



Tomas Bata University in Zlín

Doctoral Thesis Summary

**Preparation and characterisation of saccharide-based
antibacterial coatings**

**Příprava a charakterizace antibakteriálních vrstev na bázi
sacharidů**

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Abstract

This thesis for state doctoral exam aimed at development and characterization of saccharide-based bioactive coatings for biomedical applications. As a degradable, environmentally friendly polymer, polylactic acid was the material of choice for studying the effects of the coatings on the materials biocompatibility and antibacterial properties. The first part of the research focuses on preparation, characterization of glucosamine and chondroitin sulfate immobilized surface-activated PLA films, and the second part is dedicated to evaluating the chitosan and chondroitin sulfate coatings. Both experimental parts were also comprised of antibacterial activity and biocompatibility studies. Moreover, the release trend of the antibiotic lomefloxacin loaded chitosan and chitosan-chondroitin sulfate coated films were described.

Key words: biomaterials, saccharides, coating, antibacterial activity, biocompatibility

Abstrakt

Toto pojednání ke státní doktorské zkoušce je zaměřeno na vývoj a charakterizaci bioaktivních povlaků na bázi sacharidů pro aplikace v biomedicíně. Kyselina polymléčná byla použita pro studium vlivu povlaků na biokompatibilitu materiálů a antibakteriální vlastnosti jako odbouratelný polymer šetrný k životnímu prostředí. První část výzkumu je zaměřena na přípravu a charakterizaci povrchově aktivovaných PLA filmů s imobilizovaným filmem glukosaminu a chondroitin sulfátu a druhá část je věnována hodnocení chitosanových a chondroitin sulfátových povlaků. Obě experimentální části zahrnují též studie antibakteriální aktivity a biokompatibility. Mimo to byla popsána kinetika uvolňování chitosanu s obsahem antibiotika lomefloxacinu a filmů na bázi chitosan-chondroitin sulfát.

Klíčová slova: biomateriály, sacharidy, povlaky, antibakteriální aktivita, biokompatibilita

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1. INTRODUCTION

Despite all the comprehensive research and development studies, biomaterial devices and implants related infections are still a major health concern for their long-term usage. It is apparent that many microorganisms can attach to a wide range of biomedical instruments and can cause infections, which are also known as nosocomial infections. Apart from the severe prevalence and mortality rates, these infections increases the length of hospitals stays and health-care costs. Therefore, actions are needed to be taken to remove or reduce bacteria colonization by tailoring surface properties of biomedical devices that are unfavourable for microorganism attachments.

One main reason of biomaterial-associated infection is bio-inert materials that cause bacteria attachment and biofilm formation on the surface. So, biomaterial-related infections can be prevented with the usage of immobilized antibacterial agents that can inhibit bacteria adherence or kill them upon contact.

For the biomaterials that are susceptible to bacteria colonization, surface modification is an important approach in the fabrication of antibacterial devices and implants. In recent years, biofunctionalization of materials with antibacterial features focuses on the immobilization of bactericidal agents to the biopolymer surface, such as antibiotics, antimicrobial enzymes and peptides, cationic molecules, metals and so on [1]. However, there are some limitations, including burst release of the antibacterial agents, cytotoxicity issues, and multi-resistance emergence [2].

Polylactic acid has exceptionally good advantages over conventional polymers. The US Food and Drug Administration (FDA) have approved PLA products as suitable for direct contact with body liquids. The most important four advantages of this polymer are biocompatibility, processability, low cost production and renewability [3]. However, one of the major drawback of using PLA in biomedical field is their vulnerability to bacterial invasion [4]. To be able to use as a biomaterial, PLA-based materials should be both biocompatible and antibacterial. The lacking of reactive functional groups on the polymer surface makes it chemically inactive. Due to this, before any application on the polymer surface it needs to be activated.

The presented thesis is devoted to the preparation of antibacterial surfaces based on saccharides coating on PLA.

In the first section a general overview of biomaterials, PLA-based materials, biofilm formation and antibacterial coating are reported. At the end a general introduction to saccharides and the studies of them as antibacterial agent is given. In the second section, the experimental part, the method used for saccharides coating preparation and characterization, loading and release studies of a model drug and the most significant results are illustrated.

2. Theoretical Part

2.1 Biomaterials

Biomaterials have an enormous impact on medicine today. They can be defined as the materials in contact with living tissues, organisms and microorganisms with the purpose of any therapeutic and diagnostic procedures. More specifically, they can be defined as synthetic or natural any material in contact with cells, blood, tissues and biological fluids, intended to use as diagnostic, therapeutic, prosthetic and storage applications without causing any adverse effect in the living organism and its components [5].

Usage of a biomaterial in contact with biological medium is possible with the reliable and efficient properties of the materials. These properties are supported with a combination of chemical, physical, mechanical and biological characterizations. In the designing and engineering of biomaterials, the materials that have been benefited from are classified under four broad classes of materials and their combination. Those are ceramics, metals, polymers and composites. Some characteristics are required to be able to use a material in a medical manner [5]. These can be listed as;

- technical functionality,
- sufficient stability against physiological media,
- residue-free metabolism for biodegradable materials,
- high biocompatibility,
- simple processing
- sufficiently long shelf-life

Among class of biomaterials, polymers possess unique properties that make them the largest class in biomedical field [6]. The current medical applications of them include artificial hearts, vascular grafts, dental materials, lenses, cardiac valves, dialysis systems, coating materials, surgical materials, etc. [5].

The most common polymeric biomaterials used in medical field are poly(methyl methacrylate), polyacrylamide, polyethylene, poly(vinyl chloride), polypropylene, polydimethylsiloxane, poly(ethylene terephthalate), cellulose acetate and polylactic acid.

2.2 Polylactic Acid

Polylactic acid (PLA) is a highly versatile polymer commonly used in biomedical field for tissue engineering and drug delivery systems. The application field of PLA are expanding with usage in human body creating particular great global demand. This biodegradable polyester product is the second most important bioplastic of the world in 2021 [7].

PLA comprises lactic acid monomers connected through the ester bonds, which makes it classified as an aliphatic polyester. These lactic acid monomers are derived from natural and 100% renewable resources, like corn, starch, sugarcane or tapioca roots. [8,9]. In the production of PLA, many different polymerization processes take place, such as ring opening, polycondensation, azeotropic dehydration and enzymatic degradation. Nowadays, ring opening polymerization and enzymatic degradation processes are the most used techniques in PLA production.

PLA is one of the most promising bioplastic and offers an alternative to traditional metallic and ceramic biocompatible materials, thanks to its unique combination of properties. These are (i) biodegradability (ii) processibility (iii) good mechanical properties and (iv) eco-friendliness [10].

For the successful implementation of PLA in biomedical applications, besides its good mechanical performance, controlled surface properties are also crucially important. However, PLA has some limitations for biomedical applications such as low wettability and being lack of reactive side-chain groups [11]. PLA is an intrinsically hydrophobic polymer, which has a static contact angle in the range of 80-85°. This surface hydrophobicity have raised the concerns of bio-incompatibility of PLA to function as a biomaterial in medicine. Moreover, although it contains methyl and carbonyl as the pendant chemical groups on the polymer backbone, lacking of reactive side-chain groups results in a chemically inert polymer surface. These major drawbacks in surface properties interferes with compatibility and surface modification properties, eliciting inflammatory response in the direct contact with biological parts due to the low cell affinity in biomedical applications and resulting a challenging step for surface modification [12].

Because PLA is bio-inert and do not exert antibacterial behavior, various methods have been applied to produce antibacterial PLA materials.

2.3 Surface Activation Methods

In general, surface treatment techniques can be classified as physical and chemical methods, which contain non-covalent and covalent attachment of functional groups, respectively. The most common example of chemical surface treatments is coating by means of wet chemistry. However, these chemical methods consist of strong oxidizing agents, acids, or solvents that may generate wastes and potentially be harmful to the environment. Although chemical surface modifications have been utilized for many years in a very effective way, physical treatments have been developed to overcome the disadvantages related to the chemical methods, such as potential undesired changes on polymer structure, rigorous process control, and environmental problems. Among these physical surface modification methods, plasma technology based treatments is growing in a remarkable way with their numerous advantages, as follows [13]:

- Without causing any changes in the bulk material, they selectively modify the outmost layers of the polymer surface;
- By choice of the gas used, the type of the inserted functional groups on the surface can be adjusted;
- A fairly homogenous modification can be accomplished over the whole surface;
- It is possible to avoid the problems of wet chemical methods, such as substance swelling, and solvent residual on the surface.

2.3.1 Plasma Treatment

Plasma technology is suitable for almost all the polymers and it is very effective to modify the surface functionality of the materials. Until 1927, three commonly states of matter had been known, which are solid, liquid and gas. A heated solid transforms into a liquid and then finally into a gas. When enough energy is supplied to gas, it becomes ionized that contains reactive chemical species such as ions, electrons, atoms, subatomic particles, neutral molecules [14].

The glow discharge is one of the cold plasma techniques that is commonly used for the thin film synthesis. The conventional excitation sources in cold plasma are direct current (DC), radio frequency (RF) and microwave. Apart from the excitation sources, the working pressure, applied energy field, gas flow rate are also important parameters that effect electron density and energy. Depending on the electron energy, various chemical reactions take place in collisional movements.

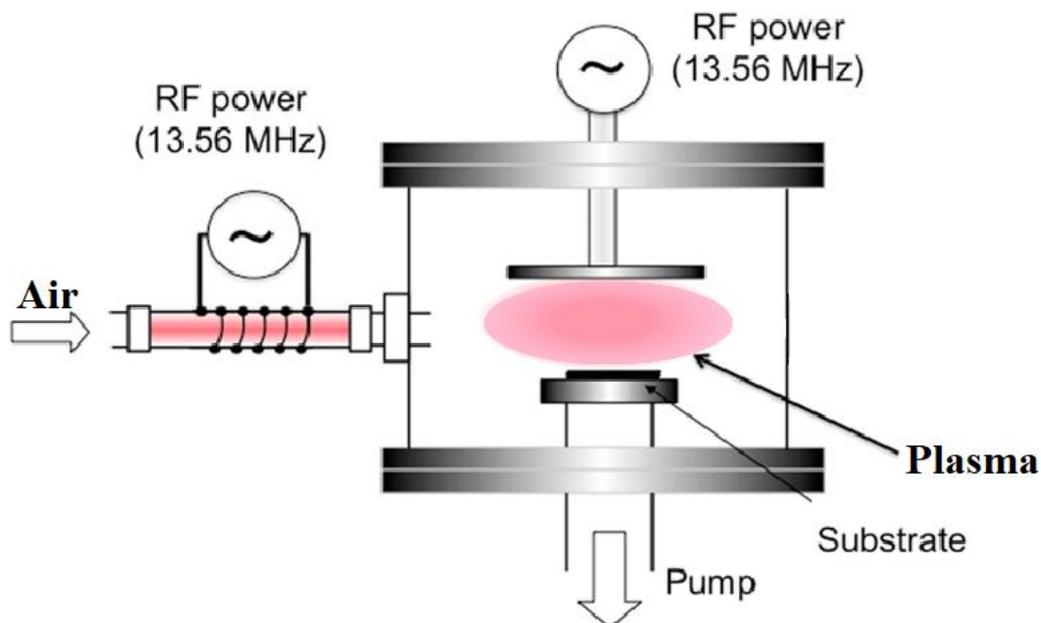


Figure 1. Low pressure Radio Frequency Plasma Technology mechanism

2.4 Antibacterial Coating

One of the major problem of using biomaterials in biomedical applications is their susceptibility to infection due to the bacteria colonization and proliferation on the material surface [15]. To prevent any possible adverse effects of bacterial infections, the design of advanced biomaterials with antibacterial properties is highly anticipated.

Apart from intrinsic susceptibility, physical and/or chemical treatments of the biomaterial surface may result in a substantial change of its bacterial infection susceptibility. For instance, a change in the surface properties of an implant, like roughness, wettability, conductivity, surface chemistry and surface energy plays a detrimental role in terms of bacterial invasion.

Antibacterial coatings are deposited on biomaterial surfaces with the purpose of reducing the implant-related infection possibilities. Depending on the delivery mechanism, they can be passive or active (Figure 2). Passive coatings can prevent bacterial attachment and/or cause bacteria killing upon contact to the material surface. The physicochemical properties of the coating, such as hydrophilicity, surface roughness, surface charge have crucial importance on the bacteria behaviour [16].

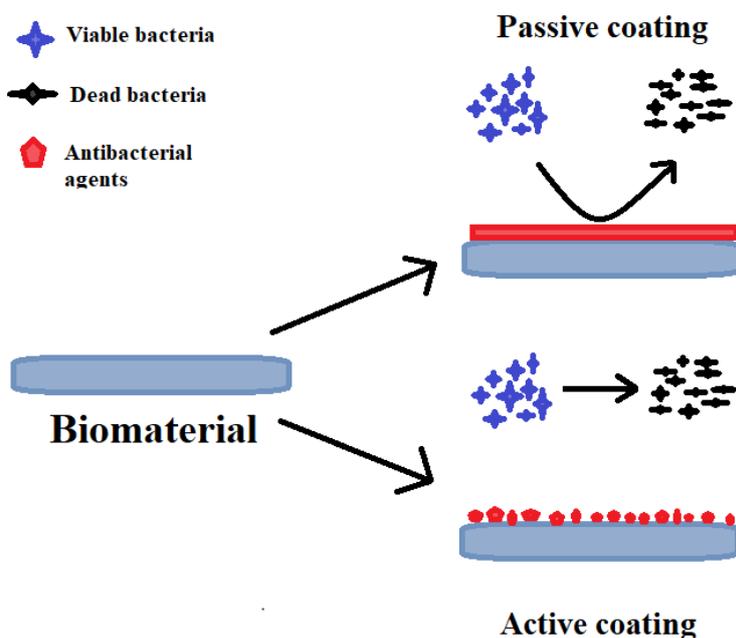


Figure 2. Schematic representation of active and passive coating mechanisms (modified after Oin 2018) [2]

In the case of active coatings, the loaded antibacterial agents release to the peripheral tissues by diffusion or dissolution. A number of antibiotics have been loaded in biopolymer coatings. Although they possess a broad bacteria-killing spectrum, optimum antibiotic release kinetics that do not cause harmful side

effects on cellular functions is difficult to achieve. More importantly, every time in antibiotic usage, development of potential drug resistance of bacteria is a challenging problem. To overcome this possibility, coatings containing non-antibiotic organic agents is a promising method. In addition, passive coatings are much more preferable as they do not induce bacterial resistance [16].

2.5 Biofilm Formation

All microorganisms need to adhere to surfaces as a part of their survival mechanism. This adherence is mostly dependent on surface-related interactions between the target biomaterial and planktonic bacteria or microorganisms. And adherence kinetics are also affected by other factors such as type of serum proteins, flow conditions [17].

With the biofilm formation, bacteria become more resistant to host defense mechanisms and antibacterial agents. Once it formed, the complex structure of the biofilm makes it extremely difficult to treat with conventional antibiotic therapies [18]. Thus, prevention of a biofilm formation and bacterial adhesion is actually preferable to any post-infection treatment. The initial 6h period in the biomaterial implantation is crucially important in the device-related infection prevention.

2.6 Saccharides

Saccharides or carbohydrates are organic compounds with the empirical formula of $C_m(H_2O)_n$. The saccharides are composed of four chemical groups: monosaccharides, disaccharides, oligosaccharides and polysaccharides. Monosaccharides are the smallest carbohydrates and the building blocks of polysaccharides by connecting through *o*-glycosidic bonds. The specific physical and chemical properties of the polysaccharides depend on the composition of monosaccharides, chain type, molecular weight, and other factors. According to their charges, saccharides can be classified as positively charged (chitosan, glucosamine) and negatively charged (e.g., pectin, chondroitin sulfate, carrageenan) polysaccharides [19]

2.6.1 Glucosamine

As a natural monosaccharide, glucosamine (GlcN) is synthesized in all organisms and derived from the substitution of the hydroxyl group of a glucose molecule with an amino group. GlcN plays a vital role in the protection of joint cartilage by promoting glycosaminoglycans (GAGs) synthesis [20]. In addition to its chondroprotective properties, it has reported that GlcN has anti-inflammatory, antioxidant, cardioprotective and antibacterial effect [20,21]. Moreover, it is stated that, GlcN increases the function of other antimicrobial compounds [22].

Veerapandian et al. reported that antibacterial activity of GlcN-Ag nanoparticles were higher than only Ag nanoparticles in all strains and this may be related to surface functionalization with GlcN, which facilitates the distribution and penetration of glycol-nanoparticles into the bacterial cell surface [23]. In a later study, they functionalized copper nanoparticles with GlcN and resulted that GlcN-CuNPs exhibited a prominent bactericidal activity against two Gram-positive and Gram-negative bacteria strains, compared to simple copper NPs and an antibiotic drug kanamycin [23].

In another study, Govindaraju et al. compared the antibacterial activity of gold nanoparticles (AuNPs) and GlcN-AuNPs [24]. They reported that while the bacterial growth was observed to be $63.25 \pm 5.45\%$ in AuNPs, at the same concentration of GlcN-AuNPs bacterial growth was observed $6.18 \pm 3.11\%$. Treatment of microorganism with GlcN grafted AuNPs resulted in greater bacterial inhibition than the only AuNPs.

Hao et al has studied the antibacterial performance of both multiwalled carbon nanotubes (MWCNT) and AgNP with and without GlcN grafting [25]. Firstly, they stated that the GlcN-AgNP showed better antibacterial activity than MWCNT-AgNPs. Additionally, it is suggested that the large number of hydroxyl groups on GlcN can enhance the capacity of AgNPs and lead to the GlcN-AgNPs to have a better bacteria killing property than MWCNT-AgNPs. Lastly, with the grafting of GlcN on MWCNT-AgNPs, the samples exerted extraordinary antibacterial performance and were effective on both bacteria species.

2.6.2 Chondroitin Sulfate

Chondroitin sulfate (ChS) is a negatively charged sulfated branched polysaccharide which is member of GAG family [26-28]. It is one of the main components of ECM of articular cartilage, which is a fibrous connective tissue in human body. Like GlcN, ChS also helps to maintain cartilage activities by retaining water and acts as a lubricant. In addition, it supports cell adhesion, proliferation, differentiation. ChS has documented as anti-inflammatory properties, and potential as an antibacterial agent [29-33]. These all properties suggest that ChS could combine many advantages as a coating on biomaterials such as vascular implants, scaffolds, bone implants, and drug delivery systems [34-37].

Antibacterial activity of ChS has been studied in some researches. Sharma et al. has studied the ChS and CS scaffolds antibacterial property and resulted that when they combined together the formed PEC showed an additive effect on the bacteria species [38].

In a recent study Cestari et al. worked on nanofibers containing silk fibroin, ChS and silver sulfadiazine. For the antibacterial activity assessment, they used zone of inhibition test and reported that concentration of ChS greatly effects the inhibition zone of the fibers [39].

2.6.3 Chitosan

Chitosan (CS) is a polycationic polysaccharide that is obtained by alkaline deacetylation of chitin (should be greater than 50%), which is the most abundant polysaccharide in nature and structural component of fungal cell walls, insect exoskeletons, and shells of crab, shrimp and lobster [40]. It has favourable properties, such as biocompatibility, biodegradability, antimicrobial and antitumor activity, minimal foreign body reaction, availability of chemical side groups for attachment and modification [36].

Chitosan is one of the most studied non-migratory antibacterial biomaterial. This polysaccharide is processed according to the needed conformation such as film casting, fiber spinning, and freeze-dried sponges and so on [41].

Many studies document the antibacterial activity and mode of action of CS. There are several possible mechanisms about the antibacterial activity of CS. However, despite the amount of literature available, the antibacterial effectiveness of CS and its derivatives against Gram-positive and Gram-negative bacteria and their mechanisms are somewhat controversial.

CS interaction with the pathogen DNA triggers inhibition of mRNA synthesis and interferes with protein synthesis [42]. In addition, it creates chelates with metals, elements and essential nutrients and cause malnutrition of bacteria.

Antibacterial activity of CS is affected by a number of factors that act in an orderly and independently manner. The most prevalent proposed mechanisms are [41,42]:

- Binding to the negatively charged bacteria cell wall and causing disruption by altering the membrane permeability
- Acting as a chelating agent and selectively binding to the trace metals that are crucial for stability of the bacteria cell wall
- Forming an impermeable layer around the cell and blocking the transportation of essential solutes

CS has differing inhibitory efficiency against gram-positive, gram-negative bacteria and different fungi. Antifungal activity of this polysaccharide is exerted by sporulation and spore germination suppression. However, the mode of action against gram-positive and gram-negative bacteria strains are rather complicated due to the differences in cell surface structures.

Goy et al. stated that *E.coli* (gram-negative) strain was more resistant to bacterial-action of CS than *S.aureus* [43]. Additionally, they showed that the concentration of polymer had the profound effect on bacteria killing property. However, in some of the other published works, the literature presents that CS generally acts stronger on Gram-negative than on Gram-positive strains.

Benhabiles et al. reported that CS oligosaccharides (COS) exhibits higher bactericidal activity than CS due to they are more soluble in water than the native polysaccharides. Moreover, they stated that CS has also better in bacteria killing than chitin due to the polycationic amines group in its structure [44].

2.7 Coating Approaches

2.7.1 Plasma post-irradiation grafting

Plasma post-irradiation grafting is a two-step technique of which the first step plasma treatment followed by polymerization reactions. While plasma treatment creates temporary, limited lifetime species, post-irradiation grafting method results in permanent effect. In this step, the activated polymer surface is brought into contact with monomers. By immersing into a monomer solution, polymerization will take place by radical polymerization reactions.

Polymeric brushes consist of monolayer of chains that has one end of which is attached to a solid substrate while the other end is freely exposed to the surrounding media [45]. Polymeric brushes have been used in many biological applications thank to their well-defined architecture, highly accessible functional groups and the ability to immobilize a variety of substances [46].

Polyacrylic acid (PAA) is a biocompatible, hydrophilic polymer that possess one carboxyl group at each monomers. Among other polymeric brushes, PAA has become a very attractive option with its hydrophilic property and reversible swelling-contracting structure [47].

The attachment of biologically active substances to the PLA surfaces through a suitable crosslinker is an alternative way to impart desired physicochemical and biological properties to the biomaterial. This crosslinker possesses easily reactive groups: hydroxyl, amino, carboxyl groups or combination of these functional groups. A suitable crosslinker should has non-toxicity, easy availability, low cost and hydrophilic properties [48].

2.7.2 Carbodiimide Chemistry

Carbodiimides are important classes of reactive organic substances that possess the heterocumulene structure $R-N=C=N-R$. They can classified as carbon dioxide diimides or 1,3-substituted urea anhydrides [49]. These organic compounds are synthesized by dehydration of ureas or thioureas.

These coupling agents are also known as zero-length crosslinkers and mainly used in crosslinking of amines and carboxylates by promoting covalent bond formation [50]. It is called as zero-length crosslinkers because after ester formation between these two functional groups, carbodiimide agents leave the reaction as byproducts of urea derivatives.

The most common carbodiimide agents are N,N-dicyclohexylcarbodiimide (DCC) and N-ethyl-N-(3-dimethylamino)propylcarbodiimide (EDC). Due to its water solubility property, using EDC is more advantageous. It can be either used alone or in combination with N-hydroxy succinimide (NHS). NHS allows EDC to form a more stable intermediate product than the o-acylisurea and improves reaction efficiency [51].

AIM OF THE DOCTORAL THESIS

The overall purpose of the work presented in this thesis is to contribute to the field of biomedical applications of biopolymers possessing antibacterial properties. In accordance with this aim, the main attention is paid to the study of saccharides as antibacterial coating material on activated polylactic acid surfaces, and evaluate their efficiency both in combination, and individually.

The main goal of this study has been subdivided into following major points:

- The preparation of uniform polylactic acid films by using compression molding technique
- The surface activation of the films through radio frequency plasma treatment and subsequent assessment of chemical and physical changes by using characterization techniques
- The introduction of saccharides onto the PLA surface through carbodiimide chemistry and/or polymeric brushes
- Investigation of the role of individual and combined saccharides on the antibacterial activity, cytotoxicity, drug loading and releasing properties of PLA films

3. Experimental Part

3.1 Preparation and characterization of immobilized glucosamine/chondroitin sulfate on polylactic acid films

Poly(lactic acid) (PLA) is one of the most produced polymeric materials, due to its exceptional chemical and mechanical properties. Some of them, such as biodegradability and biocompatibility, make them attractive for biomedical applications. Conversely, the major drawback of PLA in the biomedical field is their vulnerability to bacterial contamination. This study focuses on the immobilization of saccharides onto the PLA surface by a multistep approach, with the aim of providing antibacterial features and evaluating the bacteria-killing properties of these saccharides.

GlcN is used in biomedical applications in combination with ChS to achieve a synergistic effect [52, 53]. For example, the efficacy of GlcN and ChS in treatment of symptomatic osteoarthritis of the knee [54], stimulation of vasculogenesis and angiogenesis [55], and treatment of Kashin-Beck disease [56] have been evaluated in some research. However, fewer researches have been conducted on the possible different effects of these saccharides and their combination on the antibacterial activity and cytotoxicity.

Thus, the present study aims to develop a PLA-based antibacterial platform using two common saccharides, which also have cell proliferation and adhesion features, and also to compare the antibacterial and cytotoxic activity of GlcN and CS, individually and in combination. While clinical trials and most in vitro studies have focused on the synergistic effects of glucosamine and CS in treatment of osteoarthritis, the present study focuses on the prevention of bacterial growth on polymer biomaterials by using saccharides. For this purpose, low-pressure radio frequency plasma (RF) method was utilized to activate the PLA surfaces and the molecules of GlcN and ChS were immobilized on the surface of the PLA films to improve in antibacterial activity. The bacterial adhesion of surface modified PLA films was systematically investigated by antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

3.1.1 Preparation

Prior to compression molding process, PLA pellets were first dried in a desiccator at 60 °C overnight to eliminate humidity. Then, to obtain PLA sheets the pellets were hot-pressed at 180 °C for 20 min and then the molding plates were placed in another press for cooling. Sheets of PLA cut into samples of 25 × 25 mm for further surface treatments. After that, each side of the PLA films were treated using radio frequency (13.56 MHz), low-pressure plasma equipment by generating air plasma at power 50 W for 60 s. For the preparation of poly acrylic acid brushes, PLA films were immersed immediately into AAc solution after the irradiation step. Following the grafting reaction, the samples were immersed into

either 1 w% GlcN or 1 w% ChS solutions for 24 h. Thereafter, each sample was washed with deionised water. Subsequently, GlcN immobilized samples were lastly placed into 1 w% ChS solution. The films were then removed, washed in distilled water and left for drying overnight for further characterization.

3.1.2 Results and Discussion

Surface Chemistry and Morphology

Static contact angle measurements (Figure 3) revealed that the contact angle of untreated PLA decreased significantly to 47° after plasma treatment because of the presence of plasma-induced hydrophilic oxidative functional groups. The GlcN immobilization to PLA resulted in an increased hydrophobicity but remained lower than untreated PLA. In contrast, ChS immobilization led to a lower contact angle value, which can be connected to the more hydrophilic character of ChS. The combined immobilization of ChS and GlcN resulted in a slightly higher contact angle value than the plasma treated sample. The results preliminarily suggest successful modification of the polymer surfaces, which is supported by the difference between the elemental compositions of PLA samples.

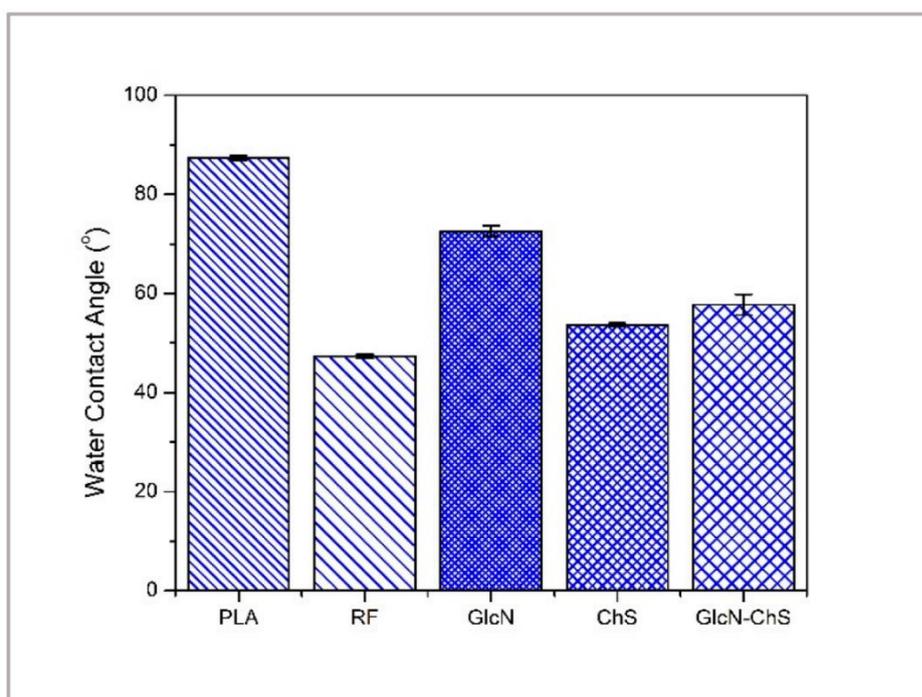


Figure 3. Water contact angles of untreated and treated PLA. Results represent mean value from three independent experiments with standard deviations.

For the analysis of surface chemical compositions of the PLA surfaces, XPS measurements were carried out before and after the treatments. Figure 3 shows

the XPS full spectra of the samples with their corresponding surface elemental compositions and N1 core level spectra. The XPS spectra (Figure 4A) for all samples show two main contributions corresponding to C(1s) at 285 eV and O(1s) located at 533 eV, due to the chemical structure of PLA. After air-plasma treatment, an increase in the peak intensity corresponding to the O(1s) transition can be clearly seen, due to the presence of oxide functional groups. In addition, a small peak that corresponds to the contribution of nitrogen, N(1s), with a binding energy of 400 eV is observed in the plasma treated sample. This nitrogen content stems from the air plasma application, which is mainly composed of oxygen (O) and nitrogen (N) radicals. After GlcN immobilization, it is expected that the nitrogen element appears on the PLA film surface. However, from the XPS spectra of GlcN immobilized PLA films (Figure 4A-d), no obvious N1s peak can be detected. This might result from the low amount of GlcN immobilized on the PLA surface. Figure 4B-c, B-e show the increase in the intensity of the nitrogen peak, which indicates ChS presence on the PLA surface.

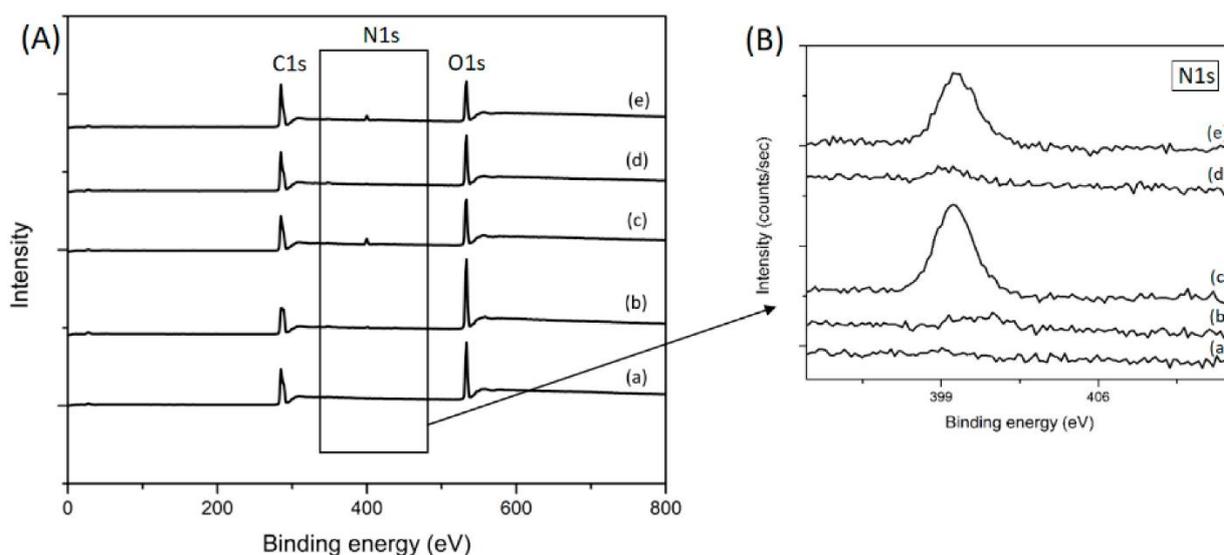


Figure 4. High resolution XPS spectra of (A) PLA films (a) untreated PLA; (b) plasma treated PLA; (c) ChS; (d) GlcN; (e) GlcN-ChS grafted films. (B) N1 core level spectra of PLA

The elemental content of carbon, oxygen, nitrogen, and sulfur in each sample is summarized in Table 1. According to the chemical structure of PLA, the increased O/C ratio in the RF samples indicates the presence of oxide functional groups just after the air plasma treatment. Moreover, untreated PLA films had no nitrogen and sulfur elements, whereas the nitrogen contents were observed for all the other samples, and sulfur contents were found for ChS and GlcN-ChS

immobilized samples. These nitrogen and sulfur contents are the proof for successful activation of the PLA surface with plasma treatment and grafting of the saccharides.

Table 1. Elemental composition of the films

Sample type	C (%)	O (%)	N (%)	S (%)	C/O
PLA	67.8	31.9	-	-	0.47
RF	60.6	38.2	0.9	-	0.63
GlcN	69.4	29.6	0.5	-	0.44
ChS	66.3	28.9	4.4	0.1	0.43
GlcN-ChS	70.7	25.1	3.6	0.3	0.36

Biocompatibility

Biocompatibility data are presented in Figure 5. It can be seen that all of the relative cytotoxicity values (%)-except for untreated PLA-are higher than 80%, independent of polymer modification. One possible reason for this phenomenon is the increased hydrophilicity of untreated PLA with various modifications (plasma treatment, grafting). The GlcN grafted samples display 85% cell viability, while the ChS immobilized samples have more than 120% viability of the cells. For the combination of these two saccharides, the cell viability increases to 148%, which shows better biocompatibility than either saccharide alone.

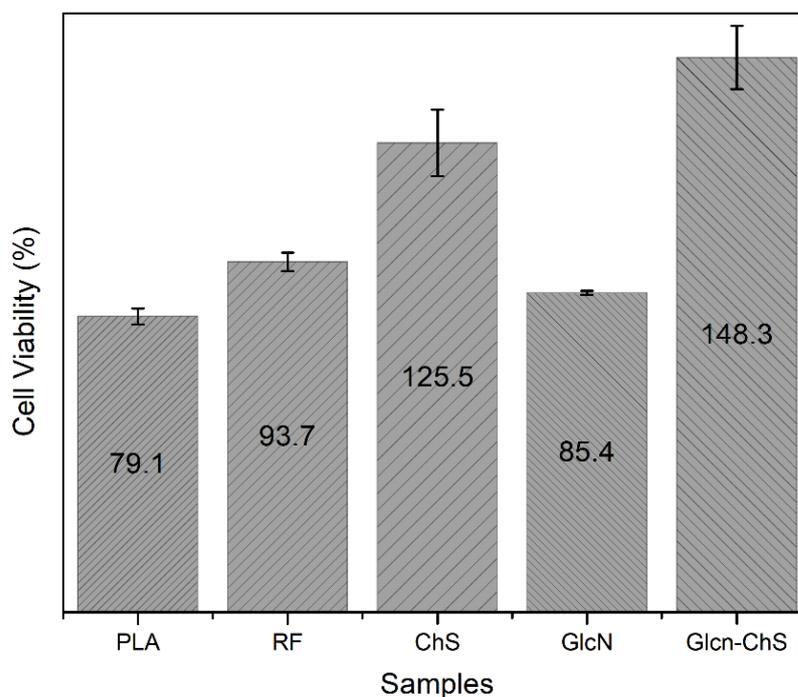


Figure 5. Biocompatibility of untreated and modified PLA samples

Other studies reported the evaluation of toxicity of chondroitin sulfate and glucosamine. Most of the studies claim little or no cytotoxicity of either GlcN or ChS for concentrations below 5.0 mg/mL [55, 56]. One study reported the combination of GlcN and ChS resulted in an increase in cellular metabolic activity in chondrocytes monolayer cultures (cell viability 139%) upon 7 days of incubation, which is consistent with our findings [57]. In another study, an enhancement in cell proliferation was found with these two saccharides [58].

Antibacterial Activity

The antibacterial activity of the PLA films was evaluated against *Staphylococcus aureus* and *Escherichia coli* strains, and analyzed by comparing the number of viable cells in the agar plates after 24h of incubation time. As shown in Table 2, untreated PLA has antibacterial activity against neither bacteria strains. After plasma treatment (RF) a similar number of viable bacteria of untreated PLA is found, which indicates the absence of any bactericidal effect before surface coating with suitable agents. While the best antibacterial activity is observed with only ChS immobilized samples against *E. coli* bacteria strains (<1 means no colonies recovered), the highest antibacterial activity against the *S. aureus* strains is exerted by only GlcN attached PLA films with 1.9 cfu/cm². The difference in counts between GlcN combined with ChS immobilized samples and separately attached saccharides are small and this combination results in a destruction of more than 99.99% of the inoculation, which generally is accepted as the definition of bactericidal agents [57, 58].

Table 2. Number of the bacteria colony on the film surfaces before and after treatments

Sample	Initial CFU	PLA	RF	GlcN	ChS	GlcN-ChS
Bacteria						
<i>S.aureus</i>						
CCM 4516	2.0×10 ⁶	1.8×10 ⁵	2.1×10 ⁵	1.9	8.8	1.1×10 ¹
N (cfu/cm ²)						
<i>E.coli</i>						
CCM 4517	2.2×10 ⁷	2.3×10 ⁶	2.2×10 ⁶	7.8	< 1	7.7×10 ¹
N (cfu/cm ²)						

Antibacterial activity of the samples was also evaluated by the calculation of their R-values. According to the standard, the samples are considered to have an antibacterial activity with the R values are equal or superior to 2. It can be seen in Figure 6. individual saccharides coatings showed higher antibacterial activity

than the combined coating. With the immobilization of ChS on GlcN grafted samples, the bacteria killing efficiency dropped. In terms of antibacterial activity, this saccharide combination did not show a synergistic effect.

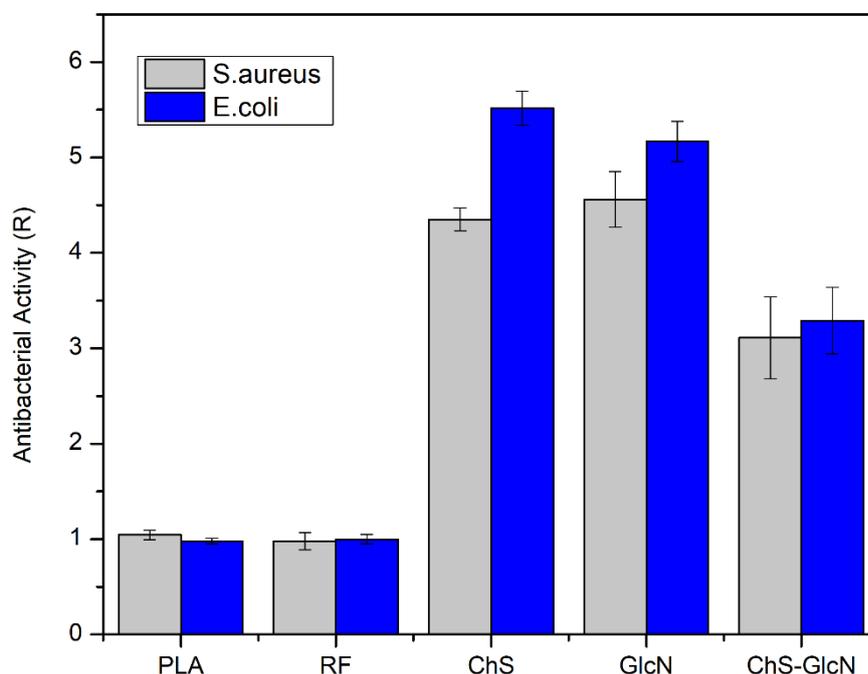


Figure 6. Antibacterial activity of the samples as R values

3.1.3 Conclusion

The antibacterial surface modification of PLA films was achieved through the immobilization of GlcN and ChS on film surfaces via plasma treatment technique, followed by AAc grafting. The contact angle and XPS results verify successful immobilization of the saccharides. In the survey scan XPS spectra increases in characteristic elements (N and S) of ChS and GlcN were observed. SEM images showed that the saccharide aggregates partially covered the PLA film surfaces. The antibacterial testing results demonstrated that PLA films coated with ChS exhibited the highest antimicrobial activity against E. coli. Besides, only-GlcN immobilized PLA films showed the best bactericidal effect against S.aureus. When combined with ChS the degree of both bacteria growth inhibition was still up to 99.99%.

This study definitely proved that the developed GlcN/ChS coated PLA films are excellent bactericide agents against representative gram-positive and gram-negative bacteria. Furthermore, the combination of these two saccharides should be highlighted in the current study, due to increased cell viability, which could make it easier to bring the developed medical devices to the market.

3.2 Chitosan/chondroitin sulfate coating on polylactic acid films for biomedical applications

Complexes of chitosan with polyanions are of undoubted interest to researcher for decades. The mixing of solutions of polycations and polyanions leads to the spontaneous formation of interpolymer complexes which also known as polyelectrolyte complexes (PEC). The PEC coatings ameliorate surface properties of the biomaterial due to the interaction between oppositely charged polymers leads to a strong surface modification. Therefore, such complexes are of great interest as potential drug delivery systems due to the easy integration of charged species into these complex particles [59, 60].

PEC coatings have gain increasing interest as ultrathin biologic reservoirs, thanks to the ability in readily coating different geometries. The success of the biomedical devices, including orthopedic implants and cardiovascular devices is bound to host tissue response [61]. Therefore, a biocompatible coating of these implanted materials to direct the host cell-surface interface response becomes detrimentally important. The saccharides have been extensively used in biomedical applications, thanks to their easy accessibility, biocompatibility, biodegradability and nontoxicity properties [62].

The introduction of drug-eluting implants into the biomedical field have resulted in a breakthrough in the treatment of many diseases by inducing healing effects in addition to their supporting task [63]. As stated before, PEC coatings has been recently explored as ultrathin drug reservoirs due to its simplicity.

Polysaccharide-based coatings have been utilized widely to deliver biomedical agents such as antibiotics, antioxidants, proteins, and peptide drugs with the formation of polyelectrolyte formation [32]. Chitosan, a cationic polysaccharide, has received great attention in pharmaceutical delivery due to its promising properties, such as it possess fine biocompatibility, low toxicity, bioresorbability, abundant availability, biodegradability. Many polyanions from different types of sources have been broadly investigated to create PEC with chitosan, including natural and synthetic polymers, metal anions and so on [29]. With the different types of preparation methods, it is possible to obtain PECs in various forms such as fibrous membranes, hydrogels, beads, films, micro/nanoparticles [29]. The improvement of the biocompatibility and drug loading ability of chitosan with PEC formation is somewhat dependent on the polyanionic materials used. Chondroitin sulfate, an acidic mucopolysaccharide, is able to form PEC with chitosan by electrostatic interactions. This anionic saccharide has a wide range of bioactivity including tissue regeneration, cell proliferation, adhesion and intercellular signaling [32]. Moreover, its low molecular weight, allows it freely diffuse through high viscosity chitosan layers, creating an ionic complexation.

The combination of natural polysaccharides to fabricate polyelectrolyte complexes develops an evolving approach for drug delivery and drug eluting implants. In the present study, the effect of the polysaccharide-based PEC coating

on the surface properties of PLA as well as antibacterial activity, biocompatibility and drug eluting properties were investigated. For this purpose, following the plasma-treatment activation of PLA surfaces the molecules of chitosan were immobilized on the surface through polyacrylic acid brushes either electrostatically or carbodiimide coupling. The PEC formation was obtained with the addition of chondroitin sulfate on chitosan coated PLA through dipping method. Lomefloxacin was used as a model drug to test the drug loading and releasing properties of PEC coated PLA films. Besides hydrophilicity and biocompatibility tests, the bacterial adhesion of surface modified PLA films was investigated by antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

3.2.1 Preparation

PLA films were prepared as previously described with compression molding technique. Briefly, dried PLA pellets were hot-pressed at 170°C with a pressure of 100 kN for 20min and then cooled. The square shaped sheets were cut into pieces of 25 × 25 mm for further surface treatments, and were rinsed with a detergent solution. Then, dried PLA films were exposed to radio frequency (13.56 MHz) low-pressure plasma treatment (PICO Diener, Ebhausen, Germany) at reactor power of 50 W for 60 seconds, under 60 Pa vacuum chamber and 20 sccm of air flow.

Immediately after plasma treatment, PLA films were immersed into acrylic acid (AAc) solution for 24 h and then placed into sodium hydroxide for neutralization. Before CS immobilization on PLA surface, samples were immersed into EDC/NHS solution for 4h for the activation of PAA carboxyl groups. Thereafter, saccharide assembly was performed as dip coating. For the drug release experiments, CS coated samples were dipped into Lomefloxacin-containing solutions. Subsequently, coated/loaded films were then washed 2-3 times with deionized water and dried overnight at 25 °C for further characterization.

3.2.2 Results and Discussion

SEM measurements were carried out to confirm the surface functionalization of the PLA film surfaces. The SEM image of the neat PLA film (Figure 7) clearly shows that the surface is homogenous and smooth after the compression molding process. It could be seen in Figure 7b that the RF plasma treatment cause rough and micro-crack formations on the PLA surface. That behavior is attributed to the oxidation and etching/ablation properties of the topmost layers of polymers during low-pressure plasma treatment.

In Figure 2c-7d, CS and CS-ChS coated PLA film surfaces can be seen, respectively. Chemical grafting of CS through EDC/NHS coupling onto post-

plasma treated polymer surface resulted in a thin layer of CS deposition on the surface and the CS immobilization subsequently introduces independent structures. Similar observation of morphological arrangements has been stated for the CS immobilization on polyacrylic acid grafted PET surfaces [64].

On the other hand, PEC formation between CS and ChS can be seen as flocculated and coalesced particles on the polymer surface. This relatively non-uniform flocculated clusters are result of the interaction of a weak polycation and a strong polyanion. Similar occurrences on coating surfaces resulting from polyelectrolyte complex formation have been reported previously. Safitri et al. studied the PEC formation between pectin and chitosan, and reported that upon the complex formation the surface was found to be covered with small granules [65]. Moreover, Cui et al. reported that the film consisted of polyelectrolyte complexes of PAA and polyethylene imine displayed layers of protrusions and porous structures [66].

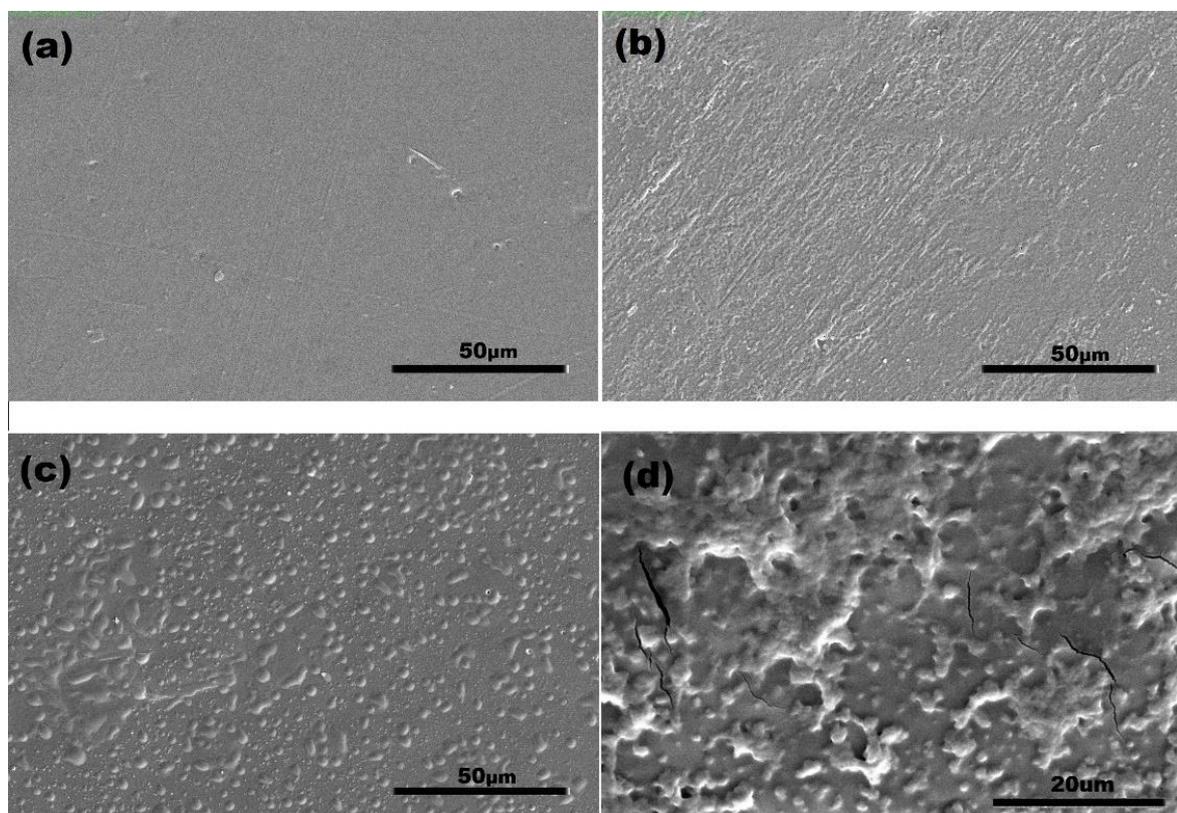


Figure 7. SEM images of the (a) untreated PLA; (b) plasma treated PLA; (c) CS; (d) CS-ChS coated films.

The effect of surface modification on hydrophilicity was analysed through static contact angle measurements (Table 3). The average of the static water contact angle for pristine PLA samples was 81.5°, which is in accordance with the literature [67]. When the samples were subjected to plasma treatment, the

contact angle reached to 46.1° resulting in hydrophilic PLA surfaces. This reduction can be attributed to the surface etching and formation of hydrophilic oxidative functional groups on the polymer surface. Upon polysaccharide adhesion, the mean contact angles of samples slightly increased due to a decline in the oxidative functional group content. When compared to electrostatically grafted surfaces, carbodiimide coupled CS samples had a higher contact angle value, which is attributed to increased amount of CS polymer chain grafted on the surface. On the other hand, ChS presence significantly reduced the contact angle to 50.9°. This decrease was expected as ChS is a more strongly charged molecule than CS [68], which increased the charge density on the surface that can result in increased hydrophilicity. Together with SEM images, these preliminary results indicate the successful modification of the PLA surfaces.

Table 3. Values for contact angle for the prepared films.

Sample type	Contact Angle (%)
PLA	81.5 ± 1.4
RF	46.1 ± 0.8
CS	62.6 ± 1.2
g-CS	69.4 ± 1.6
g-CS-ChS	50.9 ± 0.9

For further confirmation of the surface modification, XPS measurements were carried out before and after the treatments. As shown in Table 4, the atomic composition of PLA significantly changed after plasma treatment (RF), resulting in increased amount of oxygen species and presence of nitrogen content that have the effect of decreasing the relative carbon content. During the plasma treatment, the oxygen and nitrogen in the carrier gas become short- and long-lived reactive oxygen (ROS) and nitrogen (RNS) species that react with molecular chains of PLA and introduce functional groups to the polymer surface [69]. XPS results clearly revealed that sulfur (S) and fluorine (F) elements were only detected in ChS coated and drug loaded samples which proves their presence on the PLA surface.

Table 4. The atomic weight percentage of unmodified and modified PLA samples

Sample type	Composition (%)					Ratio N/C
	C	O	N	F	S	
PLA	70.9	28.4	-	-	-	-
RF	65.3	31.9	2.7	-	-	0.041
CS	77.6	19.4	3.0	-	-	0.044
g-CS	74.1	22.2	3.6	-	-	0.048
g-CS-ChS	60.9	26.6	10.1	-	0.5	0.164
g-CS-ChS-L	64.6	27.8	6.9	0.6	0.4	0.107

The grafting protocols of CS were performed with and without EDC/NHS coupling. In the case of carbodiimide coupling (g-CS), the amount of nitrogen increased to 3.6%, along with an increase of N/C ratio. These changes suggest that the coupling made it possible to graft a larger amount of CS to the PLA surface. The ratios of C/N and the amount of nitrogen in the g-CS-ChS samples reach to 0.164 and 10.1% respectively, which are significantly higher than those of CS, indicating that ChS has been grafted with CS to form a PEC complex with a higher nitrogen content. Moreover, the sulfur was found on the assembled polymer films, which was another proof of successful ChS grafting. The presence of lomefloxacin on the polysaccharide coated film surface was confirmed with the appearance of fluor species that is only found in this fluoroquinolone antibiotic drug.

Figure 8 shows the biocompatibility of pristine and modified polymer films through the direct contact study on NIH/3T3 cells. The % cell viability was calculated relative to PET film as control 100% viable. As can be seen, RF plasma treatment enhanced the biocompatibility of PLA film, which was consistent with the literature [70].

On the other hand, a slightly lower cell viability was observed for the both type of CS grafted samples when compared to the plasma treated ones. This slight reduction in viability may be related to the remnants of acrylic acid monomers. Nevertheless, the % cell viabilities of CS coated samples were higher than the untreated PLA films. Another remarkable increase in biocompatibility was seen with the carbodiimide coupling of CS with PAA brushes. This effect is probably caused by the higher amount of immobilized CS chains on surface, which is consistent with the XPS results. Moreover, EDC/NHS coupling reaction has no potentially cytotoxic residuals and has been shown in many studies that carbodiimide/succinimide reaction leads to an increase in cell proliferation [71]. This is because the carbodiimide conjugation activates the carboxyl groups on PAA brushes for direct reaction with the primary amines of CS backbone and as a result of this reaction positive charge of polymer decreases which leads less

binding affinity with the negatively charged cell membranes [72]. The highest biocompatibility was observed with the samples of ChS immobilization on CS-grafted polymer surface by electrostatic adsorption. This phenomenon could be ascribed to the masking effect of positive charge on CS with the negatively charged sulfate groups of ChS. Cytotoxicity test results were consistent with the hydrophilicity degree of the samples. The most hydrophilic samples had the highest % cell viability. As reported in literature, while proteins can maintain their conformations on hydrophilic surfaces, hydrophobicity cause protein denaturation. As a whole, the obtained results in this study indicate that chondroitin sulfate and chitosan coated PLA films do not cause toxicity in the cells and highly suitable materials for biomedical applications.

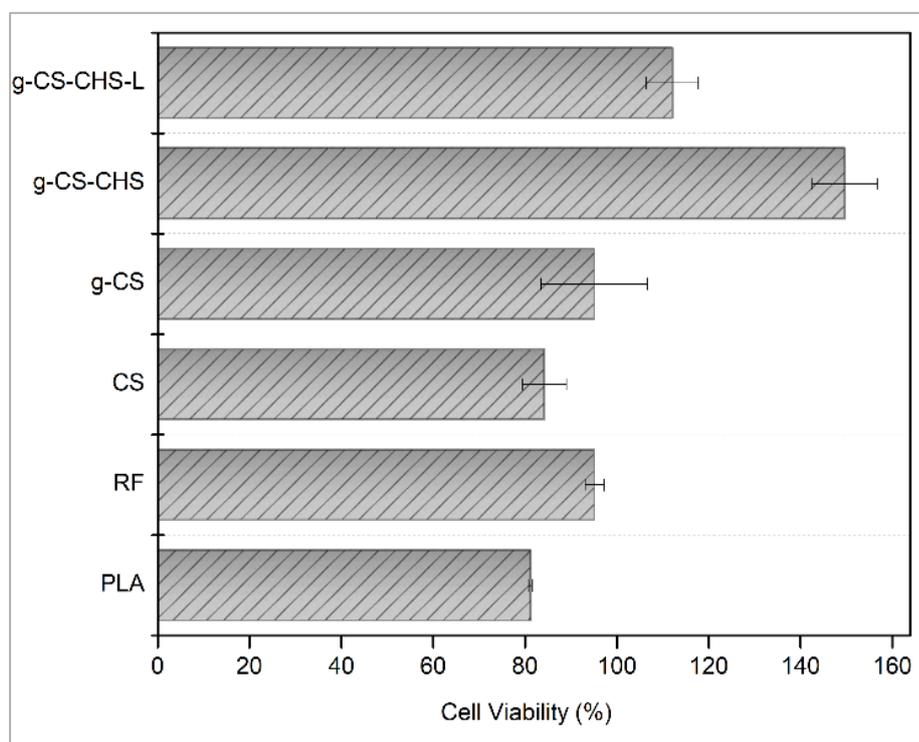


Figure 8. Viability of untreated, polysaccharide coated and lomefloxacin loaded PLA samples

Antibacterial efficacy of untreated and modified PLA films were evaluated against *S.aureus* and *E.coli* bacteria. According to JIS Z 2801 standard, the samples were considered to have antibacterial activity with the value of R equal or superior to two.

The antibacterial efficacy of the neat PLA and modified films was evaluated against *Staphylococcus aureus* and *Escherichia coli* strains, and analyzed by calculating the antibacterial activity value (R). As can be seen in Figure 9, untreated PLA did not possess an antibacterial activity against the bacteria strains. The bactericidal activity of PEC coated PLA films against both of the gram-

positive and the gram-negative bacteria strains was higher than the CS coated ones. Antibacterial property of ChS has been reported in previous studies [32]. The improved antibacterial activity with CS-ChS PEC compared to CS alone may be due to an additive effect of both ChS and CS interaction.

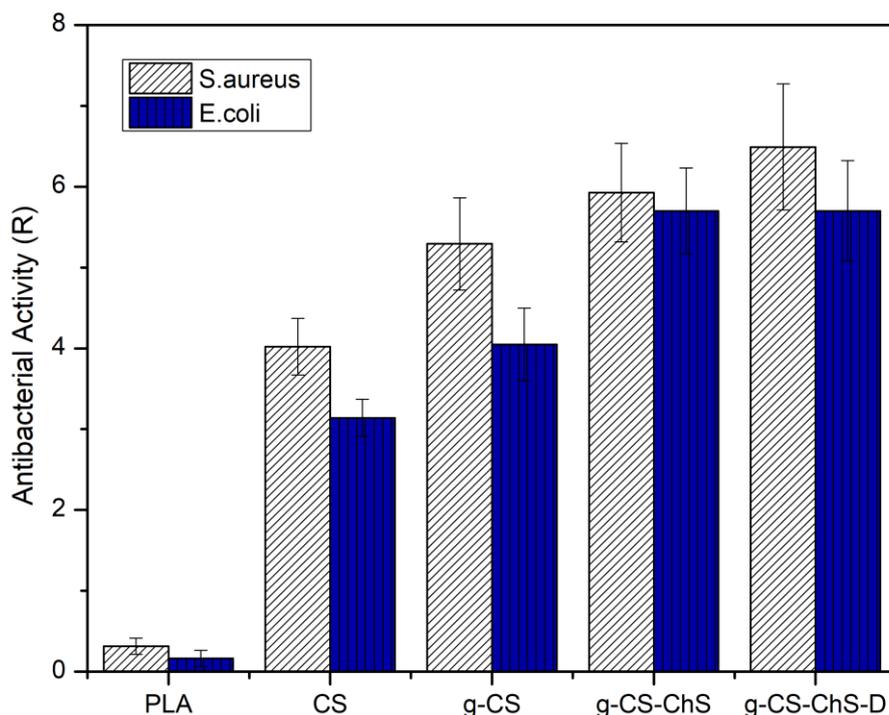


Figure 9. Antibacterial activity of the surface modified films

In addition, the CS samples grafted through carbodiimide chemistry (g-CS) showed better bacteria-killing properties than the ones directly immobilized on PAA brushes (CS). This may be due to the higher amount of CS chains on the PLA surface. With EDC/NHS reaction mechanism, carboxyl groups on PAA brushes activated and formed a robust amide linkage with the amino groups on CS chains [65]. Although plenty of researches have reported the materials grafted with PAA brushes to achieve an enhanced immobilization of cationic molecules, few works paid attention to the effectiveness of carbodiimide reaction on antibacterial efficiency and biocompatibility that is applied to PAA brushes for immobilization of substances. Finally, with the antibiotic loaded samples antibacterial efficiency reached to the highest level.

To test the drug loading and releasing properties of the coating, lomefloxacin used as a model drug and results are presented in Figure 10. It can be seen, the amount of lomefloxacin loading is highest for the PEC-coated PLA films. The amount of drug adhering on the CS-coated samples doubled after CS-ChS coating.

The results were in line with the literature that shows the PEC formation between polysaccharides increases the drug loading efficiency [73].

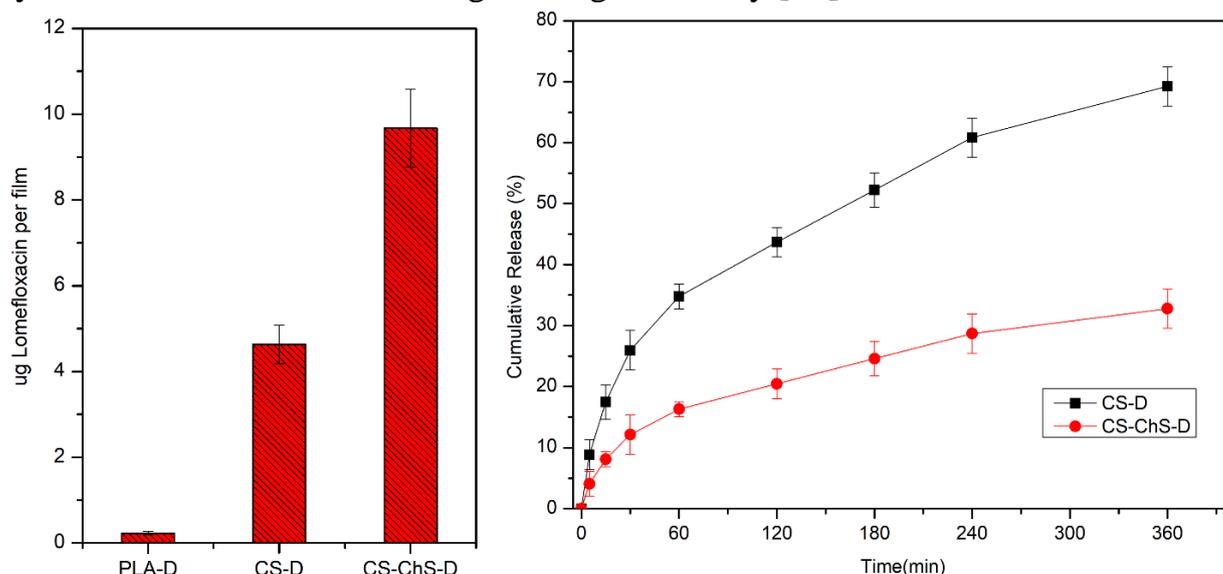


Figure 10. Drug loading and release profiles of CS and CS-ChS coated films

The polyelectrolyte complex formation between CS and ChS affects the cumulative release of the drug from the films (Figure 10-b). The CS-coated films reached 69.2% drug release rate in 4 hours, while the PEC formed films released only 32.7% of the loaded drug. The lower release rate observed by the PEC-coated films as expected. The interaction between CS chains and ChS molecules caused entrapment of the drug molecules between complex structures. About 16% of lomefloxacin released from the CS-ChS coated films during the first hour, significantly less than those of CS-coated (34.7%) ones. The burst release for both types of films at the beginning of the release time is due to the presence of weakly adsorbed drug molecules on the film surfaces. An ideal antibacterial coating for implants based on release of an antibiotic should start releasing at the time of implication, and followed by prolonged release over the time for at least some hours [74]. The presented release results showed that the PEC structure affected directly the release profile of lomefloxacin. This implies that the electrolyte complex structure between CS and ChS showed a prolonged release profile.

Possible bacteria killing mechanism of modified PLA films was examined by the zone of inhibition test. As shown in Figure 11, the samples without drug loading were lack of inhibition zones and only lomefloxacin-loaded samples (no.5) showed the bacterial inhibition zone for both of the bacteria strains. The inhibition zones for S.aureus and E.coli were 5.33 ± 0.94 and 6.67 ± 1.25 mm, respectively. In terms of surrounding clearing zone around drug loaded CS-ChS coated samples, our results have revealed that these samples shows dual mechanism with contact killing through CS-ChS molecules and release killing by

leaching of lomefloxacin from the CS-ChS coated surface. It is stated in the literature that CS does not diffuse on the agar gel [75], and our study supports this statement as no inhibition is found beyond the limits of CS coated PLA films. These experiments also showed that zone of inhibition tests alone may not be enough to prove antibacterial efficiency of the materials as the agar dilution methods indicated bactericidal activity for CS and CS-ChS coated films, in zone of inhibition tests antibacterial activity did not observed due to the lack of a clear zone around the samples. Only drug-loaded films showed a clear inhibition zone around the specimens, which indicates a diffusion related killing mechanism.

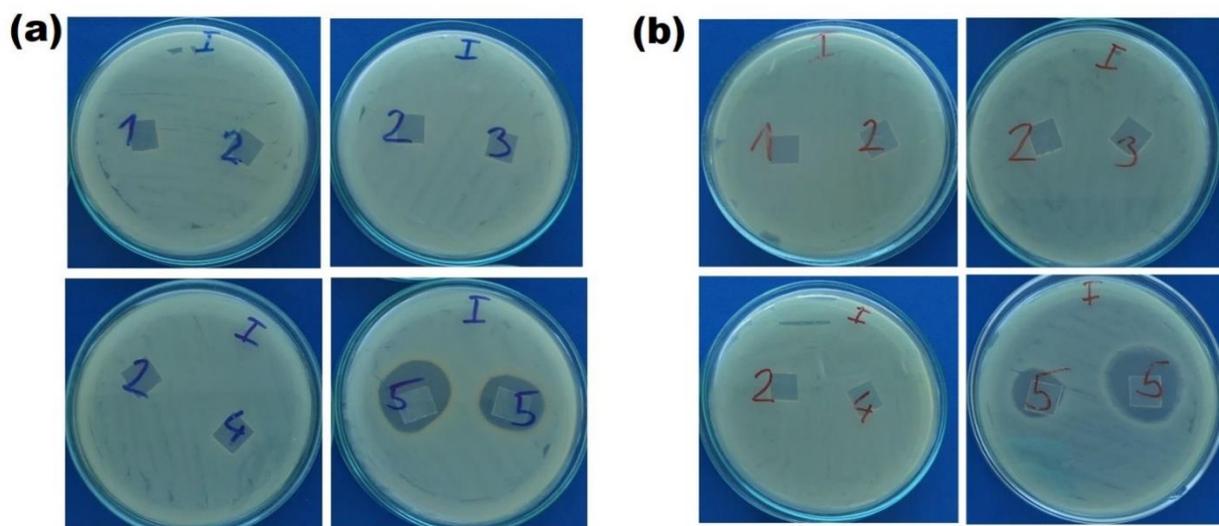


Figure 11. Drug loading and release profiles of (1) untreated PLA (2) g-CS (3) g-CS-ChS (4) CS-ChS (5) g-CS-ChS-D coated films against (a) *S. aureus* and (b) *E. coli* bacteria

3.2.3 Conclusion

In the present study, surface modification of PLA films was achieved through polysaccharides coating following the plasma-treatment. Individual and combined effects of both saccharides on antibacterial properties, biocompatibility and drug loading/releasing profiles were evaluated. Together with SEM images, XPS results and contact angle values verified the surface modification of the PLA films. The direct immobilization of CS on PAA brushes was compared with the EDC/NHS assisted coating. From the XPS results, it was clear that with the carbodiimide reaction between PAA and CS chains, the amount of immobilized CS molecules increased significantly. This increase resulted in a better antibacterial activity and biocompatibility for the films grafted through carbodiimide chemistry between the brushes and CS molecules. Moreover, the films coated with CS-ChS exhibited better bactericidal property and higher cell viability than the CS-coated film. It was clear that the combined coating of both polysaccharides resulted in significantly higher bioactivity. Besides, from the results of drug loading study, it could be concluded that the drug amount was mainly dominated by the CS-ChS interaction. The release profile showed that PEC formation between these two polysaccharides resulted in the prolonged drug-release rate. Finally, it is proved that CS and CS-ChS coatings on PLA films possess contact killing ability while drug loaded films showed zones of inhibition, which indicates a dual killing mechanism that covers both release and contact killing routes.

SUMMARY OF WORK

Developing and evaluating antibacterial surfaces is a serious matter, especially to prevent potential adverse effects that may arise related to the surface colonization of bacteria on biomaterial surfaces. Depending on the infection type, this bacterial invasion has direct and indirect implications for health of patients. More than half of the hospital-associated infections worldwide are related to the adhesion of bacteria cells to biomedical devices and implants. To prevent these infections, it is crucial to modify biomaterial surfaces to develop antibacterial property.

In this view, polylactic acid film surfaces modified with saccharides coating to exert antibacterial and biocompatible features. In this matter, glucosamine, chondroitin sulfate and chitosan immobilized PLA surfaces were investigated for their individual and combined antibacterial efficacy against *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli*. (*E.coli*).

In the first study, plasma-mediated surface activated PLA films were coated with aforementioned saccharides through PAA brushes. XPS, SEM micrographs and contact angle analysis confirmed the successful modifications of the PLA surfaces. The combined effects of saccharides coating on surface hydrophilicity and biocompatibility were evident, in particular, a noticeable increase in the percentage of cell viability compared to the individual saccharides coatings. Moreover, our results showed that all saccharides coatings have bactericidal effect on both bacteria strains. The GlcN-ChS coating resulted in a slightly less antibacterial activity than the individual coatings; however, the growth inhibition was still up to 99.99%. It can be concluded from this study that even though these saccharides showed an additive effect on biocompatibility, in terms of antibacterial activity, this saccharides combination did not show a synergistic effect.

In the second part of the study, CS and ChS were chosen as antibacterial coating. Plasma treated PLA surfaces were coated with CS in two ways. In direct coating method, PLA films were immersed into CS solution without any prior treatment. This method was based on electrostatic interactions between PAA brushes and CS molecules. In the second route, first carboxyl groups on PAA brushes were activated through carbodiimide agents and then those treated samples were immersed into CS solution for a covalent coupling between amine groups of CS and carboxyl groups of PAA. As a next step for the combined saccharide coating, CS grafted samples were immersed into ChS solution. Also in this experiment, to test the drug loading and releasing efficiency of the thin film coatings, CS grafted samples were immersed into lomefloxacin-containing ChS solution. The successful modifications were confirmed by elemental composition analysis (XPS), surface topography images (SEM), hydrophilicity change (contact angle measurements). The carbodiimide coupling resulted in higher CS grafting on the PLA surface. The coatings with the polyelectrolyte complex

formation between CS-ChS showed improved activity against the bacteria than the separate coatings. Moreover, these interactions increased the lomefloxacin amount adhered on the film coatings and extended the drug release profile. Finally, zone of inhibition test confirmed that the drug loaded CS-ChS coating showed a dual killing mechanism which include contact and release killing.

As prepared surface modified-saccharide coated materials exhibit its active way of functioning, they may be in future used as a prevention against hospital-associated infections that may arise from bacteria-invaded biomedical materials usage.

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LIST OF ABBREVIATIONS, SYMBOLS AND UNITS

PLA	Polylactic acid
CS	Chitosan
ChS	Chondroitin sulfate
GlcN	D-glucosamine
RF	Radio Frequency
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
GAGs	Glycosaminoglycans
ECM	Extracellular matrix
S.aureus	Staphylococcus aureus
E.coli	Escherichia coli
MhZ	Megahertz
Pa	Pascal
W	Watt
AAc	Acrylic acid
CCD	Charged-coupled device camera
SEE	Surface Energy Evaluation System
SEM	Scanning electron microscopy
XPS	X-ray photoelectron spectroscopy
UV-Vis	Ultraviolet visible
PAA	Polyacrylic acid
N	Nitrogen
O	Oxygen
C	Carbon
S	Sulfur
°C	Celcius degree
PEC	Polyelectrolyte complex
mm	Milimeter
h	Hour
s	Second
w%	Weight percentage
Mw	Molecular weight
EDC	(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
NHS	N-Hydroxysulfosuccinimide sodium salt
PET	Polyethylene terephthalate
DP	Direct polycondensation
ROP	Ring opening polymerization

NADH	Nicotinamide adenine dinucleotide
ADP	Adenosine diphosphate
ATP	Adenosine tri-phosphate
HMW	High molecular weight
UV	Ultraviolet
IR	Infrared
DC	Direct current
DBD	Dielectric barrier discharge
K	Kelvin
AuNPs	Gold nanoparticles
MWCNT	Multiwalled carbon nanotubes
DD	Deacetylation degree
NaOH	Sodium hydroxide
COS	Chitosan oligosaccharide
LPS	Lipopolysaccharide
DCC	N,N-dicyclohexylcarbodiimide
LOMO	The lowest excited state
HOMO	The highest ground state
SEs	Secondary electrons
NB	Nutrient broth
PP	Polypropylene
Cfu	Colony forming unit

Příprava a charakterizace antibakteriálních vrstev na bázi sacharidů

Preparation and characterisation of saccharide-based antibacterial coatings

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