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Review article

# Polysaccharide-based antibiofilm surfaces

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ABSTRACT

Surface treatment by natural or modified polysaccharide polymers is a promising means to fight against implant-associated biofilm infections. The present review focuses on polysaccharide-based coatings that have been proposed over the last ten years to impede biofilm formation on material surfaces exposed to bacterial contamination. Anti-adhesive and bactericidal coatings are considered. Besides classical hydrophilic coatings based on hyaluronic acid and heparin, the promising anti-adhesive properties of the algal polysaccharide ulvan are underlined. Surface functionalization by antimicrobial chitosan and derivatives is extensively surveyed, in particular chitosan association with other polysaccharides in layer-by-layer assemblies to form both anti-adhesive and bactericidal coatings.

**Statement of Significance**

Bacterial contamination of surfaces, leading to biofilm formation, is a major problem in fields as diverse as medicine, first, but also food and cosmetics. Many prophylactic strategies have emerged to try to eliminate or reduce bacterial adhesion and biofilm formation on surfaces of materials exposed to bacterial contamination, in particular implant materials.
Polysaccharides are widely distributed in nature. A number of these natural polymers display antibiofilm properties. Hence, surface treatment by natural or modified polysaccharides is a promising means to fight against implant-associated biofilm infections. The present manuscript is an in-depth look at polysaccharide-based antibiofilm surfaces that have been proposed over the last ten years. This review, which is a novelty compared to published literature, will bring well documented and updated information to readers of Acta Biomaterialia.



**Contents**



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## 1. Introduction

It is now well recognized that bacteria attach to solid supports to form structured communities called biofilms, defined as biopolymer matrix-enclosed microbial populations adhering to each other and/or surfaces [1]. Biofilms occur on both inert and living supports in all environments [2]. They affect many industrial and domestic domains [3] and are responsible for a wide range of human infections [1]. Considering the ever increasing number of implanted patients, biofilm-associated infections of indwelling medical devices are more particularly a major public health concern. Examples of implants that can be affected by biofilm formation are catheters (intravascular, urinary), mechanical heart valves, vascular prostheses, pacemakers/defibrillators, ventricular assist devices, coronary stents, neurosurgical ventricular shunts, cerebrospinal fluid shunts, neurological stimulation implants, joint prostheses (hip, knee, ...), fracture-fixation devices, breast, inflatable penile, cochlear and dental implants, ocular prostheses and contact lenses, intrauterine contraceptive devices [4–6]. Bacteria commonly isolated from biofilm-infected implants include the gram-positive *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus mutans*, and the gram-negative *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* ([7]; see also [8] for a more detailed list including fungi and yeasts). Biofilm-associated infections are particularly problematic because sessile bacteria are much more resistant to antibiotics and biocides than their planktonic counterparts [9]. Hence, the treatment of biofilm infections needs high concentrations of disinfectants or antibiotics, which may cause severe environmental damages and multiresistance emergence. In this context, prevention of biofilm formation is actually preferable to any post-infection treatment.

At the biomaterial surface level, two main strategies are currently proposed to oppose biofilm formation, i.e., the development of anti-adhesive or bactericidal surfaces (Fig. 1) – the use of biofilm-degrading agents [11] being still in its infancy. Surfaces that are mainly repellent are characterized by a decrease in the number but no significant loss in viability of attached bacteria. Anti-adhesive properties of inert materials can be improved by modifying surface characteristics known to affect microbial cell adhesion, namely surface topography (roughness) and physicochemistry (surface free energy, hydrophilic or hydrophobic, cationic or anionic behavior) [12–15]. A physical treatment of the surface such as plasma irradiation followed or not by attachment of anti-adhesive molecules or polymers, is commonly applied for that purpose [16]. However, sustained cell adhesion on implanted materials is required for suitable tissue integration of permanent implants such as vascular grafts or joint prostheses. Hence, the properties of such implant surfaces must balance between repellency against bacterial cells and adhesiveness for tissue cells, controlling the "race for the surface" [17,18] between bacteria and tissue cells. Killing effect of the surface against attached and/or suspended bacteria is highlighted by a decrease in adherent cell viability and/or the number of viable suspended cells. As shown in Fig. 1, bacterial killing properties can be achieved by non-covalent immobilization of an antimicrobial agent through direct incorporation in the biomaterial bulk or deposition on the surface (previously modified or not), leading to progressive release of the drug in the surrounding medium. Another way consists in covalent binding (i.e., with no leakage) of an antibacterial compound to the biomaterial surface to yield a contact-killing coating. The first method has been widely used in commercial devices such as catheters that are heparinized for thromboresistance and loaded with antimicrobials (e.g., $Ag^+$ ions, chlorhexidine, benzalkonium chloride, minocycline-rifampicin) [19]. The covalent method presents the advantage of avoiding potential toxic effects of classical biocidal compounds and loss in efficiency due to a limited reservoir capacity of the biomaterial [20]. Moreover, both strategies could be mixed to elaborate infection-resistant biomedical materials with synergic anti-adhesive and bactericidal properties.

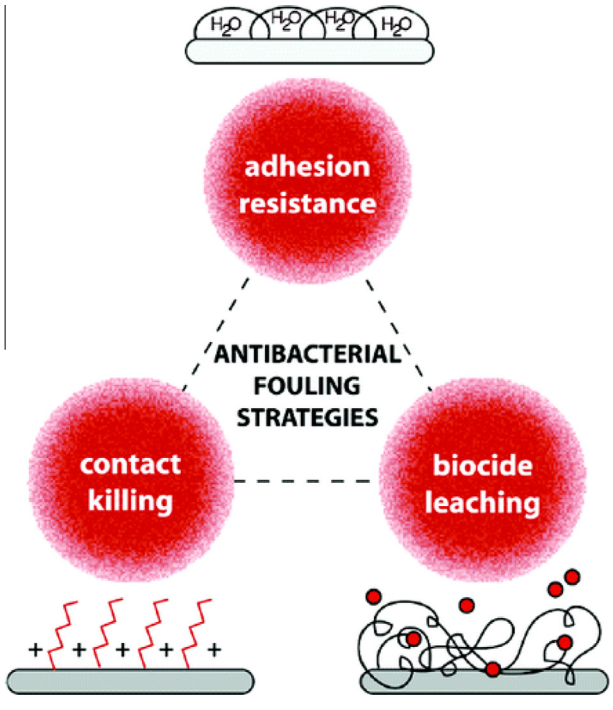



**Fig. 1.** Main strategies for antibacterial surface design. Taken from [10].

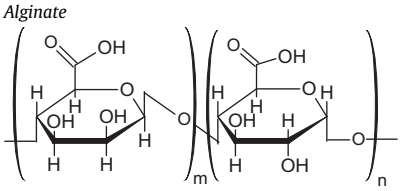
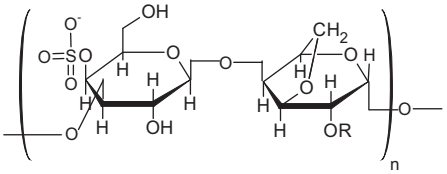
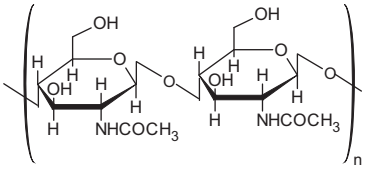
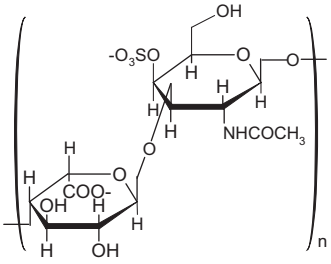
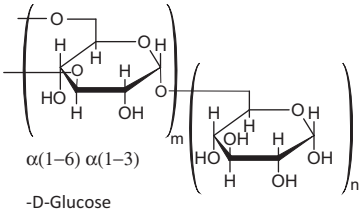
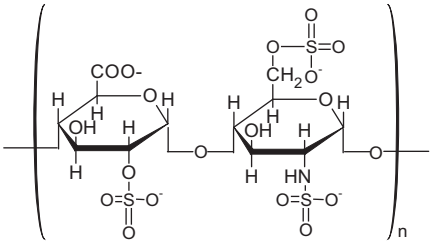
One of main features of biofilm formation is the production of an extracellular matrix composed of 90% water and 10% extracellular polymeric substances [21]. The latter are mainly composed of polysaccharides and proteins, but also include nucleic acids, lipids and other biological macromolecules. Their components mediate cell-to-cell and cell-to-surface interactions that are necessary for biofilm formation and stabilization [21]. Some observations also suggest that some bacterial extracellular polysaccharides might inhibit and/or destabilize the biofilm (see [22,23] and references therein). However, none of antibiofilm exopolysaccharides identified so far exhibits antibacterial activity. Most of them act as surfactant molecules, modifying the physical characteristics of bacterial cells and abiotic surfaces [23]. On the other hand, several bacterial exopolysaccharides have been shown to display antimicrobial efficiency [24–27], as have been chitosan, a chitin derivative [28], and a number of polysaccharides of algal [29,30], fungal [26,31] and plant [32,33] origins.

Hence, modified polysaccharides are being developed as bacteria-repellent and/or -killing coatings for material surfaces exposed to biofilm formation. The following is an in-depth look at polysaccharide-based antibiofilm surfaces that have been proposed over the last ten years, focusing in particular on bactericidal coatings that mainly involve chitosan and its derivatives.

## 2. Anti-adhesive surfaces

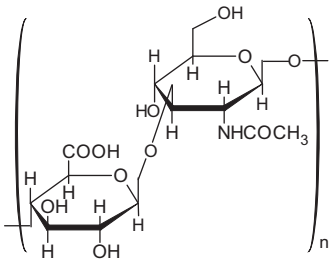
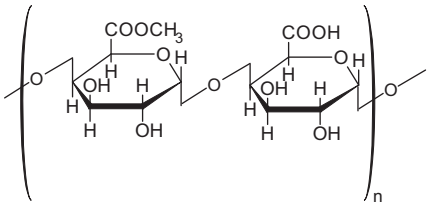
Prevention of bacterial adhesion on surfaces through anti-adhesive coatings is one of the simplest, potentially cost-effective ways to avoid biofilm formation. Bacterial adhesion is a complex process which is affected by many factors including – as stated above – the physical and chemical characteristics of material surface, but also bacterial cell properties (e.g., hydrophobicity

**Table 1**
Main polysaccharides used in antibiofilm coatings.

| Polysaccharides | Origins |
|---|---|
| *Alginate*<br>  <br>(1 → 4) Linked β-D-mannuronic and α-L-guluronic acid residues | Brown algae (Phaephyta), i.e., *Laminaria*, *Macrocystis*, *Lessonia*, *Ascophyllum*, *Sargassum* and others.<br>Bacterial fermentations (*Azotobacter vinelandii, Pseudomonas aeruginosa, P. mendocina*). |
| *Carrageenans*<br>  <br>D-galactose residues linked alternatively via α(1 → 3) and β(1 → 4) linkages (R=H, κ-carrageenan; R=$SO_3^-$, ι-carrageenan) | Red algae (Rhodophyta), mainly *Chondrus*, *Eucheuma*, *Gigartina* and *Kappaphycus* species. |
| *Chitin/Chitosan*<br>  <br>Chitin consists of β(1 → 4)linked N-acetyl-D-glucosamine units. Partial N-deacetylation of chitin yields chitosan. | Shell of marine invertebrates |
| *Dermatan sulfate*<br>  <br>D-iduronate and *N*-acetyl-D-galactosamine-4-sulfate linked by β (1 → 3) bonds. | Extracellular matrix of animal tissues (bovine trachea, pig nasal septum, chicken keel, shark fins and fish cartilage). |
| *Dextran*<br>  <br>α(1–6) α(1–3)<br>-D-Glucose<br>α(1–6)-D-Glucose<br>Main chains consist of α(1 → 6)-linked D-glucose residues while side chains begin from α(1 → 3) linkages. | Bacterial fermentation<br>(*Leuconostoc mesenteroides, Streptococcus mutans*). |
| *Heparin/heparan sulfate*<br>  <br>D-glucuronate-2-sulfate and *N*-sulfo-D-glucosamine-6-sulfate linked by α(1 → 4) bonds (glucosamine groups are less sulfated in heparin sulfate). | Porcine intestinal linings. |

(*continued on next page*)

**Table 1** (*continued*)

| Polysaccharides | Origins |
|---|---|
| *Hyaluronic acid*<br>  <br>Disaccharide units of D-glucoronic acid and *N*-acetyl-D-glucosamine linked at 1,3- and 1,4-positions, respectively. | Rooster comb.<br>Streptococcal fermentation |
| *Pectins*<br>  <br>α(1 → 4)-linked D-galacturonic acid residues bearing free or methylated carboxyl groups. | Wastes from fruit juice production (citrus peel, apple pomace), sugar-beet pulp |

and surface charge) and environmental factors such as the bulk medium composition (ionic strength, presence of organic substances) and flow conditions [12–15]. Adhesion of bacteria to negatively charged surfaces under physiological pH conditions may be affected by electrostatic repulsion forces since the net electrostatic charge of most bacterial cell walls is negative at neutral pH [34]. It has also been frequently observed that hydrophilic, low surface energy materials are less prone to bacterial adhesion than hydrophobic ones, though contradictory results do exist [15]. It is generally admitted that hydrophilic surfaces in contact with media containing organic molecules such as proteins oppose the formation of a conditioning film harboring adhesion sites for bacteria – limiting specific adhesion/attachment of bacteria and subsequent biofilm development [13,15]. Anionic polysaccharides with hydrophilic properties have been consequently considered possible candidates to elaborate anti-adhesive surfaces.

### 2.1. Hyaluronic acid

One of the most studied polysaccharides as a biofilm repelling coating is hyaluronic acid (Table 1) [35–37]. In 1999, Morra and Cassineli [35] demonstrated non-fouling properties of glass surfaces modified with hyaluronic acid covalently bound to a first layer of poly(ethyleneimine). Displaying hydrophilic characteristics (contact angle of 22°), this coating reduced adhesion of *S. epidermidis* and *E. coli* by several orders of magnitude compared to the unmodified glass slide. Harris and Richards [36] investigated *S. aureus* adhesion on titanium (a metal currently used as implant material in orthopedic and dental applications) surfaces, displaying differences in roughness (resulting from varying polishing treatments) and grafted or not with hyaluronic acid. Showing no clear dependence on surface roughness, bacterial adhesion was significantly reduced by the coating. In the same way, adhesion of *S. aureus* on Ti foils functionalized with hyaluronic acid-catechol was lower than on pristine substrates [37]. The bacteria-repelling properties of hyaluronic acid have been recently illustrated by a reduction in adhesion of *S. aureus* cells to hyaluronic acid-coated Ti surfaces [38] and poly(methyl methacrylate) intraocular lenses [39] compared to untreated surfaces. A graft copolymer derivative of hyaluronic acid bearing amino and carboxyl groups showed better prevention of *S. aureus* adhesion on Ti disks than the pristine hyaluronic acid hydrogel [38]. However, many commercial hyaluronic acid-based coatings currently available (e.g., Hydak® from Biocoat Inc, Horsham, Pa. and Incert®-S from Anika Therapeutics Inc., Bedford, Mass.) are mainly designed to minimize tissue attachment (e.g., post-surgery adhesions) on implants.

### 2.2. Heparin

Heparin is another natural polysaccharide of animal origin whose anti-adhesive properties have been extensively investigated. Heparin is commonly used as an antithrombotic coating in implanted devices that are in contact with blood, in particular catheters and stents. The Bioline Coating® from Maquet Cardiopulmonary GmbH, Rastatt, Germany – a subsidiary of Getinge AB, Göteborg, Sweden, the Bioactive Surface CBAS® from Carmeda AB, Upplands Väsby, Sweden – a subsidiary of W.L. Gore and Associates, Inc., Newark, Del., and the Trillium® biosurface from Medtronic, Inc., Minneapolis, Minn., are some HP-based antithrombotic coatings available on the market. This negatively charged, linear polysaccharide (Table 1) has been immobilized on material surfaces via various physical or chemical strategies including electrostatic deposition, layer-by-layer self-assembly and covalent attachment [40]. Bacterial adherence to heparinized commercial devices, e.g., ureteral [41,42] and biliary [43] stents, central vein [44] and dialysis [45] catheters, has been assessed *in vitro* [41,42] or *in vivo* [43–45]. Most studies highlighted anti-adhesive effects of heparin coatings [19] though Lange et al. [42] noted no significant difference in the number of bacteria adhered to heparin-coated stents and non-coated controls (see also [46]).

### 2.3. Other polysaccharides

Some other polysaccharides have also shown anti-adhesive properties against bacterial cells. Xu et al. [47] elaborated

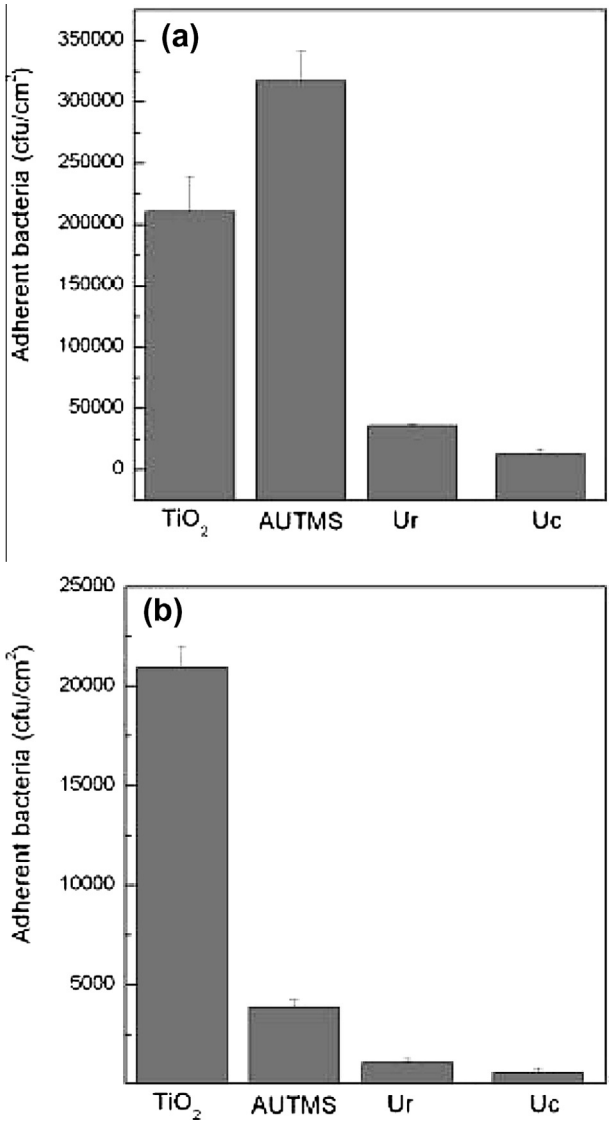



**Fig. 2.** Number of *S. epidermidis* cells adhered on various titanium substrates (8 mm × 8 mm) after (a) 1h30 and (b) 24 h of contact with a bacterial suspension [$10^6$ Colony Forming Units (CFU)/mL]. Error bars indicate standard deviations from (a) three independent CFU counts and (b) ten AFM images (100 μm × 100 μm). Ti substrates: $TiO_2$, unmodified; AUTMS, modified by 11-aminoundecyltrimethoxysilane; Ur/Uc, created from *Ulva rotundata*/*Ulva compressa* after modification by 11-aminoundecyltrimethoxysilane. Adapted from [48].

copolymers of poly(urethane) and dermatan sulfate with different degrees of substitution. Also known formerly as chondroitin sulfate B, dermatan sulfate is a glycosaminoglycan polysaccharide whose structure is close to that of chondroitin sulfate (it contains iduronic acid in place of guluronic acid) (Table 1). The *in vitro* adhesion of proteins, mammalian and bacterial cells (*E. coli*) on all copolymer films significantly decreased compared to unmodified poly (urethane). Gadenne et al. [48] have shown the ability of ulvan coatings to inhibit initial adhesion of gram-positive (i.e., *S. epidermidis*) and gram-negative (i.e., *P. aeruginosa*) bacteria on titanium plates. Polysaccharides extracted from *Ulva rotundata* and *U. compressa* were covalently immobilized on Ti surfaces which had been previously functionalized by self assembled monolayers of 11-aminoundecyltrimethoxysilane. The inhibition of *S. epidermidis* adhesion was more particularly marked, with a reduction in the number of adhered bacteria up to 97% after contact for 1h30 (Fig. 2a). Furthermore, in the same study [48], these authors have demonstrated by atomic force microscopy (AFM) imaging that such polysaccharide coatings affect the spreading of bacteria and could limit bacterial colonization for a long time (24 h) (Fig. 2b). Ulvan is a sulfated polysaccharide which is extracted from green algae that invade the Brittany coast. Its structure shows great complexity and variability, with main repeating disaccharide units containing glucuronic or iduronic acid ([49]; see also [50,51] for detailed structural features). Recently, the same authors have tried to find a relationship between molecular parameters of ulvans and their anti-adhesive properties [52]. From this study it seems that surface hydrophilicity, surface roughness and negative charges are not decisive parameters, while polysaccharide molecular weights and experimental immobilization conditions play a key role.

Dextran, a branched homopolymer of glucose (Table 1) excreted by growing cultures of *Leuconostoc mesenteroides* [53], is one of the few bacterial exopolysaccharides produced by fermentation at an industrial scale. Dextran and its derivatives have been widely investigated for their antifouling properties limiting protein and animal cell adhesion [54–56], but much more rarely tested as bacterial-repellent coatings. One can mention the work by Shi et al. [57]. In this study, the surface of Ti alloy substrates was functionalized by covalent grafting of oxidized dextran via a first layer of dopamine. *S. epidermidis* and *S. aureus* adhesion on functionalized surfaces was reduced twice compared to that on pristine substrates. Further attachment of the bone morphogenetic protein-2 (bone growth factor) to dextran did not affect bacterial repellency but promoted osteoblast function – a positive feature for successful osseointegration of Ti alloy implants. Beside dextran, a number of microbial exopolysaccharides, in particular from marine environments, offer promising opportunities as anti-adhesive coatings. As mentioned earlier, some of them have been shown to inhibit biofilm formation and disrupt established biofilms with no biocidal effect [23], which might be advantageous in biomedical applications by preventing the emergence of bacterial resistance [22]. However, the variety in composition and structural characteristics of microbial exopolysaccharides, the difficulties inherent in the standardization and optimization of fermentation conditions are impeding their short-term practical development. Until the advent of efficient biofilm-repellent surfaces using, in particular, these newly identified natural macromolecules, the elaboration of bactericidal or, better still, both bactericidal and anti-adhesive coatings remains a challenge.

## 3. Bactericidal surfaces

There exist a few polymeric materials capable of killing bacterial cells upon contact. A survey of synthetic polymers displaying bactericidal activity "by themselves" or after chemical modification or incorporation of antimicrobial compounds has been published a few years ago [58]. Natural polymers exhibiting bactericidal properties in their native or modified form are much scarcer. The carboxymethylated form of a linear β-D-glucan extracted from the wood-decay fungus *Poria cocos* (*Wolfiporia extensa*) was used by Wang et al. [59] to coat poly(urethane) discs via covalent binding. Modified poly(urethane) discs inhibited the growth of *P. aeruginosa* in culture medium. Besides the well-documented, commercially developed heparin-based coatings loaded with antimicrobials [19], some recent works have tested hydrogels made of hyaluronic acid derivatives as antibiotic releasing coatings. In particular, a patented derivative of hyaluronic acid grafted with poly(D,L-lactic acid) (DAC®, Novagenit, Mezzolombardo, Italy) [60], loaded with vancomycin, has been tested *in vivo* against bacterial colonization of Ti intramedullary nails by inoculated methicillin-resistant *Staphylococcus aureus* [61].

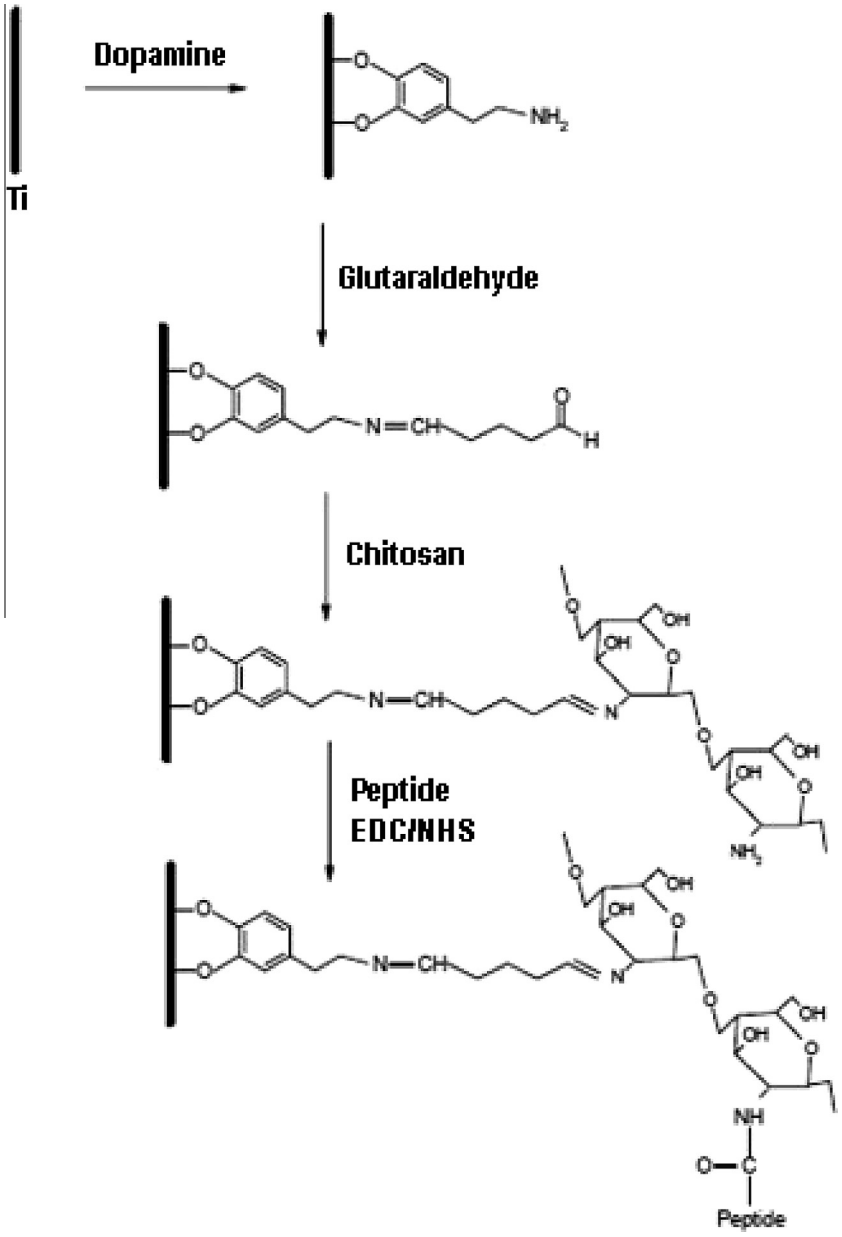



**Fig. 3.** Immobilization of chitosan and RGD peptide on Ti substrates. Amine coupling agents for covalent binding of RGD peptide: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NHS, N-hydroxysuccinimide. Taken from [78].

The antibacterial efficacy of the vancomycin DAC® coating, highlighted by these preliminary experiments, remains to be confirmed. Hydrogel coatings based on chemically modified hyaluronic acid showing bacterial killing properties have also been proposed [39,62]. The bactericidal effects were obtained via hyaluronic acid modification by covalent grafting of nisin, an antimicrobial peptide [62], or binding of lysozyme, a tear protein with antibacterial and anti-inflammatory functions [39]. As concerns polysaccharide-based coatings with bacterial killing properties, however, chitosan and its derivatives occupy a hegemonic position.

Indeed, a number of antimicrobial coatings based on chitosan and derivatives have been proposed over the past ten years. Chitosan (Table 1) is a natural polymer obtained by deacetylation of chitin, the second most abundant polysaccharide found in nature after cellulose – present in particular in the exoskeletons of arthropods (crustaceans and insects) [63]. Chitosan offers excellent biodegradability, biocompatibility, non-toxicity and processability properties, allowing a wide range of applications in the biomedical area [64–67]. It also displays antimicrobial properties [28,63,68] that are generally attributed to its polycationic nature – the positively charged amine groups of glucosamine interacting with negatively charged constituents of microbial cell membrane, causing the leakage of intracellular components [63]. Recent studies have revealed the antibiofilm effects – including inhibition of biofilm formation and/or reduction in survival of mature biofilm – of chitosan solutions [69–71] and nanoparticles [72]. These properties, allied with its film-forming ability, have made chitosan an efficient packaging material for food preservation [73,74]. They also explain the extensive use of this polysaccharide and its derivatives in antimicrobial surfaces of implant materials.

### 3.1. Chitosan in polymer blends

Direct incorporation of an antimicrobial drug, e.g., an antibiotic, is the simplest means to confer bacterial killing properties to a substratum, e.g., a biomedical polymer. Recently illustrated with

commercial hyaluronic acid-based hydrogel coatings (see above), this strategy has also been followed by Tan et al. [75] using chitosan and a quaternized chitosan derivative as bactericidal compounds to yield poly(methyl methacrylate)-based bone antibiofilm cement. Quaternized chitosans are water-soluble derivatives that display in solution enhanced antibacterial activity compared with unmodified chitosan and inhibit biofilm formation and growth [76,77]. In the quoted study, chitosan and hydroxypropyltrimethyl ammonium chloride chitosan (HACC) with varying degrees of substitution were mixed with poly(methyl methacrylate) (PMMA) and the antibiofilm efficiency of polymer blend discs was compared with that of gentamicin-loaded PMMA. The number of *S. aureus* and *S. epidermidis* cells adhering on gentamicin-loaded- and HACC–PMMA discs was significantly lower than on crude and chitosan-loaded PMMA. On the other hand, the HACC–PMMA cement displayed enhanced contact killing of bacteria compared to gentamicin-loaded-PMMA but PMMA loading with unmodified chitosan did not improve the bactericidal efficiency. Therefore, the inhibition of biofilm formation on HACC–PMMA discs was due to the bactericidal properties of the surface. However, like gentamicin, chitosan and HACC were released from cement discs immersed in buffer.

### 3.2. Covalent grafting of chitosan

In a series of papers, Shi et al. [78,79] and Hu et al. [37] described the functionalization of commercial titanium (and Ti alloys) pieces by chitosan or carboxymethyl chitosan – the *O*-carboxymethylated derivative, more bactericidal than chitosan in solution [80] and preventing bacterial biofilm formation [81]. In these works, Ti surfaces were first grafted with dopamine by adsorption from aqueous solution. Then chitosan/carboxymethyl chitosan was covalently attached to dopamine through a glutaraldehyde linker (Fig. 3). The polysaccharide coatings were further functionalized by attachment of proteins designed for promoting tissue integration of implants, i.e., the cell-adhesive arginine-glycine-aspartic acid peptide (RGD) (Fig. 3) [78], the bone morphogenetic protein-2 (BMP) [79], and the vascular endothelial growth factor [37]. The antimicrobial efficacy of coatings was evaluated by exposing pristine and functionalized Ti specimens to dense bacterial suspensions (*S. aureus* and *S. epidermidis*) and counting adsorbed cells after contact for a few hours. In all three studies, the numbers of bacterial cells on modified Ti substrates were noticeably lower than on uncoated controls, as illustrated by Fig. 4 for the carboxymethyl chitosan-BMP coating, with a higher proportion of dead bacteria on coated surfaces (observed by fluorescence microscopy). While favoring osteoblast attachment and functions, osteoinductive proteins exerted no significant effect on bacterial adhesion. Later on, the same team functionalized silicone sheets with carboxymethyl chitosan via a poly(dopamine) layer (resulting from self-polymerization of dopamine) [82]: the modified silicone substrates also showed anti-adhesive and bactericidal properties against *E. coli* and *P. mirabilis* cells.

Chitosan has also been bound to Ti surfaces via silanization procedures. In a two-step process detailed by Martin et al. [83], the silane molecule triethoxysilylbutyraldehyde [4-(triethoxysilyl) butanal] (TESBA) was first immobilized on the metal surface (Fig. 5). Then chitosan was covalently bound to TESBA through the amino and aldehyde groups of chitosan and TESBA, respectively, to form an imine link. This functionalization process has been recently applied to commercial Ti foils by Renoud et al. [84] who investigated the chemical, mechanical and biological (i.e., biocompatibility and antibacterial efficiency) properties of the chitosan coating. As concerns the latter point, relevant to the present review, the polysaccharide coating did not inhibit fibroblast proliferation, but the presence of coated metal foils in nutrient

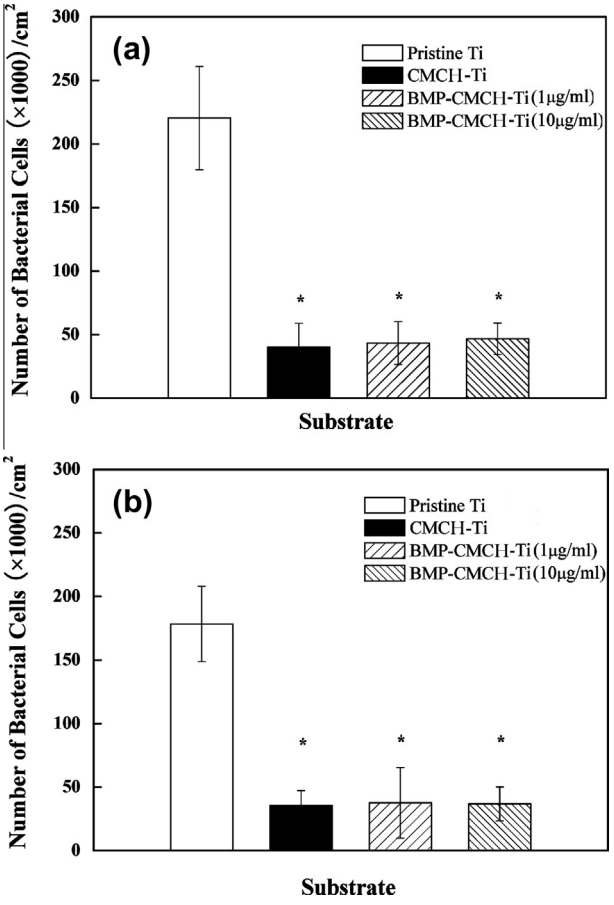



**Fig. 4.** Number of adherent cells of (a) *S. aureus* and (b) *S. epidermidis* per $cm^2$ on surfaces of pristine and functionalized Ti substrates. (*) Denotes significant differences ($p < 0.05$) compared with the pristine Ti. CMCH, carboxymethyl chitosan; BMP, bone morphogenetic protein-2. Taken from [79].

medium reduced the growth of *Actinomyces naeslundii* and, to a lesser extent, *Porphyromonas gingivalis* – two bacterial species belonging to oral microflora and frequently involved in dental implant infections. In this work, however, adhered bacteria were not enumerated.

### 3.3. Chitosan grafting via polymer brushes

Another approach to graft chitosan on surfaces is the use of polymer brushes [85]. Chitosan has been immobilized on various polymer surfaces, including poly(ethylene) [86], poly(propylene) [87], poly(ethylene terephthalate) [88] and poly(vinyl chloride) [89]. The surface of the polymer substrate was first activated by plasma treatment or γ-ray irradiation to generate functional groups such as carboxyl, hydroxyl, peroxy and epoxy groups. Then polymer chains of poly(acrylic acid) [86,88,89] or poly(N-isopropylacrylamide) [87] were synthesized by graft polymerization initiating from these active groups. Finally, chitosan was attached covalently to polymer brushes bearing carboxyl or amino groups. The surface-modified polymers were shown to display antibacterial activity against bacterial strains of medical interest, i.e., *E. coli*, *P. aeruginosa* or *S. aureus* (Fig. 6). As concerns poly (acrylic acid)-chitosan covered poly(ethylene) samples [86], however, their cell-killing efficiency was due to chitosan leakage from the coating, showing weak attachment of the polysaccharide to poly(acrylic acid) brushes – leakage being impeded by crosslinkage with glutaraldehyde (Fig. 6). Surface immobilization of chitosan via polymer brushes using this so-called surface-initiated

polymerization "grafting from" strategy [85] has also been achieved on non-polymer substrates such as stainless steel [90]. In this study, the metal surface was first coated with barnacle cement that was next functionalized with alkyl bromide initiator to enable formation of poly(2-hydroxyethyl methacrylate) (PHEMA) brushes by atom transfer radical polymerization using a two-step process (Fig. 7). Owing to the antifouling and antibacterial properties of PHEMA and chitosan, respectively, the stainless steel surfaces with chitosan-coupled PHEMA brushes exhibited both anti-adhesive and cell-killing properties against *E. coli*. The attachment of polymer brushes to a surface via a "grafting to" approach, according to which a preformed polymer is attached to the surface, has been reported by Lee et al. [91] using a silicon oxide substrate and chitosan modified with quaternary ammonium salts (Fig. 8). The silicon oxide surface was silanized with 3 -glycidoxypropyl-trimethoxysilane to generate epoxy groups. Then quaternized chitosan was grafted to the silica substrate via covalent links between epoxy groups and (primary) amino groups of chitosan (Fig. 8). The cationic polymer layer, displaying pH-dependent swelling, noticeably reduced *S. aureus* colonization [91] and shear resistance of established *S. aureus* biofilms [92] compared to control substrates.

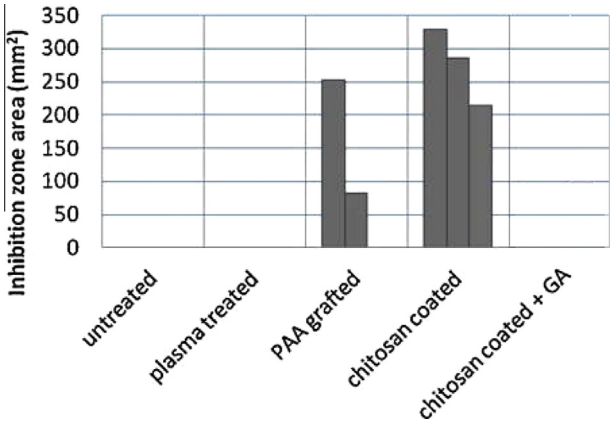



**Fig. 6.** Assessment of antibacterial activity of poly(acrylic acid)-chitosan modified poly(ethylene) foils against *S. aureus* by the inhibition zone method (diffusion test). Circular samples (8 mm diameter, 20 μm thick) were placed on nutrient agar plates inoculated with $10^6$ bacteria and incubated for 24 h at 37 °C. Each column represents the inhibition zone area for one experiment out of three. PAA, poly (acrylic acid); GA, glutaraldehyde. Adapted from [86].

### 3.4. Chitosan in layer-by-layer architectures: anti-adhesive and bactericidal coatings

Chitosan, as a cationic polyelectrolyte, has also been combined with anionic polymers via layer-by-layer self-assembly [10,93], giving rise to multilayered architectures. The coating process is based on the alternate deposition of polyanions and polycations that adsorb spontaneously on each other through weak interactions, mainly electrostatic interactions between opposite charges (Fig. 9). The polyelectrolyte multilayer (PEM) structure can be further functionalized by incorporation of active compounds and its properties adjusted by the deposition conditions and the choice of the outermost layer (i.e., anionic or cationic) [93]. Owing to its well-known properties, quoted above, chitosan is probably the most widely used polysaccharide in layer-by-layer films [95]. In PEM structures, chitosan and derivatives have been associated with varying anionic polymers, more particularly polysaccharides such as heparin [96–100], hyaluronic acid [93,101,102] and alginate (Table 1) [103–105], but also pectin [89,106], κ-carrageenan (Table 1) [107] and others [108,109]. PEM-coated substrates include Ti and Ti alloys [100,101], glass and silica [107], synthetic polymers like poly(propylene) [106], poly(styrene) [97], poly(vinyl

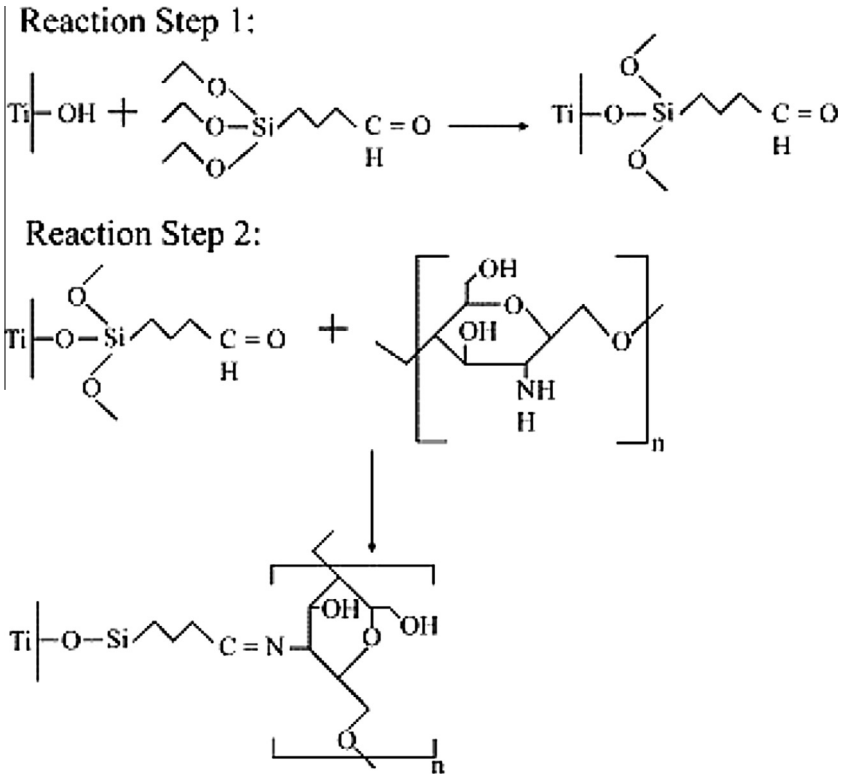



**Fig. 5.** Reaction steps involved in the binding of chitosan to titanium substrates via silanation with triethoxsilylbutyraldehyde (TESBA): (1) TESBA deposition; (2) reaction of TESBA with chitosan. Taken from [83].

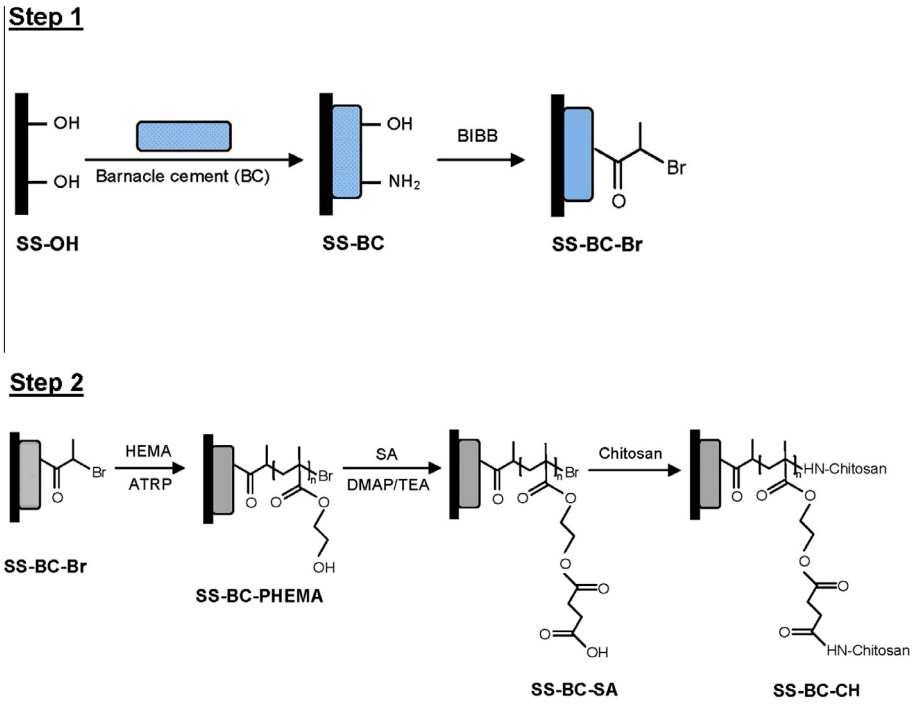



**Fig. 7.** Chitosan grafting on stainless steel (SS) surface via poly(2-hydroxyethyl methacrylate) (PHEMA) brushes. Step 1. Immobilization of alkyl bromide ATRP (atom transfer radical polymerization) initiator via barnacle cement (BC). BIBB, 2-bromoisobutyryl bromide. Step 2. Surface-Initiated ATRP of 2-hydroxyethyl methacrylate (HEMA) and chitosan (CH) immobilization. DMAP, 4-(dimethylamino)pyridine; TEA, trimethylamine; SA, succinic anhydride. Adapted from [90].

chloride) [89], poly(urethane) [108], poly(L-lactic acid) [109] or poly(ethylene terephtalate) [98,99], and animal solid tissues [96]. As a general rule, the main role of chitosan is to confer long-lasting antimicrobial activity to these multilayered films whereas associated anionic polysaccharides are chosen for their anti-adhesive properties. For instance, Bratskaya et al. [107] compared the antibacterial and anti-adhesive properties of varying chitosan-based coatings against two *E. faecalis* strains isolated from clogged biliary stents: chitosan alone, covalently grafted on the glass substrate, and chitosan combined with κ-carrageenan in PEM films. PEMs displayed better anti-adhesive properties than the chitosan layer owing to electrostatic repulsion between sulfo groups of κ-carrageenan (Table 1) and negatively charged enterococcal cells. On the other hand, the ability of PEM coatings to kill bacteria upon adhesion ("contact killing") was significantly lower than that of covalently grafted chitosan. Only chitosan-terminated PEMs showed higher killing efficiency than pristine glass surface. Therefore, these multilayer coatings offered a good compromise between the expected antibacterial activity of chitosan and its detrimental, adhesion-promoting effects towards negatively charged bacteria (via positively charged amino groups) [107]. Cui et al. [102] reported PEM hollow microcapsules whose envelope consisted of hyaluronic acid-quaternized chitosan multilayer. These microcapsules displayed contact-killing efficiency against *E. coli* while retaining their biocompatibility – showing potential as drug carriers for antibacterial delivery systems. Wang et al. [108] associated chitosan with lentinan to modify poly (urethane) surfaces. Lentinan, a mushroom polysaccharide isolated from *Lentinus edodes*, is a (1 → 3)-β-D-glucan having (1 → 6)-glucosyl side groups that exhibits antitumor activity [110]. Lentinan was first sulfated and poly(urethane) aminated before PEM deposition via the layer-by-layer assembly technique which yielded chitosan or lentinan as topmost layer (Fig. 10). PEM coatings significantly inhibited the growth of *P. aeruginosa* cultures and reduced fibrinogen adsorption and platelet adhesion. To reinforce the antibacterial potency of chitosan-based PEMs, one way consists to introduce bactericidal compounds in the multilayer structure. This conventional strategy has been applied to PEM coatings designed for blood-contacting materials, where chitosan was associated with an antithrombotic polysaccharide. Silver nanoparticles were incorporated into chitosan–heparin [99] and chitosan–dextran sulfate [109] deposited on poly(ethylene terephtalate) and poly(L-lactic acid) surfaces, respectively. These polymers are widely used in cardiovascular implants and vascular tissue engineering. Chitosan–heparin multilayer complemented with silver nanoparticles displayed enhanced antimicrobial activity against *E. coli* compared to silver-free PEM [99]. The bactericidal efficiency of Ag–chitosan–dextran sulfate PEM against *S. aureus* increased with the number of bilayers deposited [109]. Takeoka and his team [104,105] developed an original antibacterial nanosheet designed for wound dressing, based on chitosan–alginate PEM loaded with an antibiotic (i.e., tetracycline). Tetracycline was sandwiched between a poly(vinyl alcohol) layer, as a transparent and hydrophobic protecting barrier, and the chitosan–alginate PEM. Nanosheet pieces were placed in contact with wounded tissue *in vivo*, the polysaccharide multilayer facing the wound. The chitosan–alginate PEM, ensured diffusive delivery of the antibiotic towards the wound, preventing bacterial infiltration and improving survival of mice affected by cecal puncture [104] or *P. aeruginosa* burn-wound infection [105].

Among biomedical materials, titanium and Ti alloy implants, widely used in orthopedics and dental surgery, are more particularly faced with the "race for the surface" [13,14] implying bacteria and tissue cells, i.e., biofilm formation and tissue integration [111]. To enhance bone cell attachments on hyaluronic acid-chitosan PEM-functionalized Ti, Chua et al. [101] immobilized the cell-adhesive arginine-glycine-aspartic acid peptide on the outermost PEM surface. The presence of this peptide actually improved osteoblast adhesion, proliferation and function, while PEM with peptide conjugation retained high antibacterial efficacy against *S. aureus*.

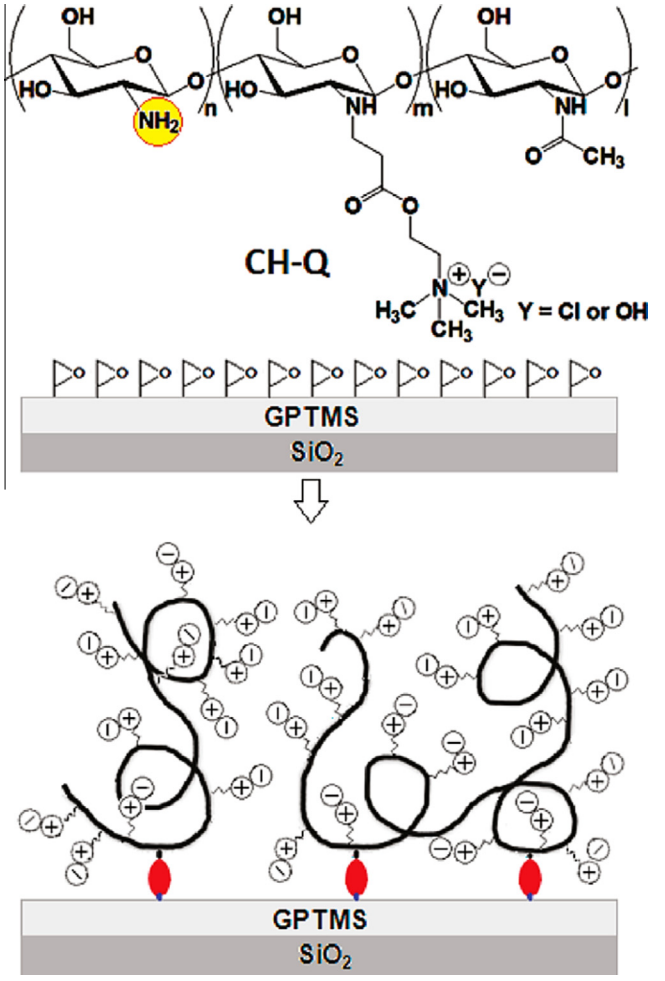



**Fig. 8.** Immobilization of quaternized chitosan (CH-Q) brushes on silicon oxide surface via a "grafting to" approach. (Top) The chemical structure of water-soluble chitosan modified with quaternary ammonium salts. CH-Q is "grafted to" epoxide-derivatized silicon oxide surfaces via the primary amine functional groups of CH-Q (yellow circle). GPTMS, 3-glycidoxypropyl-trimethoxysilane. (Bottom) Red ovals represent the covalent grafting of the primary amine of CH-Q with the epoxide group. The quaternary ammonium cations and counteranions ($Cl^-$ or $OH^-$) are denoted as plus and minus, respectively. Taken from [91]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

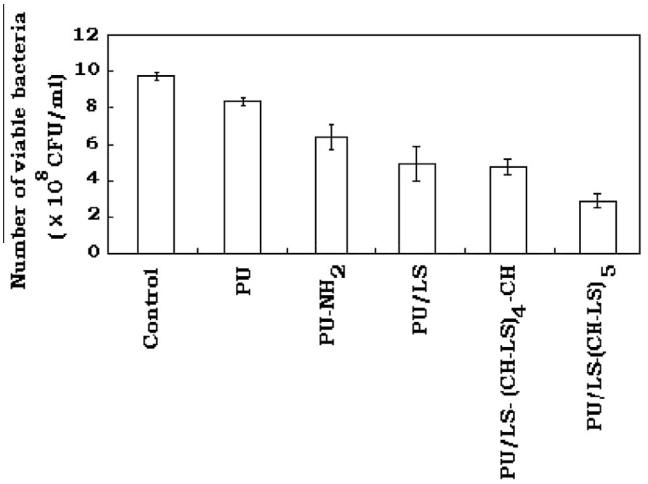



**Fig. 10.** Inhibition of bacterial growth by PEM-coated poly(urethane) (PU) films. Aminated PU ($PU/NH_2$) was covered with PEMs of chitosan (CH) and lentinan sulfate (LS). The bar chart shows the number of viable and cultivable *P. aeruginosa* cells (CFU) in the medium after incubation for 24 h in the presence of modified PU and unmodified PU films. Initial bacterial concentration was about (5.0–10.0) × $10^5$ CFU/ml. Control, growth in the absence of PU sample; PU/LS, aminated PU covered with LS monolayer; PU/LS–$(CH–LS)_4$–CH, aminated PU modified by five LS–CH bilayers, yielding CH as topmost layer; PU/LS–$(CH–LS)_5$: PU/LS modified by five CH–LS bilayers, yielding LS as topmost layer. Adapted from [108].

To favor endothelialization of nickel/titanium alloy (nitinol) covered with chitosan–heparin PEM, Schweizer et al. [100] proposed to increase PEM surface hydrophilicity by mineralization using calcium phosphate – leading to decreased contact angle compared to bare substratum and untreated PEM. However, neither antibacterial efficiency nor cell tissue adhesiveness of mineralized coatings was tested in this study. More recently, Almodovar et al. [96], in search of a synthetic periosteum, modified sheep bone substrates by chitosan–hyaluronic acid multilayer deposition. These coatings promoted the attachment of ovine mesenchymal stem cells and showed significant antibacterial activity against *S. aureus* and *E. coli*. However, adsorption of the cell-adhesive protein fibronectin to PEM exerted no noticeable influence on adhesion of mesenchymal stem cells – which might be due to the presence of adhesion ligands on bone surface, able to interact with osteoblasts though covered by the PEM.

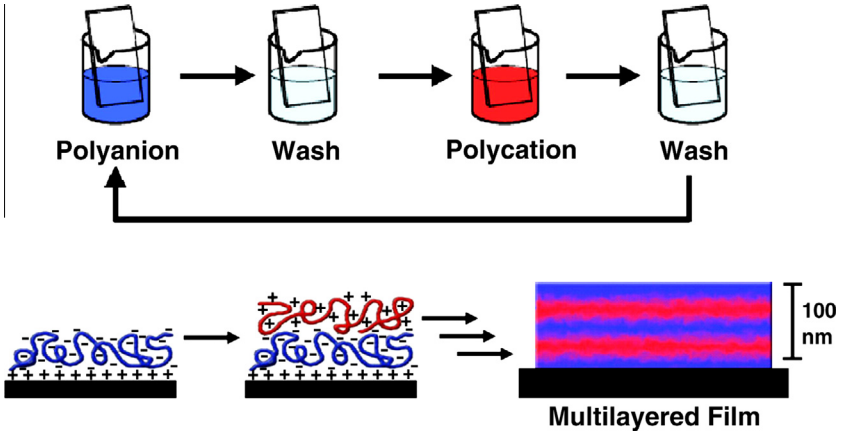



**Fig. 9.** Layer-by-layer deposition of oppositely charged polyelectrolytes on surfaces. (Top) Fabrication proceeds by iterative dipping of a substrate into dilute aqueous solutions of cationic and anionic polymers. (Bottom) Electrostatic interactions guide the assembly of multilayered polyelectrolyte assemblies. Taken from [94].

## 4. Conclusion

A number of surface coatings based on polysaccharides have been proposed over the past ten years to confer antimicrobial properties to materials intended for implantation – metals and polymers, essentially. Polysaccharides offer flexible, biocompatible platforms for designing coatings to protect surfaces from infection. The most promising assemblies combine anti-adhesive and bactericidal efficiencies to prevent biofilm formation on implanted devices. When tissue integration of the foreign body is required, additional functionalities may be added to the polysaccharide-based composite coatings. To date, the majority of published studies make use of chitosan and derivatives in bactericidal formulations. Future extensions will involve microbial polysaccharides [22,23] which, together with antimicrobial peptides [112,113], will be the cornerstone of bio-inspired antibiofilm coatings.

Antibiofilm properties of these functionalized surfaces have been essentially tested *in vitro*, although *in vivo* experiments are an obvious prerequisite for any practical application. *In vitro* bacterial adhesion tests on modified surfaces, evaluation of their killing efficiency against suspended bacteria and their ability to oppose biofilm formation/development have been performed in synthetic media ranging from simple salt media to conventional culture broths. However, many body fluid components interact with implanted devices, modifying the physicochemical characteristics of material surfaces [13,14]. Proteins carrying a net negative charge such as human serum albumin – the most abundant blood plasma protein – may adsorb on hydrophobic, negatively charged polysaccharide coatings [114], affecting the anti-adhesion potential of coated surfaces. Ionic interactions between chitosan – positively charged via its amine groups – and these negatively charged proteins [115] may also affect the expected bactericidal efficiency of chitosan-based coatings. Many patents devoted to antibiofilm surfaces involving chitosan and derivatives have been published over recents years (e.g., [116–120]), in which different types of functionalized chitosan are used alone [116–118] or combined with anionic polymers in multilayer coatings [119,120]. The antibiofilm properties of chitosan-modified material surfaces remain to be tested in biological fluids before any industrial development. Reported *in vivo* experiments are actually very scarce. In addition to the already mentioned work by Giavaresi et al. [61] using vancomycin–DAC® coated Ti intramedullary nails, studies by Martinez et al. [121] and Cobrado et al. [122] can be quoted, where chitosan-treated (by incubation in a chitosan solution) poly(ethylene) [121] or poly(urethane) [122] venous catheters were implanted *in vivo* applying murine device-associated infection models. The conception and implementation of *in vivo* models, requiring close collaborations between biomedical engineers and hospital practitioners (microbiologists, immunologists, and surgeons) are the very way towards the development of both innovative and practically relevant antibiofilm surfaces.

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