Surface modification of stainless steel for biomedical applications: Revisiting a century-old material

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Abstract

Stainless steel (SS) has been widely used as a material for fabricating cardiovascular stents/valves, orthopedic prosthesis, and other devices and implants used in biomedicine due to its malleability and resistance to corrosion and fatigue. Despite its good mechanical properties, SS (as other metals) lacks biofunctionality. To be successfully used as a biomaterial, SS must be made resistant to the biological environment by increasing its anti-fouling properties, preventing biofilm formation (passive surface modification), and imparting functionality for eluting a specific drug or capturing selected cells (active surface modification); these features depend on the final application. Various physico-chemical techniques, including plasma vapor deposition, electrochemical treatment, and attachment of different linkers that add functional groups, are used to obtain SS with increased corrosion resistance, improved osseointegration capabilities, added hemocompatibility, and enhanced antibacterial properties. Existing literature on this topic is extensive and has not been covered in an integrated way in previous reviews. This review aims to fill this gap, by surveying the literature on SS surface modification methods, as well as modification routes tailored for specific biomedical applications.

*Keywords***:** stainless steel; surface modification; biofunctionalization; bioactivity.

1. Introduction

Metals have been used in various biomedical applications for a century owing to their excellent mechanical properties (high strength-toughness and fatigue resistance) and inertness [1, 2]. Stainless steel (SS), titanium (Ti), and cobalt-chromium-molybdenum (CoCrMo) alloy are the most extensively used materials in biomedical engineering because of their biocompatibility and mechanical properties [2, 3]. Ti and its alloys have superior biocompatibility, excellent strength and corrosion resistance, thus being more often used in implants for hard tissue replacement (hip joints and dental implants) compared to other metals [4]. For implants which require high wear resistance (e.g. artificial joints) CoCrMo alloys are typically the metal of choice [2]. Stainless steel (SS) is an iron-based alloy with at least 12% chromium which allows it to resist rust formation in unpolluted atmosphere [5]. Most implants used in cardiovascular, orthopedics, dentistry, craniofacial surgery, and otorhinology applications are made of SS [2]. Introduction of the first SS (18-8) as a bone implant in 1920s has widened clinical use of metallic materials [2]. As a bone implant material, SS has been used for about a century [6]. It has been used both as a permanent (artificial joints) and as a temporary implant (plates, medullary nails, screws, pins, sutures and steel threads and networks used in fixing fractures) [7]. Low carbon AISI 316L SS (complying to ASTM F138 and F139) has high molybdenum (2-3%) and chromium (17-20%) and low carbon content (less than 0.03%), which increases its local and intergranular corrosion [7]. Moreover, 316L is known for its good ductility, work hardenability and fatigue properties [8]. Compared to the two commonly used metals, SS has a lower cost [9] and its demand is also still high in developing countries [10, 11]. SS is a most cost-effective choice of material for orthopedic implants; this is due to its comparatively low cost, availability, ease of manufacturing, and reasonable corrosion resistance [12].

The biological environment in the human body is very harsh on metals and can lead to protein adsorption, biofilm formation, and corrosion. Despite its wide use as a biomaterial and its general good biocompatibility [9], SS does not have inherent biofunctional properties such as blood compatibility, osteoconductivity, and bioactivity [2]. Hence, these surface properties are normally targeted when performing surface modification of SS. When unmodified, SS surface is hydrophobic (with a high contact angle of 86.32±4.5˚ as reported in [13]) and hydrophobic surfaces tend to attract the adsorption of proteins. Earlier studies have shown the susceptibility of SS for biofilm formation and protein adsorption [14]. It is believed that adsorption of organic molecules such as proteins on the surface leads to biofilm formation, which in turn can lead to corrosion or itself be a source of bacterial contamination [15]. Moreover, additional bioactivity, like release of drugs or capturing specific cells, might be desirable. For this, SS has to be tethered with an active compound (for example, drug or antibody). To introduce the above mentioned desired properties without sacrificing important bulk characteristics [16], SS surface is modified through various coatings and biofunctionalization methods.

Due to the importance of SS in biomedical applications, the use of SS has been partially discussed in a review on surface modification of metallic biomaterials [17]; the use of a particular type of SS (nickel-free nitrogen containing austenitic SS) in biomedicine has also been reviewed [18]. However, there are no comprehensive reviews on the surface modification of SS for biomedical applications. This review article aims to provide a critical analysis on the functionalization/surface modification of SS by reporting and discussing the research conducted in this area. The paper is divided into two main sections. The first section focuses on relevant properties of SS, such as surface roughness, corrosion resistance, and biofunctionality. The second part discusses the rational for the surface modification of SS in the context of its

biomedical applications, including blood compatibility, osseointegration, anti-infection and functionalized endovascular stents.

2. Surface modification of SS

Surface modification of SS includes laser treatment, plasma modification, chemical and electrochemical treatment to obtain SS with certain properties such as roughness, hydrophilicity and corrosion resistance. Other surface modification techniques employ different physico-chemical methods and linker molecules and are used to render SS with various functional groups for further attachment of biomolecules required for different applications.

2.1. Roughness and wettability

Surface roughness and surface wettability play an important role in biomaterials performance. Surface roughness calculation is arbitrary since it depends on the method used, area measured and techniques used after the measurements (leveling and filtration) [3]. For instance, similar R_a (arithmetical mean deviation of the assessed profile) values can be obtained from surfaces with very different surface features (and hence functionality), when they are filtered under the same conditions [19]. Table 1 shows different methods which can be chosen to obtain a certain surface morphology according to the literature survey. Rough or smooth surface can be obtained by various means: electrochemical methods [10, 20-22], plasma methods [23], or severe shot peening [24].

Sharp edges and burrs can cause thrombus formation and neointimal hyperplasia on stents once implanted. Bhuyan *et al.* [21] identified optimal electropolishing conditions for real stents able to produce a surface with improved mechanical properties (minimized thickness reduction and pitting corrosion) at the desired surface roughness (100 nm). The effect of different surface treatments (polishing, aluminum oxide blasting, and hydroxyapatite (HA) coating) on osteoblast-like cells was recently evaluated by Zhang *et al.* [3]. This study revealed that rough surfaces were better than polished ones in terms of promoting cell morphology phenotype (flattened shape and complete spreading of cells) and adhesion. Among three treatments used, hydroxyapatite coating had superior results in supporting cell adhesion, but cell viability was reduced in the long-term (7 days) on HA-coated samples (reduced absorbance in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay). This is probably due to the change of the microenvironment for cell proliferation. Plasma spray of HA results in the formation of calcium phosphate and metastable compound with reduced crystallinity. These properties are thought to promote adhesion of osteoblast cells. However, excessive dissolution of calcium phosphate and metastable compound affect the long-term stability of the HA coating. Lower crystallinity may reduce pH of the medium and thus increase cytotoxicity of the coating in the long run. Another reason for a reduced long-term viability of cells on the surface might be due to an increased confluency of the cells. This study was important in demonstrating that different surface modification techniques can produce similar surface morphology, but with a difference in chemical composition, or similar chemical composition, and varied surface morphology [3]. In the case of vascular implants, the extent of surface roughness was found to affect the expression of three genes of human umbilical vein endothelial cells (HUVECs) as indicators of cell injury and activation. McLucas *et al* [25] tested three types of surfaces: roughened (by sand blasting; details were not shown), polished (600, 800 and 1200 grit silicon carbide paper followed by polishing cloths (3 μm) and sol-gel alumina suspension (0.06μm)) and as received SS (surface roughness average root mean square (rms) 95.8 ±5.7 nm). Surface roughness (rms) was 40.9 ± 1.7 nm for polished samples and 671.8 ±

27.8 nm for roughened samples, as estimated by atomic force microscopy (AFM). Cells were shown to be injured and activated when roughened (upregulation of three genes) and polished (upregulation of two genes) as opposed to as received SS and control sample (no SS). This may be an indication that polishing (at least resulting in this surface smoothness) may not be necessary for stents given their roughness is similar to the as received sample. This study is important in establishing a new way of initial testing of biomaterials for cell response. However, having more samples with varying surface roughness would give more valuable information in choosing a target surface roughness (and hence surface treatment).

Table 1

Methods used to obtain a rough or smooth SS surface.

Method	Characteristics of the obtained surface	Initial specimen	Obtained roughness	Technique used to measure roughness	Reference
Rough surface					
Severe short peening	Induced grain refinement; martensitic phase transformation; compressive residual stresses	316L R_a* = 137.27 ± 44.66 nm	R_a* = 290.87 ± 116.08 nm	Atomic force microscopy	[24]
Electrochemical grain-boundary etching	Microstructured surface for drug coating	316L electro-polished	R_z = 9.8 and 15.8 µm	Laser perthometer	[20]
Radio-frequency plasma irradiation	Increased Cr and Fe oxides for further coating (silicon rubber)	316L electro-polished and acid etched	S_a = 1.96 ± 0.94 nm, S_{rms} = 2.06 ± 1.34 nm	Atomic force microscopy	[23]
Smooth surface					
Electropolishing	Minimized pitting corrosion; increased surface Cr and Ni	304 and 316L R_a = 220 and 110 nm	R_a = 69-100 and 60-100 nm	Interferometer	[21]
Electropolishing	Increased corrosion resistance against disinfecting agents and NaCl	316L pickled R_a* = 0.12 ± 0.02 µm	R_a* = 0.078 ± 0.03 µm	Numerical assessment	[22]
Electropolishing and acid dipping	Increased corrosion resistance and Cr oxides	316L S_a = 161.34 ± 57.15 nm; S_{rms}= 206.58 ± 70.06 nm	S_a = 0.96 ± 0.29 S_{rms} = 1.71 ± 0.78 nm	Atomic force microscopy	[10]

R_a* - the arithmetical mean deviation of the assessed profile; R_a - average surface roughness; R_z - average of the highest peaks and the lowest valleys; S_a - arithmetic mean surface roughness; S_{rms} - root mean square surface roughness.

A study by Hilbert *et al.* [22] found no influence of surface roughness on bacterial adhesion on SS, while other studies indicated minimal bacterial adhesion at R_a = 0.16 µm that increased when the surface was smoother or rougher than this value [15]. Although both works studied similar R_a values, the contradictory results could possibly be explained by the different SS types (304 and 316L; latter having lower content of carbon and chromium [26]) used, bacteria tested (the common one being only *Pseudomonas aeruginosa*), and method for measurement of roughness (optical and numerical assessment). Schlisselberg *et al.* [27] showed that surface roughness alone was not the only factor of increased or decreased biofilm

formation, but it was a combination of the chemical composition and surface treatment used. Recent work by Bohinc *et al.* [28] demonstrated that bacteria adherence increased with respect to increasing roughness, independent of the technique used to change the surface roughness (3D polishing, brushing, grinding, and electropolishing). An increase in effective surface area led to increased bacterial adhesion. Based on these results, it seems plausible that both surface roughness and surface chemistry have an effect on bacterial adhesion, and it depends on the method used to obtain the specific surface and also on the type of bacteria tested.

In addition to the previously mentioned methods, plasma cleaning and blowing with gas are also used to modify SS surface. For example, radio-frequency plasma irradiation under pure oxygen atmosphere was used to enrich oxides on SS surface before spraying an inorganic polymer, silicone rubber, resulting in a uniform and strong layer. In comparison to electropolished and electropolished plus acid-etched surfaces, plasma treated surface had higher concentrations of surface oxides. Surface roughness was the highest in electropolished samples and the lowest in electropolished plus acid etched ones [23].

Distribution of surface irregularities on the surface is also critical in biomaterials, especially those interacting with cells [29]. Hence, creating a micro- and nanostructured surface is another important method in surface modification of SS and novel techniques are being developed to create surfaces with controlled topographies. For example, laser treatment has been employed in a number of studies to modify the surface of SS. Using femtosecond laser, Oberringer *et al.* [30] were able to engineer a SS surface that reduced the differentiation of myofibroblasts, being this beneficial in the prevention of restenosis. Micro- and nanofeatures produced by this method had no effect on proliferation of endothelial cells (ECs), but the effect of other interfering factors, such as hydrophilicity and oxygen content, were not fully studied. Through a technique using high repetition rate and low pulse energy femtosecond laser, Kam *et al.* [31] controlled the wettability of SS making it either hydrophilic or hydrophobic (with contact angle of water varied from 0◦ to 113◦). By varying the scan speed, various micro-conical structures with different morphology, size and density, were created. Femtosecond pulse makes the surface rough and the irregularity formed on the surface results in the local variation of absorbance which leads to the formation of micro-cones. Nucleation and growth process of micro-cones depends on the dynamic balance between redeposition and ablation process. Further studies on the effect of these structures on protein adsorption or cell adhesion on the modified surfaces are needed for a optimized use of this technology. Severe shot peening is another mechanical method that can be used to form a nanoscale layer with increased surface roughness and wettability [24]. This technique was claimed to be a promising low-cost technique but it still requires a specialized equipment which may not be available in most of the surface modification laboratories. This method might also be difficult for real implants due to their complex geometrical structures without adapting rotation of the samples during surface modification.

2.2. Corrosion resistance

Corrosion resistance is an important characteristic of metallic biomaterials. More than 90% of all retrieved SS implants (which failed) occurred due to corrosion attack, by pitting or crevice corrosion [32]. A number of physical and chemical methods have been used to increase the corrosion resistance of SS (Table 2).

Chemical composition and the presence of surface oxide layers on SS play an important role in their corrosion resistance. 316L SS is not susceptible to intergranular corrosion due to its low carbon content. It is protected against corrosion by a spontaneously formed oxide layer; this layer enhances properties of metals including increased corrosion resistance and inertness

in biological fluids, passivation, improved wear, and adhesion characteristics [33, 34]. Different acid treatment methods (Piranha [13, 14, 35-37], sulfochromic acid [38, 39] and nitric acid [40]) have been widely used to obtain SS surface rich in hydroxyl groups. Although no study comparing these pretreatments was found, one can assume that acid treatment conditions, shown in Table S1, would lead to the formation of hydroxylated surfaces due to their previous effectiveness in various applications.

Table 2

Methods used for improving corrosion resistance of SS.

Method	Coating	Corrosion resistance analysis	Advantages	Disadvantages	Reference
Physical methods					
Plasma immersion ion implantation and deposition	TiO film	PDP (SBF) at RT)	No peeling and delamination; can be used for different shapes	Specialized/ expensive equipment required; cytotoxicity not tested	[43]
Closed field unbalanced magnetron sputtering	Ti-Cu	PDP and EIS (Hank's solution/SBF)	Uniform continuous; compact coating; antibacterial properties	Expensive equipment required	[44]
Radio frequency magnetron sputtering	Nanostructured Zr_2CN	PDP (PBS)	Stable coating; strong adhesion; improved blood compatibility	Expensive equipment required; Low deposition rate	[45], [46]
Direct current (DC) and radio frequency glow discharge (RFGD)	Trimethylsilane	PDP and EIS (PBS)	Combines well-recognized stability of RFGD and adhesion to metallic substrates of DC	Needs RF power source, DC power supply, mass flow controller and other equipment	[47]
Chemical methods					
Electropolishing and acid dipping	NA	PDP (Ringer solution at 37°C)	Homogenous; smooth surface	Rough surface defects cannot be removed	[10]
Electrodeposition (pulse current deposition)	Polyaniline-graphene oxide	PDP and EIS (3.5% NaCl solution at RT)	Compact; uniform coating	Probable not uniform thickness	[48]
Plasma assisted chemical vapor deposition	TiN	PDP (Hank's solution at RT)	Enhanced surface hardness; not cytotoxic; uniform coating	Coating of sharp-edged geometries might be difficult	[49], [50]
Sol-gel spin coating	Polypyrrole-strontium hydroxyapatite	PDP (SBF)	Good adhesion of lower inorganic layer; low defect density of the upper organic-inorganic layer	Requires high sintering temperatures; difficult to control porosity; chemical and phase composition	[51], [46]

Abbreviations: EIS – electrochemical impedance spectroscopy; PDP – potentiodynamic polarization; RT – room temperature; SBF – simulated body fluid; NA – not applicable.

One of the early studies on the electropolishing of SS for corrosion resistance was first performed in the 1980s, and continues to be used widely in SS modification [41]. Two methods were compared by Latifi *et al.* [10]: (i) electropolishing (phosphoric and sulfuric acids as electrolyte solutions) and (ii) electropolishing with acid dipping (nitric and hydrofluoric acids). Both treatments resulted in corrosion resistant surfaces. However, more chromium oxides were formed after electropolishing and acid dipping in hydrofluoric acid, while after electropolishing alone hydroxide layers were increased [10]. Direct current anodization in the presence of sulfuric acid with hydrogen peroxide was performed on SS to obtain nanoporous chromium-rich oxide films with the size range of 5-20 nm. The modified surface had improved hydroxyapatite deposition [42].

Ceramic materials, especially hydroxyapatite, have also been widely used for having anti-corrosive properties together with its osteostimulative properties, excellent biocompatibility and similarity (in composition and structure) to bone [52]. Hydroxyapatite substituted with Sr or Sr/Mg has been electrodeposited on SS previously electropolymerized with conducting polymers of polypyrrole and poly(3,4-ethylenedioxythiophene), respectively [52, 53]. Both of these bilayers significantly improved anti-corrosive properties of the modified surface as opposed to one layer only. Another ceramic coating, composed of fluorapatite (hydroxyapatite with incorporated fluoride ions) and niobum filler, was plasma sprayed on SS and led to improved corrosion resistance and a claimed improvement in biocompatibility [12]. However, biocompatibility in this case was only indicated by the corrosion resistance test and no cytotoxicity or *in vivo* studies were performed. Ceramic coatings can also be prepared by sol-gel method which produces homogenous coatings and can be exploited for complex-shaped surfaces. Double layer thin film, consisting of inorganic ($ZrTiO_4$) and organic-inorganic ($ZrTiO_4$-polymethyl methacrylate), were spin-coated on SS and showed excellent corrosion resistance. Inorganic layer provided a good adhesion to the surface, while the upper one reduced physical defects of the bottom layer resulting in a less porous upper layer resistant to corrosion [51]. Sol-gel spin coating was also exploited to introduce bioactive glass/zirconium titranate anti-corrosion coating [54]. Although not intended for an increase of corrosion resistance *per se* but for formation of a bioactive surface, SS modified with nanostructured forsterite [55] made it more resistant to Cl^- ion attack in the SBF. Potentiodynamic polarization corrosion test (Fig. 1a) of the coating showed a reduced corrosion current density for the modified surface implying an improvement of uniform corrosion resistance due to coating. Scanning electron microscopy (SEM) analysis of the two surfaces shows (Fig. 1b, c) deep pits on the untreated samples and a milder and more uniform attack of the forsterite coating serving as a barrier against corrosive medium.

Silane and composite silane can also be used for improving corrosion resistance. Silane coupling agents (SCAs) (*see* section *Silane-based agent*s) have reactive terminal functional groups, which enable them to improve adhesive strength between metals and polymers [56]. Hosseinalipour *et al.* [57] developed a crack-free hybrid coating on SS with improved corrosion resistance. The coating was composed of tetraethylorthosilicate and 3-methacryloxy-propyltrimethoxysilane with their ratio being the most important factor in forming of this highly adhesive film. Although the authors do not show SEM images and FTIR (Fourier transform infrared) spectroscopy results for the untreated sample, the results for the silane-coated samples proved a successful coating. SEM images reveal the presence of a hybrid coating on the surface, while FTIR analysis showed the presence of groups attributed to both organic and

inorganic components of the hybrid coating. The coating served as a good barrier (crack-free and adherent) between electrolyte and SS surface and no cytotoxic effect was observed on L929 cells (mouse fibroblasts). Trimethylsilane coating was deposited using both radio-frequency glow discharge (RFGD) and direct current (DC) thus combining the advantages of stability of RFGD with the ability of DC to form metal-adherent coatings. This coating had improved corrosion resistance and could be a promising way to block the release of ions into the bloodstream [47]. One might also consider using composite silanes for improving SS corrosion resistance. Carbon nanotubes applied on silanized SS surface had a better corrosion resistance compared to silane alone [58].

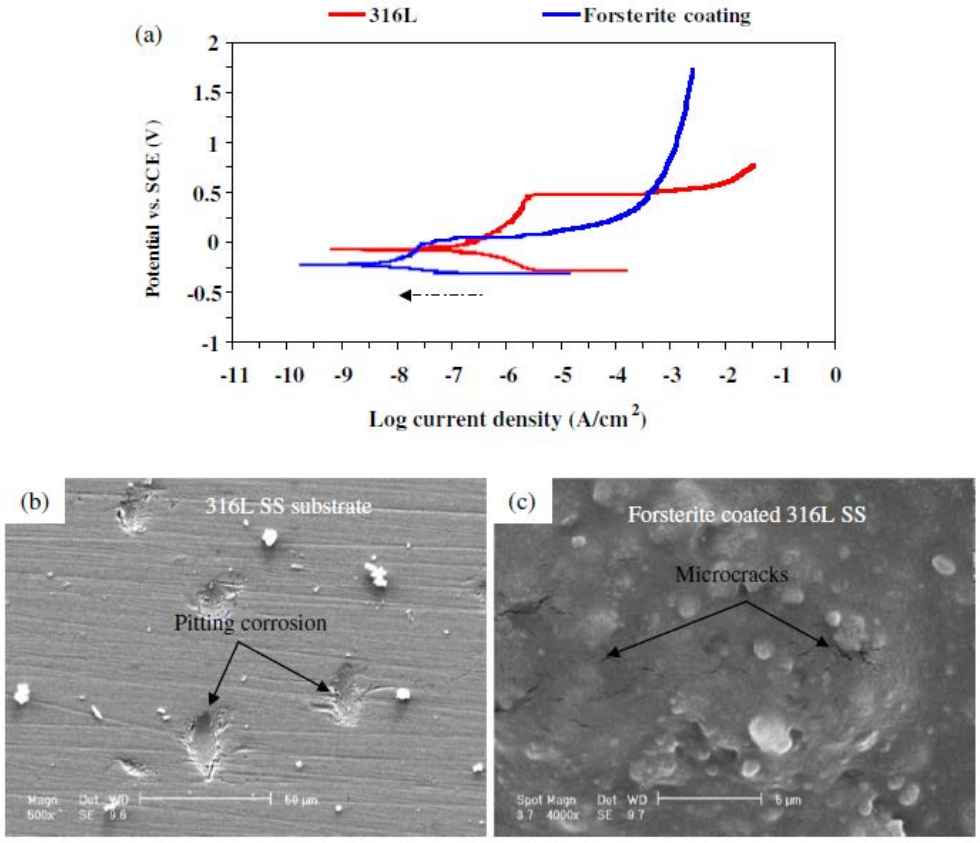

Fig. 1. Corrosion resistance of uncoated 316L SS and forsterite coated 316L SS samples in simulated body fluid: (a) the results of potentiodynamic polarization test; corrosion morphology of (b) uncoated 316L SS (obvious dip pits implying localized severe corrosion) and (c) forsterite coated 316L SS samples (few microcracks implying a milder and uniform corrosion attack). Reprinted from [55] with permission from Elsevier.

Apart from ceramic materials or SCAs, other materials (including composite ones) have been explored to improve corrosion resistance of SS. Composite coating consisting of a polymer (polyaniline) and graphene oxide [48] was electrodeposited on SS and showed enhanced corrosion resistance (higher corrosion inhibition efficiency and protection efficiency) compared to each of them alone.

Another way to increase the concentration of surface oxides consists in depositing metal oxides (other than SS). This is done, for example, by depositing tantalum oxide on SS implants with physical vapor deposition (PVD) to make it more resistant to corrosion [59]. Park *et al*. [49] coated titanium nitride on the metal surface deposited by plasma assisted chemical vapor deposition, and this resulted in improved mechanical properties, such as surface hardness, and

corrosion resistance, and was shown to be cytocompatible according to standard cytotoxicity test (ISO10993-5) and namely an elution test was used [49]. Titanium ethylene glycol-coated nanoparticles were plasma sprayed on SS. The functionalized surface showed improved properties, such as increased hydrophilicity and corrosion resistance [60]. Sirconium carbonitride magnetron sputtering of SS [45] improved its corrosion resistance and hemacompatibility. Electrochemical tests showed corrosion resistance of the formed nanocrystalline coating in phosphate buffered saline at body temperature.

Comparing different surface modification techniques for improving the corrosion resistance of SS (Table 2), most of the studies used potentiodynamic polarization (PDP) to measure the corrosion resistance of the resulting surfaces (Fig. 1a), while a few used more elaborate techniques (electrochemical impedance spectroscopy or EIS). PDP is shown as a current density (in logarithmic scale) dependent on the applied potential measured in the corroding media [61]. Lower current densities in comparison to the untreated samples are usually the evidence of the improved corrosion resistance of the modified surface as shown by [55, 57]. PDP is a good technique to study the effect of an inorganic layer or organic compound on corrosion behavior of the surface (relative susceptibility to localized corrosion) [47]. However, it gives a snapshot of corrosion behavior rather than an average value, and only a single scan can be made due to the destructive nature of the test [62]. EIS, on the other hand, is non-destructive and powerful technique used to test barrier property and corrosion resistance of the coatings on metals. It allows to test corrosion behavior over a longer period of time [47]. Combining the two techniques can provide a better understanding on the anti-corrosion properties of the modified surface. The medium used in these tests depend on the final application of the modified surface. Unmodified 316L SS has different corrosion resistance in various solutions: it is higher in NaCl than in SBF or Hank's solution [63]. Acid treatment offers an easy way of increasing corrosion resistance when other methods requiring special instruments are not available. Some of the chemical methods such as electropolishing and electrodeposition might be more advantageous compared to some physical methods, which require more sophisticated instrumentation as in plasma assisted chemical vapor deposition. Most of these studies used SS substrate but not real implants or stents (except [47]) thus needing an additional study to assess variables as deformation (e.g. expansion for stents) or shape itself (differential coating forming on different shapes). Only a limited number from the above studies tested *in vitro* cytotoxicity of the functionalized surfaces. Gopi *et al.* [53] incubated autoclaved surface modified samples (at 121°C) with human osteosarcoma cells but did not mention how this treatment could affect the cytotoxicity; also uncoated samples were not used or not mentioned; Hosseinalipour *et al.* [57] used mouse fibroblasts to analyze cytotoxicity of the modified surfaces but used coverslips covered with a sol-gel and an untreated cover slip as a control surface instead of real SS covered with hydrogel. None of these works tested the modified surface *in vivo* as a substrate implying the need for further studies.

2.3. Functional groups

There are a number of studies exploiting native oxide layers on the SS surface to introduce new functionalities such as biomolecules. However, coupling biomolecules on a metal stent is not straightforward due to the need of incorporating linker molecules [64]. Typically, the metal surface requires an ad-layer of functional groups, such as amines, carboxyls, or quinones [65]. In the case of SS, various functional groups have been incorporated either by silanization or coating with dopamine or via self-assembled monolayers (SAMs).

2.3.1. Dopamine

Dopamine was first identified from marine mussels as a molecule having both catechol and amine groups; these functional groups allow their adhesion to a wide range of materials. Dopamines were able to form a polydopamine film on a variety of surfaces: noble metals, metals with surface oxides (including stainless steel), oxides, semiconductors, etc. It was identified to contain two functional groups: catechol (3,4-dihydroxy-L-phenylalanine) and amine (lysine) groups which are important for adhesion to many types of materials [66]. Later use of dopamine includes its copolymerization with hexamethylendiamine to produce a surface with primary amines for linking molecules with carboxyl groups. An amine-rich surface could be produced by simply dipping the metal in the copolymer solution that was then used for successfully tethering heparin on the surface [67]. The effect of pH (pH 4.5 and 8.5; pH 8.5 being a typical marine environment pH) was investigated for immobilization of epidermal growth factor. Reaction at a higher pH resulted in a much thicker layer of dopamine on the surface because at higher pH dopamine is easily oxidized to form melanine-like aggregates (after multi-step reactions), which organize into tightly adherent structures on the surface. Moreover, this surface was rougher and had more amine groups available [68]. Polydopamine-coated SS was used to graft 2-hydroxyethylmethacrylate (HEMA) by irradiation with ^{60}Co-γ-rays. Grafting HEMA resulted in a smooth surface with increased hydrophilicity and corrosion resistance and lower platelet adhesion [69]. Overall, dopamine functionalization provides an easy way of functionalizing SS with amine/quinone groups for further attachment of molecules. However, only limited chemical linkers/molecules can be grafted on dopamine-modified surface since it produces amine/catechol groups only (Table 3).

Table 3

Benefits and limitations of the linkers used for surface modification of SS.

Linker	Advantages	Disadvantages
PEG	Hydrophilic; antifouling; non-toxic; non-immunogenic; homo- and bifunctional PEGs are available or can be synthesized	Non-reactive with SS; need additional groups to make reactive with SS
SCA	SS-reactive; many functional groups available; can generate surface containing more than one SCA type; bifunctional SCAs are available	Additional techniques for an efficient silanization; different SCAs might have different surface reactivity
SAMs	Ease of modification; range of terminal groups	Less stable; additional techniques for an efficient attachment (electrodeposition, glow plasma discharge)
Dopamine	Easily used in surface modification	Limited functional groups (amine/catechol only)

2.3.2. Silane-based agents

Silane-coupling agents (SCAs) are silicon-based materials which have a general formula of $R'(CH_2)_nSi(OR)_3$, where R' being an organofunctional group and R is a hydrolysable alkoxy group [70]. Their alkoxy groups are converted to silanol groups (SiOH) when silanes are mixed with water/ethanol solution. They form hydrogen bonds with surface hydroxide groups on metals, while the excess of silanol groups form siloxane network (Si-O-Si). This network is chemically stable and shows resistance to corrosion [58].

Silanization can result in a surface with a greater variety of functional groups depending on the SCA used. Different functional groups resulting from SCA coupling and SCA types used are listed in Fig. 2. While some surfaces can be made non-reactive for antifouling surfaces

(mPEG-IPTS [13, 71] and hexamethyldisilane [36]), others can be used to functionalize surface with various groups: amine, carboxyl, epoxide and thiol. These surfaces can be further tethered with different moieties for various purposes: grafting PEGMA (for reducing protein adsorption) [72], ionic liquids (for antibacterial properties) [73], or antibodies (to capture cells) [16]; various polymers (for reducing protein adsorption) [74] and N-halamine (for antibacterial properties) have also been used [75]. The type of SCA is also an important factor to consider because of their different hydrolysis rate. For example, methoxy groups were found to have a higher hydrolysis rate compared to ethoxy groups (Fig. S1) and can react directly with a metal during an incomplete hydrolysis [76]. SCA usually binds with the substrate with their methoxy or ethoxy groups while the nature of functional groups can be different.

Various strategies were employed to make SS more suitable for silanization by increasing the density of surface oxides. Ni-free SS samples oxidized in air at 527 °C [77] and 316L SS heated at 500 °C [78] were shown to be successfully silanized with (3-aminopropyl)triethoxysilane (APTES). In another study, SS was deposited with an aluminum oxide layer before silanization with APTES and grafting with 2-methacryloyloxyethyl phosphorylcholine [79] or tantalum oxide for collagen immobilization [40]. Both aluminum and tantalum oxide layers increased the surface hydroxides and aided further silanization of SS. Some SCAs were bound on electrochemically passivated SS [74, 76, 80]. It was shown that Fe oxides were better attached fixed to APTES than Cr oxides [77].

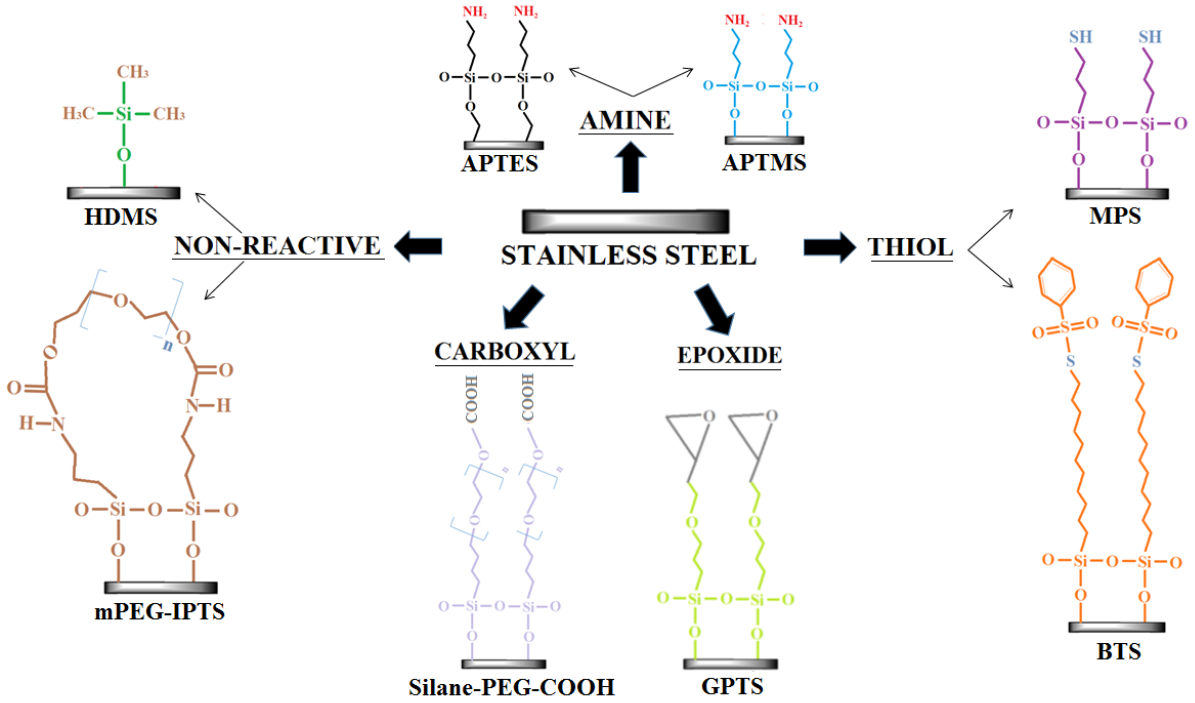

Fig. 2. Silanization of SS using different SCAs to generate various functional groups. APTES – (3-aminopropyl)triethoxysilane [77]; APTMS – (3-aminopropyl) trimethoxysilane [76, 81]; BTS – S-(11-trichlorosilylundecanyl)-benzene-thiosulfonate [16]; GPTS – glycidoxypropyltrimethoxysilane [74, 75]; HDMS – hexadimethylsilane [36]; mPEG-IPTS (methyl-polyethylene glycol - 3 - isocyanato-propyltriethoxysilane [13, 71]; MPS – (3-mercapto-propyl)trimethoxysilane [72, 73, 81]; Silane-PEG-COOH [80].

It is possible to render SS with more than one functional group via SCA. Co-adsorption of APTMS (3-aminopropyl)trimethoxysilane) (used for passivation) and (3-mercaptopropyl) trimethoxysilane (MPS) (for thiol groups) was studied by Vuori *et al.* [81] after hydroxylation by

electrochemical treatment. MPS was dispersed in APTMS and, interestingly, with increasing concentrations of MPS, its uptake on the surface did not increase linearly; this may be due to the differences in their hydrolysis and condensation rates. Therefore, it might be easier to use SCA with the same or similar end groups.

In some studies, molecules were silanized before being grafted on the surface. Silanized methoxy-PEG (MW 2000) was synthesized using IPTS (3-isocyanatopropyltriethoxysilane) and grafted on the acid treated surface [13]. The modified surface showed decreased fibrinogen adsorption, reduced platelet activation and adhesion, improved adherence and proliferation of HUVECs. A simplified version of this method involves using bifunctional PEG with silane and other functional groups instead of silanizing molecules, as demonstrated by Hynninen *et al.* [80].

2.3.3. Other linkers

Poly(ethylene glycol) (PEG) is one of the most commonly used molecule in surface modification of SS for promoting antifouling properties (reduction of protein adsorption) and coupling other molecules. Most important characteristics of PEG include its hydrophilicity, non-toxicity, non-immunogenicity and flexibility [61]. Due to the relatively low concentration of surface oxides on SS, it is challenging to couple PEG molecules directly on the SS surface [82] and often SS has been silanized first before grafting PEG [72], [74]. In other cases, PEG was directly synthesized to have a silane group, as discussed above. Amine groups were introduced on SS by physical adsorption of polyethylene imine (PEI) and then bifunctional PEG (aldehyde group and methoxy-group) was grafted on this surface. To maximize the surface coverage, the reaction was performed at the lower critical solution temperature of PEG. This surface was resistant to adsorption of a model protein (β-lactoglobulin), but was not repellant to bacteria (*Pseudomonas sp.* and *Listeria monocytogenes*) indicating inhibition of protein adsorption was not the only prerequisite for inhibiting bacterial attachment [82]. However, this study has some limitations: only one type of protein (β-lactoglobulin) was studied while other proteins present in media or secreted by bacteria were not studied; these proteins could possibly bind to PEG and promote bacterial attachment and/or cause instability of PEI-PEG interaction. However, PEG is well-known anti-fouling molecule and most probably make the surface repellent to protein attachment. Although successful grafting of PEG on PEI surface has been demonstrated by X-ray photoelectron spectroscopy, a method commonly used for surface chemical composition analysis, it may be possible that the treatment before bacterial adhesion tests led to leaching of PEG from the surface.

Surface modification with SAMs has the benefits as ease of modification and a range of terminal functional groups. SAMs made of aliphatic acids can serve as linkers to attach molecules of interest. Phosphonic acids (dodecylphosphonic and phosphoundecanoic acid) were attached on electropolished and mechanically polished SS and methyl- and carboxyl-terminated SAMs were formed as a result. Since long-term stability of monolayers in physiological conditions is often required, stability tests were performed. It was shown that carboxyl groups were more stable on a surface polished by both methods. Interestingly, desorption of SAMs from both surfaces was similar [83]. To functionalize the SS surface with two drugs (perphenazine and ibuprofen) two SAMs (SAM of 16-mercaptohexadecanoic acid and 11-mercapto-1-undecano) were prepared to bear carboxyl and hydroxyl functional groups. Lipase catalysis was utilized to attach drugs to SAMs and alleviate steric hindrance of an organic reaction on the surface. The catalytic activity of lipase was not affected by the SAMs, and its non-specific binding on the surface was not observed [84]. Nanofunctional alkanethiol SAMs with hydroxyl and carboxylic groups at the termini composed of 11-mercaptoundecanoic acid

and 11-mercapto-1-undecanol were prepared and characterized. Oxidative and physiological stability of alkylthiol SAM from 1-dodecanethiol as a model was studied [85]. They were oxidized in two weeks and after 3 weeks SAMs were desorbed from the surface. Overall, stability of alkylthiol SAMs is enough for their use in coronary stents.

2.4. Assisted deposition of molecules/ions

There are plenty of examples where the surface of SS was modified using methods such as electro and plasmadeposition that allow the surface modification with different molecules. These techniques make possible the functionalization of SS with chemical functionalities, otherwise, difficult or impossible to attach or to obtain more reproducible results.

Surface modification with SAMs can also be accomplished with the assisted technologies such as plasma or electrochemical methods. Six polymers were deposited on SS by glow discharge plasma polymerization to add functionalities to SS. This study is important as a platform for developing coronary stents with covalently coupled bioactive agents [36]. Alkanethiol SAMs with carboxyl and hydroxyl groups were prepared on SS surface using glow plasma discharge method [85]. Electrochemical methods were also applied for SAM formation on SS. One of the first examples of potential assisted SAM formation on SS was performed by Shustak *et al.* [86]. Here, SAMs were prepared from carboxylic acids (decanoic, myristic, palmitic, and stearic acid) after electrochemical activation of the surface in an organic aprotic solvent with low amounts of water. Compared with SAM formation under open-circuit potential, this method allows to better control the interface, lowers the kinetic barrier of the assembly process and shortens the time required for the formation of monolayers.

Electrodeposition of silanized molecules has also been attempted. Okner *et al.* [61] developed an one-step sol-gel electrodeposition method by using synthesized poly (ethyleneglycol)-di(N-triethoxysilylpropylcarbamate (PEG-diIPTS) as a sol-gel precursor. Application of moderate potential changes the pH on the electrode surface catalyzing the condensation of the hydrolyzed precursor. Thickness of the film depended on the time of exposure and potential of electrodeposition. They could obtain hydrophilic, mostly smooth and uniform surface. Carboxyl group bearing surface was developed by electrochemical deposition of mercaptoundecanoic acid by polarization of SS coupons as working electrodes; after activation with n-hydroxysuccinimide and mM1-ethyl-3-(3-dimethylaminopropyl] carbodiimide (EDC), fibronectin was covalently linked [87]. In this case, stable film was formed as shown by polarization modulation infrared reflection adsorption spectroscopy after 6 h of sonication (in 0.1 M NaOH, 0.16 M NaCl and in denaturing ethanol successively).

Several methods were used to assist the mild deposition of biomolecules, otherwise difficult to achieve. Plasma has used not only to increase the number of surface oxides but has also been used to render SS with new functional groups. Plasma polymerization can be used as a versatile method for modification of various surfaces with polymers providing a good surface coverage and functional groups. Amine-rich allylamine film was prepared via plasma polymerization for further covalent binding of heparin [88]. Vacuum thermal treatment of plasma polymerized stents improved cross-linking of the polymeric chains. A flexible film was formed after this treatment as seen by no crackling or peeling from the struts during balloon expansion (Fig. 3) as opposed to samples without heat treatment. Showing the effect of deformation on the coating is an important part of surface modification which is a step closer to implementing a technique in clinical practice. An example of rendering SS with carboxylic groups was done by plasma enhanced chemical vapor deposition (CVD) [89], while on other examples the metal was directly biofunctionalized with plasma deposition. The extracellular matrix protein tropoelastin was covalently linked on acetylene polymerized SS [90]. This protein

forms blood contacting surface of vessels and mediates growth of ECs and regulates infiltration of smooth muscle cells. Three conditions of plasma polymerization (the choice between acetylene surfaces created in the presence of nitrogen, argon or nitrogen/argon) were studied to find the optimal one for the retention of enzyme activity (model protein). Thus, the enzymatic activity of horseradish peroxide (HRP) on these surfaces was studied, and the treatment under nitrogen/argon atmosphere was found to have the greatest retention of HRP activity after 10 days and was chosen further. Covalent coupling of tropoelastin was proved by enzyme-linked immunosorbent assay (ELISA) using anti-elastin antibodies and anti-mouse antibodies conjugated with HRP before and after washing with sodium dodecyl sulfide (SDS), which removes physiosorbed protein leaving covalent monolayer intact. The protein formed a porous monolayer on the surface, as predicted by quartz crystal microbalance quantification comparing the masses of monolayer protein before and after SDS washing. However, no SEM or other characterization were used to show the porous monolayer. HUVECs were able to attach and proliferate on the tropoelastin-coated metal as compared to uncoated SS and plasma-coated SS. However, SDS is a denaturing agent and its effect on covalently bound tropoelastin is unknown; and bound anti-elastin antibodies proved by ELISA could only mean the retention of the binding site of the antibody to the protein; hence, the retention of tropoelastin function in this study was not fully investigated.

Fig. 4 summarizes different methods used to incorporate functional groups onto SS depending on the nature of the molecule to be attached to the surface. If the main purpose is to attach molecules with readily available carboxyl groups (e.g. heparin for blood compatibility or enzymes for reducing biofilm formation) one might consider coating SS with PEI or dopamine by physiosorption (simple methods; usually no specialized equipment required), or silanization with APTES/APTMS (more stable coatings, but requires specialized equipment for improved results), coating with allylamine (more stable coatings, but might require specialized equipment). Molecules having free hydroxyl groups can be attached to readily-made SAMs composed of aliphatic acids (but stability can be an issue) or can be silanized first (synthesis can be time-consuming, but reduces overall steps in functionalization) [61]. Ester groups, via trisuccinimidyl citrate [91], or carboxyl groups, can be added to SS to attach molecules with amine groups.

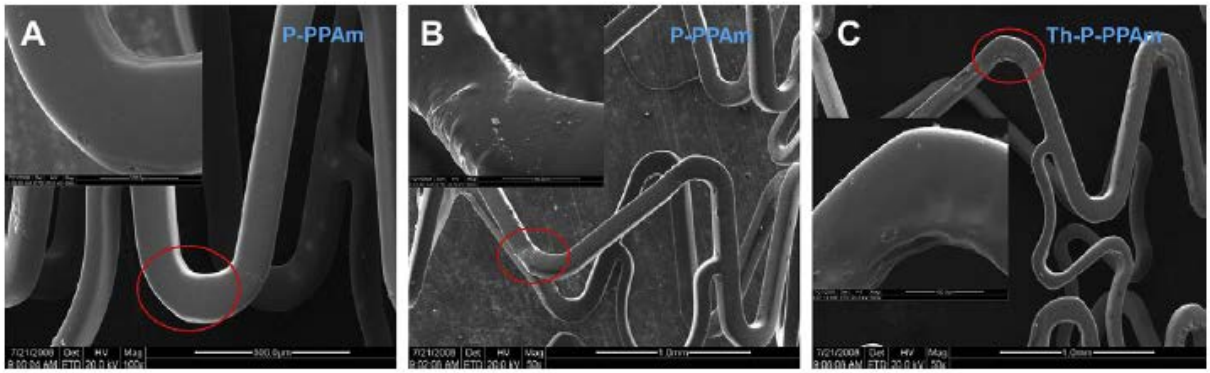

Fig. 3. SEM micrographs of 316L SS stents coated with the pulsed-plasma polymeric allylamine (P-PPAm). (A) pre-expansion image of stent with coating with no heat treatment: homogeneous, continuous and smooth coating was seen; (B) Stent with coating without heat treatment after dilation: P-PPAm coating could not resist destruction at the corner of the struts, and cracking or peeling occurred; (C) post-dilation images of stent with the coating with heat treatment. Reprinted from [88] with permission from Elsevier.

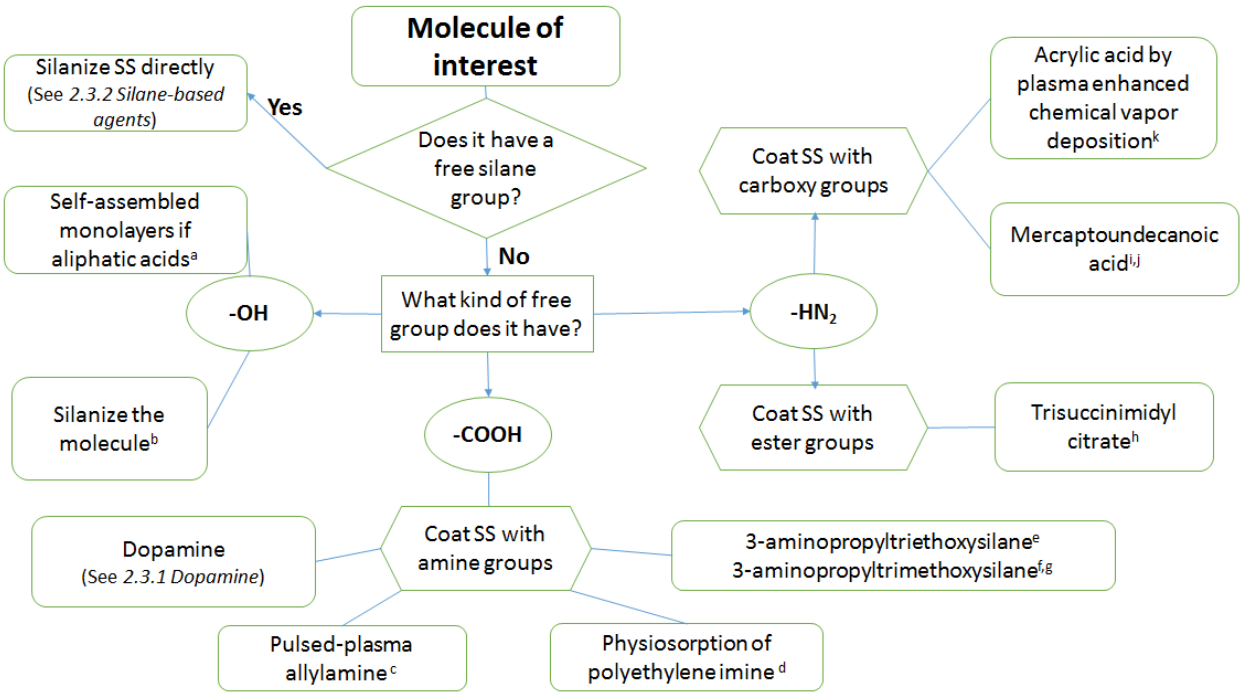

Fig. 4. A summary of different methods to functionalize SS surface depending on the molecule of interest. a) [83]; b) [61]; c) [88]; d) [92]; e) [77] f) [76]; g) [81]; h) [91]; i) [87]; j) [85]; k) [89].

3. Rational for the functionalization of SS

SS has been used in a variety of biomedical applications: dental prostheses, orthopedic fixation plates, vascular stents and guidewires. Biofunctionalization of SS for combating infection, improving osseointegration, increasing blood compatibility and for tackling restenosis problems, as well as for developing drug eluting stents will be discussed in following sections.

3.1. Prevention and management of infection of medical implants

Biofilm formation on the surface of implants remains a serious problem for metallic materials, and it can be reduced by two approaches: passive and active surface modification. The former involves changing the surface properties (especially hydrophobicity) with molecules that do not have antimicrobial properties *per se*, but affect bacterial adhesion, while the active modification entails the use of biological molecules with a specific interaction [93]. Antibiotics, peptides, enzymes, and ions with antibiotic activity have been used for this purpose, and these are summarized in Table 4.

SS bearing only SAMs (both of hydrophobic and hydrophilic nature) were not efficient in reducing the growth of *Staphylococcus aureus* compared to the antibiotic-linked surface [93]. The two antibiotics used, vancomycin and gentamicin, reduced the number of colonies on the SS specimens though with different modes of action: gentamicin was effective in the "early time points" (2 and 6 h) and vancomycin had the best effect in the long term (6 - 48 h). In another study [39], two antimicrobial peptides were grafted on chitosan-coated surface via dialdehyde, glutaraldehyde or terephtalaldehyde crosslinkers. SS prepared using this three-step protocol reduced adhesion of *L. ivanovii*. However, probably due to the "constrained mode" of immobilized peptides, the full antimicrobial effect of the surface could not be obtained.

SS has been coated with different ions given their antibacterial effect. Cu together with Ti were coated onto SS [44], Cu being an antibacterial agent. Ti was used to enhance the

adhesion of Cu onto SS surface (increasing bactericidal effect), increase the hardness and as a stabilizer to prevent intergranular corrosion of SS. Ionic liquids, organic salts made of discrete cations and anions, were also grafted on SS after silanization to render it with antibacterial properties. Compared with silanized surface, ionic liquid functionalized surface manifested decreased level of *Escherichia coli* adhesion [73]. Si implanted SS surface was found to inhibit bacterial adhesion while maintaining its biocompatibility, as shown by reduced adhesion of *S. epidermidis* and *S. aureus* and viability of human mesenchymal stem cells, respectively. Reduction of bacterial adhesion was attributed to increased surface nanoroughness and Si content [94]. While the previous study used ion sputtering to deposit Si ions, other methods used included coating with *in situ* prepared silver nanoparticles (hybrid organic-inorganic sol-gel material doped with silver) to combat infections while furthering the development of controlled release of antibiotic agents [95]. Magnetron sputtering was also used to generate TiN and TiN/Ag films on SS and TiN/Ag had a higher anti-listerial effect than TiN alone [96].

Another way of producing antibacterial SS is tethering the surface with enzymes. Lysozyme has the benefit of being a natural enzyme in the body (no leaching of toxic antibacterial agents to the host) and being more specific relative to antibiotics and quaternary ammonium compounds. It can hydrolyze bacterial and fungal cell walls. Lysozyme functionalized on chitosan (having antibacterial properties itself) surface showed to be stable and exhibited antibacterial properties against *S. aureus* (Fig. 5). Immersion in PBS for 10 days showed high stability of the grafted layer [14]. Using PEG moieties to attach lysozyme, as shown by Yuan *et al.* [98], led to both antifouling and antibacterial surface. Some studies used two types of enzymes, trypsin, and lysozyme, immobilized on amine groups of PEI. Enzymes immobilized in this way retained their enzymatic activity and featured enhanced activity, when increasing the distance away from the surface oxide layer; the surface has a potential to reduce biofilm formation [92].

Methods for obtaining improved antibacterial properties used different surface modification approaches, mostly including plasma-based methods (ion implantation and magnetron sputtering of antibacterial ion species) and grafting of antibacterial compounds (enzymes and peptides). Although requiring specialized equipment, the first set of methods has less steps in fabrication compared to often multiple steps used in the latter case. *In vitro* adhesion tests of the modified surfaces were also performed differently in the above studies. Often the modified and unmodified samples (differing in surface area in the studies) were placed in Petri dish and certain amount of bacteria (which was different in both amount (growth phases) and type of species used) was dropped for incubation (time was different). Attached bacteria were then recovered after washing/sonication/centrifugation (various conditions as well) and further grown and counted. Employing standardized tests would allow for a direct comparison between the studies. A more advanced approach was used by Zhao *et al.* [97], when they used a flow chamber designed to study bacterial attachment in the dynamic setting. Using systems which mimic the real clinical environment of the modified materials is important in the fast clinical translation of the research.

Table 4

Active surface modification of SS to prevent infection.

Antibacterial agent (type of molecule)	Proposed mechanism of action	Method of functionalization	Bacteria	Results obtained	Reference
Magainin I and nisin (peptides)	Permeabilization of cell membranes	Grafting on aminated surface (physiosorbed chitosan)	*Listeria ivanovii*	Reduced bacteria adhesion	[39]
Vancomycin, gentamicin (glycopeptide, aminoglycoside antibiotics)	Inhibition of cell wall synthesis and protein synthesis	Immobilized on SAMs	*Staphylococcus aureus*	Reduced bacterial adhesion and biofilm formation (long- and short-term activity, respectively)	[93]
Trypsin, lysozyme (enzymes)	Cleaving peptide bonds in proteins; hydrolysis of peptidoglycan respectively	Grafting	*Micrococcus lysodeikticus*	Reduction of bacterial adhesion and bactericidal activity	[92]
Ionic liquid (organic salts)	Probably disruption of the cytoplasmic membrane	Grafting	*Escherichia coli*	Bactericidal activity	[73]
Cu, Ti ions (ions)	Disruption of the cell wall and cell membrane	Physical vapor deposition	*Escherichia coli*	Antibacterial properties; lower toxicity and higher cytocompatibility; improved corrosion resistance; increased adhesion to SS surfaces and increased hardness of Ti-Cu coatings	[44]

Antibacterial agent (type of molecule)	**Proposed mechanism of action**	**Method of functionalization**	**Bacteria**	**Results obtained**	**Reference**
Si ions (ions) N^+, O^+ and SiF_3 (ions)	Probably change of the surface properties to make it less attractive for bacterial adhesion	Ion implantation	*Staphylococcus epidermidis, Staphylococcus aureus* (Si, N^+, O^+ and SiF_3) *Pseudomonas aeruginosa* (only N^+, O^+ and SiF_3)	Reduction of bacterial adhesion	[94, 97]
Silver nanoparticles/silica nanoparticles coated with silver (inorganic materials)	Cell lysis or inhibition of cell transduction	Sol-gel technique	*Escherichia coli*	Inhibition of DNA replication; respiratory enzymes; increasing cytoplasmic permeability	[95]

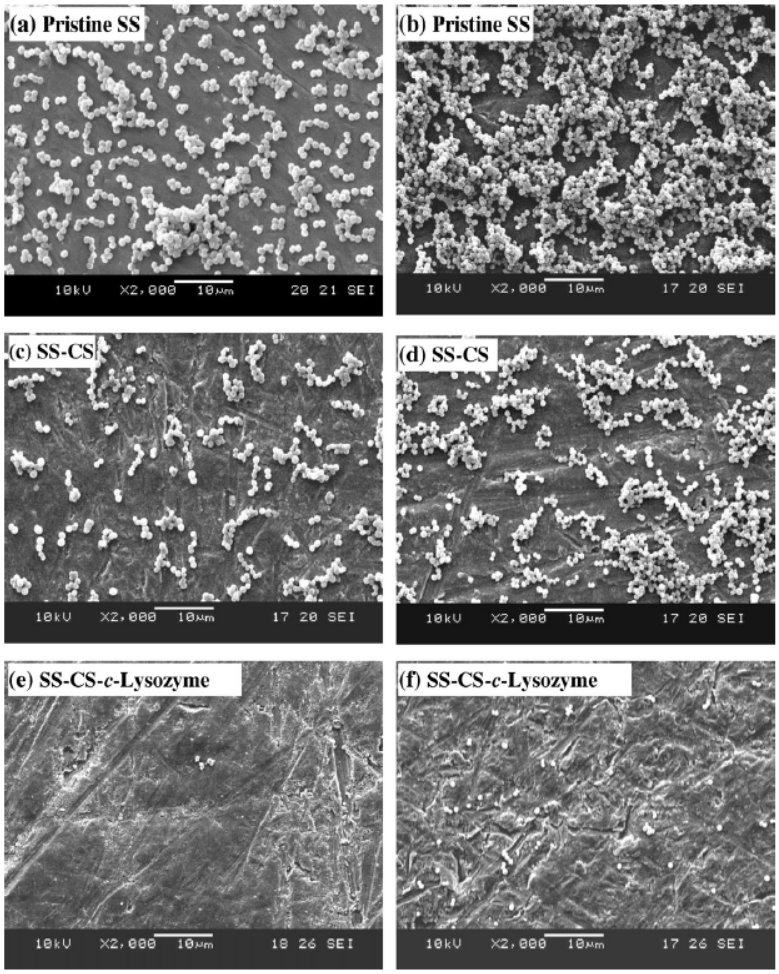

Fig. 5. Representative SEM images of the (a, b) pristine SS, (c, d) SS–CS (chitosan), and (e, f) SS–CS-c-Lysozyme surfaces after immersion in a suspension of *S. aureus* (10^7 cells/ml) for 4 and 24 h, respectively. Abbreviations: CS – chitosan, c - 1,1-carbonyldiimidazole used for activation of reactive functional groups of CS for covalent binding of lysozyme. Reprinted from [14] with permission from Elsevier.

3.2. Improving osseointegration of prosthetics

Surface properties of biomaterials (surface energy, hydrophilicity, chemical reactivity) and intrinsic properties of proteins guide their adsorption through non-covalent interactions (repulsion, hydrophobic effects). Protein adsorption, in turn, affects the behavior of osteogenic cells [1]. In orthopedics, poor wear and corrosion resistance of SS relative to Ti and CoCrMo alloys and release of Ni limits its use as temporary implants or cemented implants [99]. Various approaches for enhancing osseointegration of SS have been investigated, including ceramic coatings and modification of surface topography.

Coating metallic implants with hydroxyapatite is known to improve their resistance to corrosion and ability to interact with biological surroundings (especially bone). Hydroxyapatite has been used alone, and in different forms, and in combination with other moieties to modify SS. Hydroxyapatite nanoparticles incorporated with Zn were coated with chitosan and then spin-coated on SS. Incorporation of Zn was associated with the increased antimicrobial activity of the surface. A bone-like apatite layer was formed when the modified surface was immersed

in simulated body fluid solution [100]. A bilayer consisting of polypyrrole (as a protective and adherent interface) with salicylate (nonsteroidal anti-inflammatory drug) and strontium hydroxyapatite (to render porosity) was electrodeposited on SS, resulting in improved corrosion resistance and enhanced similarity to bone tissue, both chemically and biologically [52]. Further *in vitro* studies are needed to prove its biological similarity to bone tissue. Although the chemical composition of the film was confirmed by FTIR and surface morphology studied by SEM, no studies showing the ability of the porous structures to facilitate bone ingrowth directly into the implant were performed and bioactivity was predicted but not shown. SS coated with Mg and Sr substituted porous hydroxyapatite/poly(3,4-ethylenedioxythiophene) was prepared and showed improved corrosion resistance, adhesion, and growth of human osteosarcoma cells [53].

Apart from hydroxyapatite, other strategies are being investigated to improve osseointegration of SS biomaterials. A fragment of fibronectin, which is used for binding cells, was passively adsorbed on SS screws for improved osseointegration, as studied in rats. This surface could promote attachment and osteogenic differentiation of human mesenchymal stem cells *in vitro* or *in vivo*. Bone screw fixation and bone-implant ingrowth was improved as compared to uncoated samples [101]. However, passive adsorption of fibronectin may be a less stable and reproducible method for improved osseointegration. A recent study by Omar *et al.* [11] reported the functionalization of SS with 45S5 Bioglass® and the same bioglass with strontium as a partial substitute to calcium on hybrid organic–inorganic coating (consisting of silicon alkoxide, silicon alkyl alkoxide and colloidal silica). The obtained hybrid organic–inorganic coating could form a protective layer against media attack and the release of corrosion products. A uniform and crack-free SS coating of nanoparticulate diopside ($MgCaSi_2O_6$) had an apatite forming capability when placed in SBF [102]. Diopside was chosen because it degrades and is substituted by new bone with a good speed and forms a homogenous bonding with the bone. Transmission electron microscopy (TEM) of the synthesized diopside particles as seen in Fig.6 showed their relatively polygonal shape (average size 70 nm). The small size of the particles allows for a good apatite formation by providing a high level of interfaces for the nucleation reaction (Fig. 6). *In vitro* biomineralization studies of the modified samples in SBF reveals a formation of a uniform plates forming because of the growing apatite precipitation when soaking time is increased (from 3 days to 7).

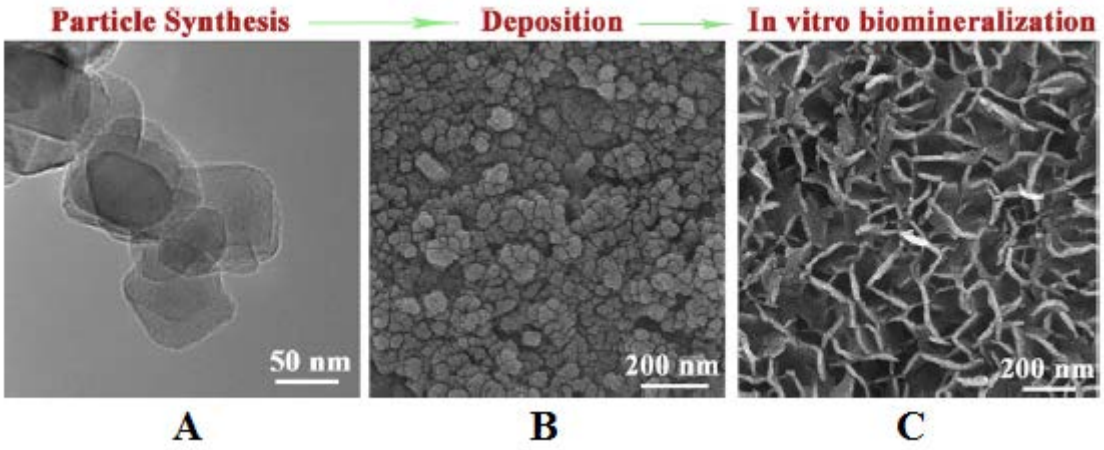

Fig. 6. (A) TEM micrograph of the powder sample of nanodiopside calcined at 700 °C for 2 h; (B) SEM micrographs of the coatings deposited on SS; and (C) SEM micrographs of the samples after soaking in the SBF for 3 days. Reprinted from [102] with permission from Elsevier.

3.3. Improving blood compatibility

Blood compatibility of blood-contacting devices is of utmost importance for their successful implantation [103]. In an earlier study, multiple layers of polyethylene imine and heparin (an anticoagulation molecule with an anti-inflammatory effect [1]) were deposited on the SS surface by dip coating from a polyelectrolyte solution. This electrostatic assembly has an advantage of being simple in performance and applicable to differently shaped stents [104]. This layer-by-layer assembled film has the drawback of being a time-consuming method and not suitable for long-term applications where stability is important. SS modified with heparin on a copolymerized coating of dopamine and hexamethylendiamine was found to be stable against hydrolysis and swelling, as proved by dynamic dissolution assay [67]. The surface had improved cytocompatibility and reduced inflammatory response. A different biomimetic approach was used to functionalize SS with heparin immobilized on SS co-polymerized with catechol-polyethylenimine. This surface was able to reduce the number of platelets attached and their activation [65]. Compared to others reports, a paper published by Yang *et al.* [88] provides a more comprehensive study of the modified surface, as it includes several *in vitro* hemocompatibility assays, EC viability test and *in vivo* analysis (canine iliac arteries). Heparin coated cardiovascular stent developed in this work led to improved hemocompatibility due to long-term anticoagulation effect, ability to promote EC adhesion and proliferation. Heparin hydrogels were used to coat SS to make the surface more blood compatible. This hydrogel was composed of chemically synthesized dopamine/tyramine linked to 4-arm poly(propylene glycol)-co-poly(ethylene glycol), which was immobilized on the metal surface by an enzymatic reaction of HRP. The modified surface was shown to reduce fibrinogen binding as seen in Fig. 7 [105].

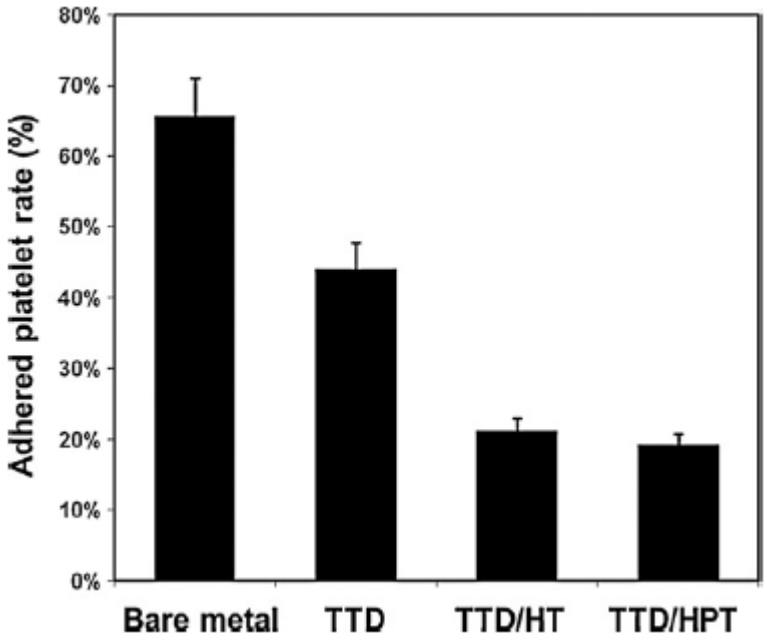

Fig. 7. *In vitro* platelet adhesion rate on the hydrogel-coated SS surfaces and bare SS surface (n = 4, mean ± S.D.). Abbreviations: TTD – Tetronictyramine/dopamine; HT- heparin–tyramine; HPT - heparin–PEG–tyramine. Reprinted from [105] with permission from Elsevier.

Together with more conventional methods, alternative ways of improving blood compatibility of SS are being studied. This include dip coating with hesperidin, one of the components in traditional Chinese medicine and also found in citruses [106], attaching alginic

acid after silanization [78] and immobilizing hyaluronan via combination of plasma reactor and electrospray [107].

Heparin is the most widely used molecule in modification of SS for blood compatibility. Several strategies have been employed to improve blood compatibility of SS implants with the most common being heparin immobilization. Activated partial thromboplastin time (APTT) assay was often used in the above studies as an indicator of the activation of the intrinsic blood coagulation system by heparin on the surface. However, incubation time of the samples with the platelet poor plasma (blood plasma with very low number of platelets), the following detection methods were different and could not be reliably compared to each other. Surface chemical structure and composition of the modified surface for improved blood compatibility was tested with different methods. It is important to study the stability of blood-contacting surface for both safety and practical reasons. Thus, obtaining covalent heparin bound surface seems superior to those obtained by physiosoption. Stable heparin film (or other film providing hemocompatibility) can guarantee the long-term blood compatible surface for a safe *in vivo* application [88]. Stability of the coatings were studied by incubating the modified samples in buffer/water in static [104] or dynamic conditions [67, 88] and observed microscopically while other studies investigated for chemical composition by X-ray photoelectron spectroscopy.

3.4. Tackling restenosis

Endovascular stents, designed to enlarge vessels and prevent stenosis, can also cause blood clotting and thrombosis. Furthermore, ECs can grow on its surface and form an encapsulation leading to stent restenosis [108]. One of the methods to tackle restenosis is to capture endothelial progenitor cells (EPCs) and reduce inflammation [109]. These stents usually favor the growth of ECs and disfavor platelet adhesion. SS stents coated with vascular endothelial-cadherin (CD144) and CD34 antibodies were compared. Increased capture of EPCs on vascular endothelial-cadherin coated stents was observed than on CD34 coated ones. The overall effect of this stent was its ability to more effectively reduce endothelization and neointima formation [110]. Larsen *et al.* [111] tested the ability of Genous^{TM} bioengineered R^{TM} (commercially available stent covered with antiCD34 antibodies) to accelerate re-endothelialization. The stent could capture cells as demonstrated by qPCR of endothelial markers and decrease of thrombogenicitiy both *in vivo* and *in vitro*. This study showed capture of EPCs in human circulation for the first time [111]. However, despite promising results of the first stent aiming to capture EPCs, antiCD34 antibodies were found to be non-specific and were also binding to smooth muscle progenitor cells resulting in neointimal proliferation. To alleviate non-specificity of antibodies against EPCs, aptamers were immobilized on SS stent [112]. This stent was more specific and could capture EPCs showing an improved viability and spreading on the modified surface as opposed to untreated samples (Fig. 8). Although this is a relatively fast two-step method of functionalization (first being plasma polymerization of allylamine), aptamers are electrostatically adsorbed and not covalently attached on the surface making the surface potentially susceptible to leaching of the ligand. Amine groups on the plasma-modified surface could be derivatized with succinic anhydride followed by activation with diisopropyl carbodiimide [113] or directly activated by *p*-phenylenediisothiocyanate [114] to covalently bind aminated aptamers for a better stability (covalent bond is formed). After activation with EDC, phosphorylated aptamers can be attached on the aminated surface [115]. Different linkers (such as PEG) could also be used to improve the attachment of the aptamer and hence a better binding to target cells [116].

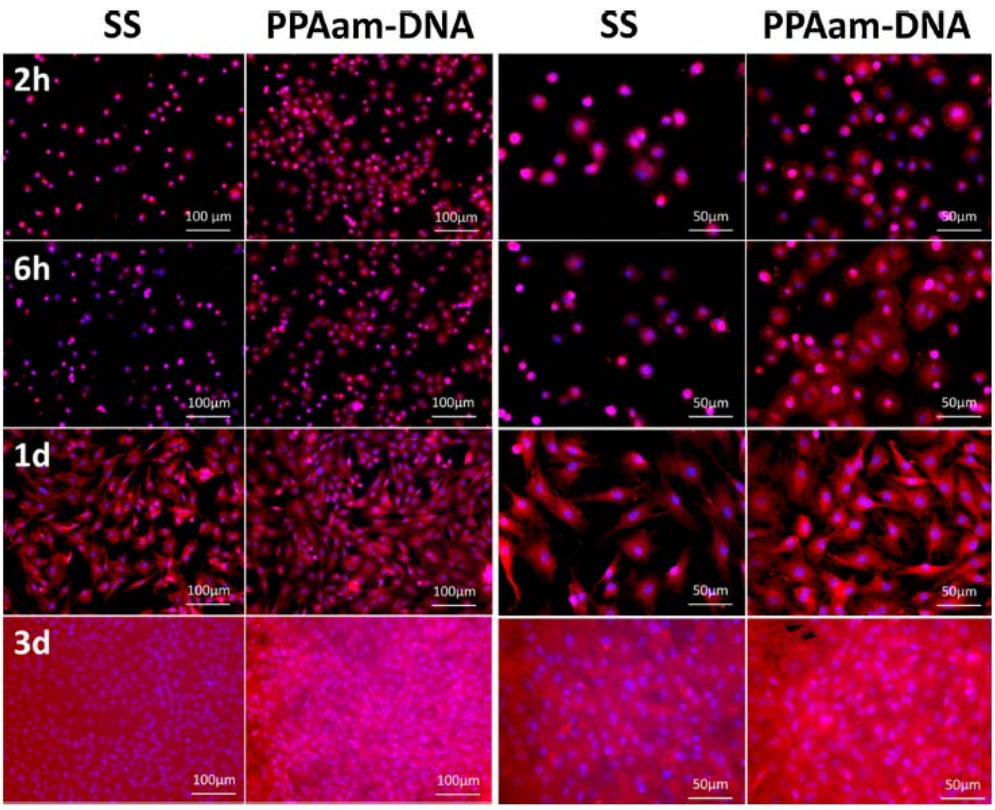

Fig. 8. DAPI (blue) and rhodamine123 (purple) staining of captured EPCs on 316L SS and SS modified with plasma polymerized allylamine (PPAm)-EPC-capturing aptamers after 2 h, 6 h, 1 d and 3 d culture under non-static condition. A much larger spreading area of EPCs on the aptamer modified surface as opposed to bare PPAm surface was seen, implying that aptamer provided a good environment for the cells to grow. No significant difference was seen after 3 days of culturing due to the high proliferation rate of the cells. Reprinted from [112] with permission from Elsevier.

A SS stent was functionalized with two types of self-assembled peptide nanofibers to promote adhesion and growth of vascular ECs. One of the peptide was used due to its ability to trigger selectively the binding and spreading of ECs compared to that of muscle cells and platelets. The second peptide served as a backbone platform that mimics the native ECM to sustain cell matrix interactions at the molecular level. Both of the peptides were linked with dopamine to attach on the SS thus forming a self-assembled layer [117]. A novel strategy of using peptides was developed considering the competition of ECs with other cells in EPC-capturing stents. Wei *et al.* [118] engineered a surface, which combines Arg-Glu-Asp-Val peptide (REDV) with phosphorylcholine because ECs bind specifically with the first and exhibit non-specific resistance to the latter [118]. Enhanced capture of target cells versus non-target was shown to be enhanced by the synergic action of the two components of the coating. Furthermore, competitive binding of target cells rather than their amount plays a crucial role in establishing pure confluent layers of cells on stents.

One of the most successful solutions to prevent restenosis has been drug-eluting stents (DESs), and a number of strategies has been employed to functionalize stents made of SS used as DESs. After sputtering gold on the metal surface, it was immersed in dimercaptosuccinic acid, a linking agent bearing thiol (to bind to the gold surface) and carboxylic groups (to bind to chondroitin 6-sulfate (ChS) after activation with a cross-linking agent), and then alternate multiple layers of ChS and heparin were formed. Release of sirolimus (drug inhibiting proliferation of smooth muscle cells) loaded on this functionalized stent could be controlled by

the number of layers on the surface, as shown by the decreased release rate and increased release time from the thick layer. The modified surface was also able to inhibit smooth muscle cell proliferation [119]. While this method is attractive due to its hemocompatibility and for a potential delivery of drugs with different release times, preparing multiple layers of ChS and heparin can be time-consuming. To control the release of sirolimus from the stent in an easier way, SS was first silanized and then covered with multiple layers of magnetic nanoparticles and gelatin. By switching the magnetic field on or off, it was possible to release and retain the drug from the surface [120]. A DES functionalized with nanoliposome loaded with heparin as a model drug, was developed by Kastellorizios *et al.* [89]. By altering the physiochemical properties of the liposome, such as lipid composition, mean vesicle size and heparin loading, it was possible to control the release of heparin. More importantly, modified surface showed good biocompatibility, as demonstrated by increased blood coagulation time. However, no cytotoxicity or *in vivo* studies were performed for this DES.

Together with Genous™ stent, a number of other stents functionalized for re-endothelialization and drug-elution are available in the market. Examples of these stents with paclitaxel, a chemotherapy drug, include Taxus® and JACTAX®. Their clinical performance and safety were compared in a 9-months study and were found to be comparable [121]. Recently, two DESs (Excel™ stent vs BuMA™ stent) based on biodegradable polymers with differing elution and adsorption kinetics were studied in a clinical trial. Despite the difference in sirolimus elution from these stents (180 versus 30 days), BuMa™ was not inferior in target lesion failure and exhibited decreased incidence of stent thrombosis as compared to Excel™ stent [122]. It should be noted that it is difficult to compare studies evaluating the release rate of drugs even from the same DES but performed in different conditions. In a recent study by Seidlitz *et al*. showed [123] that the type of different *in vitro* release setups (such as different sample volume, vessels, incubation conditions, media change, sampling time points) used strongly influenced the amount of released drug. This was also true in case of similar experimental setups and even in simple incubation (no flow system or automation) probably due to the difference in drug load between the initial samples and instability of the drug (sirolumus). When drug released from the studied settings was compared to those released *in vivo* (from published works), none of the settings showed a superiority as a predictor of *in vivo* release. *In vitro* release setups used in the above studies include immersing drug-loaded sample in 20 ml of PBS (temperature not mentioned) [120] and 5 ml PBS at 37°C [119] and measuring the absorbance of the released drug. In case of cell capturing stents, both static and non-static (shaking) conditions were used to study their cell capturing capabilities as exemplified by Qi *et al.* [112]. A more realistic approach would be using an *in vitro* flow system to better mimic the environment these stents are intended to be applied. Some of the studies used *in vivo* systems of the functionalized stents. Thus, Lee *et al.* [110] used rabbit's right and left iliac arteries to compare two stents covered with antibodies binding to two different surface markers. While Wei *et al.* [118] also used rabbits as animal models to show that competitive binding ability of ECs over SMCs (and not the number of ECs) is much more important criterion for developing an anti-restenosis stent.

4. Conclusion and Perspectives

SS remains an important biomaterial despite the emergence of new metal alloys. Surface modified SS, such as stents, are commercially available and used for anti-restenosis. Plenty of ongoing research aims to overcome inherent flaws of SS (as a foreign material) and meet its new application needs through surface modification techniques. Comparing these methods reported in the literature showed that surface treatment methods can result in

improved performance of SS with a simultaneous change of several surface characteristics important for biomedical applications. Recent trends in surface modification of SS include increasing examples of biology-inspired surfaces: whether if it is intended for bone tissue engineering [6] or improving of antibacterial properties [14]. Another emerging field is the development of hybrid coatings which offer advantages of both organic and inorganic materials on the surface. Modified types of well-known materials (such as Mg/Sr substituted hydroxyapatite [52, 53] or heterobifunctional PEG [80]) or new ways of introducing molecules on SS (such as combining advantages of RFGD and DC) are also being investigated. Different plasma methods are becoming more popular for both preparing the surface for further modification or introducing active compounds directly. It is often challenging to compare different studies aiming to obtain a modified surface for the same application because of the difference in methods they employ to characterize the surfaces. Development of standardized tests to evaluate the performance of modified SS is thus required to allow direct comparison among the various methods used to obtain modified surfaces.

Promising and emerging applications of SS could be in using it as a substrate in sorption of target analytes as a new substrate for a jacket-free stir bar [124] and diagnostic substrate in faradic impedimetric immunosensor [125] or as a guidewire to improve detection of rare cancer cells [126]. It is becoming clearer that SS of the future will most likely succeed when functionality is added without compromising its important bulk properties; for devices with a specific active compound (antibiotic, enzyme, or antibody), it is crucial to have both non-fouling properties to reduce non-specific binding together with retaining specific activity of the compound. In drug eluting stents, one of the future perspectives is developing stents with modulated release of drugs to reduce the side effects of high doses and to obtain a desired physiological effect.

Conflict of interest

The authors declare no conflict of interests.

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