Q. Ge, L. Zhang, X. Wang, "Fabrication and Characterization of Chitosan/Alginate Multilayer Composite Membrane Supported by a Porous Poly(Acrylonitrile) Substrate for Pervaporation Dehydration of Alcohol", Journal of Membrane Science and Research, 3, 272-280, 2017. **DOI**: 10.22079/jmsr.2016.18586.

[8] A. Acevedo-Fani, L. Salvia-Trujillo, R. Soliva-Fortuny, O. Martín-Belloso, "Layer-by-Layer Assembly of Food-Grade Alginate/Chitosan Nanolaminates: Formation and Physicochemical Characterization", Food Biophys, 12:299–308, 2017. **DOI**:10.1007/s11483-017-9486-3.

[9] C. Bilbao-Sainz, B.S. Chiou, K. Punotai et al., "Layer-by-Layer Alginate and Fungal Chitosan Based Edible Coatings Applied to Fruit Bars", J Food Sci, 83 (7): 1880–1887., 2018. **DOI**: 10.1111/1750-3841.14186.

[10] M.T. Cook, G. Tzortzis, V.V. Khutoryanskiy, D. Charalampopoulos, "Layer-by-layer coating of alginate matrices with chitosan–alginate for the improved survival and targeted delivery of probiotic bacteria after oral administration", J. Mater. Chem. B, 1, 52, 2013. **DOI**: 10.1039/C2TB00126H.

[11] J. Hatami, S.G. Silva, M.B. Oliveira et al., "Multilayered Films Produced by Layer-by-Layer Assembly of Chitosan and Alginate as a Potential Platform for the Formation of Human Adipose-Derived Stem Cell aggregates", Polymers, 9, 440, 2017. **DOI**:10.3390/polym9090440.

[12] J. Zhou, G. Romero, E. Rojas, L. Maa, S. Moya, C. Gao, "Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles for antifouling protection and Folic acid binding to achieve selective cell targeting", J. Colloid Interface Sci, 345, 241–247, 2010. **DOI**: 10.1016/j.jcis.2010.02.004.

[13] K. Zhang, H. Cui, H. Jiang et al., "The current status and applications of ureteral stents", Int J Clin Exp Med;13(4):2122-2133, 2020. ISSN:1940-5901/IJCEM0106761.

[14] A.V. Krasnoslobodtsev and S.N. Smirnov, "Effect of water on silanization of silica by trimethoxysilanes," Langmuir, 18, 8, 3181–3184, 2002. **DOI**: 10.1021/la015628h.

[15] A.B.D. Nandiyanto, R. Oktiani, R. Ragadhita, "How to Read and Interpret FTIR Spectroscope of Organic Material", Indones. J. Sci. Technol., 4, 1, 97-118, 2019. **DOI**:10.17509/ijost.v4i1.15806.

[16] P.T. Hammond, "Form and Function in Multilayer Assembly: New Applications at the Nanoscale," Adv. Mater., 16, 15, 1271–1293, 2004. **DOI**: 10.1002/adma.200400760.

[17] V. Boyd, O. Cholewa, and K. Papas, "Limitations in the use of fluorescein diacetate/propidium iodide (FDA/PI) and cell permeable nucleic acid stains for viability measurements of isolated islets of Langerhans," Curr Trends Biotechnol. Pharm., 2, 2, 66–84, 2008. PMCID: PMC2931281.

[18] L. Netuschil, E. Reich, and M. Brecx, "Direct measurement of the bactericidal effect of chlorhexidine on human dental plaque.," J. Clin. Periodontol., 16, 8, 484–8, 1989. **DOI**:10.1111/j.1600-051x.1989.tb02324.x.

[19] E.Y. Seo, T.S. Ahn, and Y.G. Zo, "Agreement, Precision, and Accuracy of Epifluorescence Microscopy Methods for Enumeration of Total Bacterial Numbers," Appl. Environ. Microbiol., 76, 6, 1981–1991, 2010. **DOI**: 10.1128/AEM.01724-09.

[20] S. Sagar, N. Narasimhaswamy, J. D'Souza, "Providencia Rettgeri: An Emerging Nosocomial Uropathogen in an Indwelling Urinary Catheterised Patient", J Clin Diagn Res., 11(6): DD01–DD02, 2017. **DOI**: 10.7860/JCDR/2017/25740.10026.