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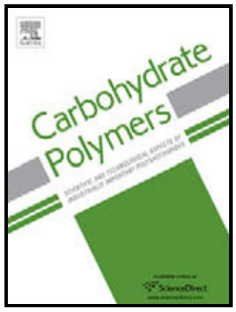

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PII: S0144-8617(20)31660-X

DOI: https://doi.org/10.1016/j.carbpol.2020.117487

Reference: CARP 117487

To appear in: *Carbohydrate Polymers*

Received Date: 15 April 2020

Revised Date: 1 November 2020

Accepted Date: 2 December 2020

Please cite this article as: Kumari S, Tiyyagura HR, Pottathara YB, Sadasivuni KK, Ponnamma D, Douglas TEL, Skirtach AG, Mohan MK, Surface functionalization of chitosan as a coating material for orthopaedic applications: A Comprehensive Review, *Carbohydrate Polymers* (2020), doi: https://doi.org/10.1016/j.carbpol.2020.117487

Surface functionalization of chitosan as a coating material for orthopaedic applications: A Comprehensive Review

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Highlights

- Recent progress related to the surface modification of biomedical implant material, i.e.
- CS-based coatings is reviewed.
- CS- based coatings result in better cell adhesion, spreading, proliferation and bioactivity
- etc.
- Improved mineralisation was achieved by incorporating inorganic particles (eg. CaP,
- HaP, BG) into the CS coatings.

Abstract

Metallic implants have dominated the biomedical implant industries for the past century, for load-bearing applications while the polymeric implants have shown great promise for tissue engineering applications. The surface properties of such implants are critical as the interaction of implant surfaces, and the body tissues may lead to unfavourable reactions. Desired implant

properties are biocompatibility, corrosion resistance, and antibacterial activity. A polymer coating is an efficient and economical way to produce such surfaces. A lot of research has been carried out on CS-modified metallic and polymer scaffolds in the last decade. Different methods such as electrophoretic deposition, sol-gel methods, dip coating and spin coating, electrospinning, *etc*. have been utilized to produce CS coatings. However, a systematic review of chitosan coatings on scaffolds focussing on widely employed techniques is lacking. This review surveys literature concerning the current status of orthopaedic applications of CS for the purpose of coatings. In this review, the various preparation methods of coating, and how the surface functionalities are helpful in determining the efficiency of coatings will be discussed. Effect of nanoparticle additions on the polymeric interfaces and in regulating the properties of surface coatings are also investigated in detail.

Abbreviations: γ-PGA; poly (γ-glutamic acid), n-Ag: Silver nanoparticle, n-Au: Gold nanoparticles, nBG: nano-Bioactive Glass, nHAp: nano-Hydroxyapatite, Ag^{+}: Silver ion, Ag: Silver, Alg: Alginate, Au: Gold, AA: Acetic Acid, AC-EPD: Alternative Current Electrophoretic Deposition, ALP: Alkaline Phosphatase activity, AZ91: Magnesium alloy, AZ91D: Magnesium alloy with small amount of iron, BDG: beta-1,3/1,6-D-glucan , BG: Bio Glass, Ca: Calcium, CaO_2: Calcium dioxide, $Ca_3(PO4)_2$: Calcium Phosphate, Cr: Chromium, C-CS: Carbooxymethyl Chitoan, C-HAp, Carbonated Hydroxyapatite coatings, C-MWCNT: Carboxy Multiwalled Carbon nanotubes, CA: Cellulose Acetate, Collagen; Coll, CNT: Carbon Nanotubes, CS: Chitosan, CVD: Chemical Vapor Deposition, DA; Degree of Acetylation, DC-EPD: Direct Current Electrophoretic Deposition, DI: Deionized Water, DLA: ethylene Dilinoleate, EPD: Electrophoretic Deposition, Gel: Gelatin, GO: Graphene Oxide, GS: Gentamicin Sulphate, HA: Hyaluronic acid, HAp: hydroxyapatite, HNT: Halloysite Nanotubes, ITO: Indium Tin Oxide, LBL: Layer-by-Layer, LP: Laponite, mAb;

Monoclonal Antibodies, M-HAp: Mineralized Hydroxyapatite, MAO: Microarc Oxidized, MW-CNT: Multi-walled Carbon Nanotubes, NiTi: Nickel-Titanium Shape Memory Alloy, P: Phosphorous, Pt: Platinum**,** PBS; Phosphate Buffer Solution, PC: Polycarbonate, PDA: poly Dopamine, PE: Polyethylene, PEI: poly(ethylene imine), PET: Poly(ethylene terephthalate), PVA: Poly vinyl alcohol, PVP: poly (vinyl pyrollidone), R_p: Polarization Resistance, Se: Selenium, Si: Silicon, Sr: Strontium, SrO: Strontium Oxide, SA: Sodium Alginate, SBF: Simulated Body Fluid, SCE: Saturated Calomel Electrode, SEM: Scanning Electron Microscope, SMA: Shape Memory Alloy, SS; Stainless Steel, Ti: cp-Titanium, Ti-6Al-7Nb: Titanium-6Aluminum-7Niobium, Ti-6Al-4V: Ti-6 Aluminum-4 Vanadium, Ti-24Nb-2Zr: Titanium-24Niobium-2Zirconium, TNT: Titanium Nanotubes, Zn: Zinc, VEGF: Vascular endothelial growth factor, ZnO: Zinc Oxide, W: Wollastonite.

1. Introduction

Metals were the primary choices for biomedical implants for almost a century now (Q. Chen & Thouas, 2015). For stable orthopaedic implants, metals were widely applied due to their long-term load-bearing capacities and mechanical properties *viz.*, high tensile and compressive strength, high yield and fatigue strength, appropriate ductility, hardness and high toughness (Saini, Singh, Arora, Arora, & Jain, 2015). However, these metal-based materials do not possess bio functionalities like bone conductivity and bioactivity. This necessitates the design of biocompatible and antibacterial implants, which is of great interest at present.

Biodegradable synthetic polymers have been used to overcome the shortcomings of metals especially in tissue engineering, gene therapy, regenerative therapy and controlled drug delivery applications requiring biodegradability(Rajan, Murugan, Ponnamma, Sadasivuni, & Munusamy, 2016; Sumathra, Sadasivuni, Kumar, & Rajan, 2018; H. Tian, Tang, Zhuang, Chen, & Jing, 2012). The use of synthetic polymers for biomedical applications is only a few

decades old. The physical characteristics of the polymeric materials can be altered based on clinical applications and can be easily manipulated. Furthermore, synthetic polymers are highly processable. However, their mechanical properties are unsuitable for load-bearing applications, and they lack the ability to bond with living tissues (Edgar et al., 2016).

Two disadvantages, common to both metallic and synthetic polymeric implant materials, are the absence of proper binding sites for cells and lack of antibacterial activity. Interaction between an implant material and the surrounding tissue is determined by the surface characteristics. Many novel and innovative strategies have been developed over the decades to modify implant surfaces. The surface modification improves the specific surface property required for different clinical applications while retaining the excellent bulk attributes. One current strategy is to alter the surface by coating the implants. This exploits the biological properties of the coatings while retaining the bulk properties (e.g. mechanical properties) of the substrate materials.

Numerous researches have come out on developing organic, inorganic, and hybrid coatings for orthopaedic applications. Many of these have been inspired by the inherent structure of bone to mimic the natural Extracellular Matrix (ECM) (S. Wu, Liu, Yeung, Liu, & Yang, 2014), whose major components include a mineral phase in the form of hydroxyapatite (HAp), collagen (Coll), non-collagenous proteins and proteoglycans (Reznikov, Shahar, & Weiner, 2014). Coatings based on HAp, collagen, beta-1,3/1,6-D-glucan (BDG) and other biopolymers (alginate, proteins, polynucleotides, etc.) have also been investigated (Ciobanu & Harja, 2019; Faria, Henriques, Souza, Silva, & Carvalho, 2020; Hameed et al., 2019; Jaafar, Hecker, Árki, & Joseph, 2020; Peltzer*, Delgado, & Wagner, 2018; J. Song, Winkeljann, & Lieleg, 2020; H. Tian et al., 2012)

Chitosan (CS) is a natural polysaccharide, used widely in biomedical implants, scaffolds, drug delivery systems, biosensors and other biomedical applications nowadays

(Archana, Dutta, & Dutta, 2013; Boccaccini, Keim, Ma, Li, & Zhitomirsky, 2010; D'Almeida et al., 2017; D & N, 2018; B. Li et al., 2019; J. Tian et al., 2016). Its immense significance in biomedical applications is because of the biocompatibility, acceptable hemocompatibility, antibacterial properties, positive host response and sufficient mechanical strength (F Pishbin, Simchi, Ryan, & Boccaccini, 2011; Puppi, Chiellini, Piras, & Chiellini, 2010; Sadasivuni et al., 2020; Sencadas et al., 2012; Sutha, Kavitha, Karunakaran, & Rajendran, 2013). CS is a natural cationic biopolymer derived from partial deacetylation of chitin. CS has received considerable interest for biological, industrial, textiles, food (Elsabee & Abdou, 2013), and pharmaceuticals applications due to its non-toxic nature and biodegradability (Sadasivuni et al., 2020; Singh et al., 2019).

CS is applied as a coating material to different types of alloys/scaffolds by following many methods such as Electrophoretic deposition (EPD) (Höhlinger, Heise, Wagener, Boccaccini, & Virtanen, 2017; Qi et al., 2018), Layer-by-Layer (LBL) deposition (R. Chen et al., 2017; R. Huang et al., 2015), dip coating methods (Reddy Tiyyagura et al., 2016), casting and spin coating methods. In addition, the surface of chitosan is functionalized to enhance the interaction towards the scaffolds. Various coating methods basically depends on the extent of the coating. The spin coating has great scope in biomedical applications, whereas electrospun fibre formation is mostly appropriate in tissue engineering. Though, solution casting is simple in approach has several disadvantages such as poor bonding strength between coat and substrate. Being a simple deposition process, dip coating is adopted for smaller substrates, whereas film growth can be controlled in the LBL method. Despite its versatility, the EPD coating technique is not expected to make headway due to its use of expensive and hazardous solvents. This review discusses the current advancements of CS coatings on scaffolds, i.e., metallic, polymeric etc., along with the methods. A critical comparison between the methods is done to investigate the influence of experimental conditions and mode of coatings on the

final biocompatibility. All methods are discussed on the basis of surface modifications and the final efficiency of CS coatings, for orthopaedic applications. It is believed that, this review will provide basic understanding of the surface functionalization/modification of CS coatings and the knowledge in depth about the correlation between surface characteristics and the implant efficiency.

2. Deposition of Chitosan on Scaffolds

CS, which is originally derived from chitin (figure 1), is cationic in aqueous solutions and has excellent film-forming properties. Many methods are adopted for deposition of various polymeric, ceramic, and composite coatings on scaffold substrates for biomedical applications. EPD, LBL, casting, dip, and spin coatings are the most widely utilized techniques. Depending on the surface coating method, the final mechanical properties of the coatings like the adhesive strength, thickness etc. vary, which influences the functionality of implants (Honarkar & Barikani, 2009). This section specifically addresses each coating method used for CS coatings on implants, with special emphasis to the surface modifications, and the influence of coating efficiency on targeted orthopaedic applications.

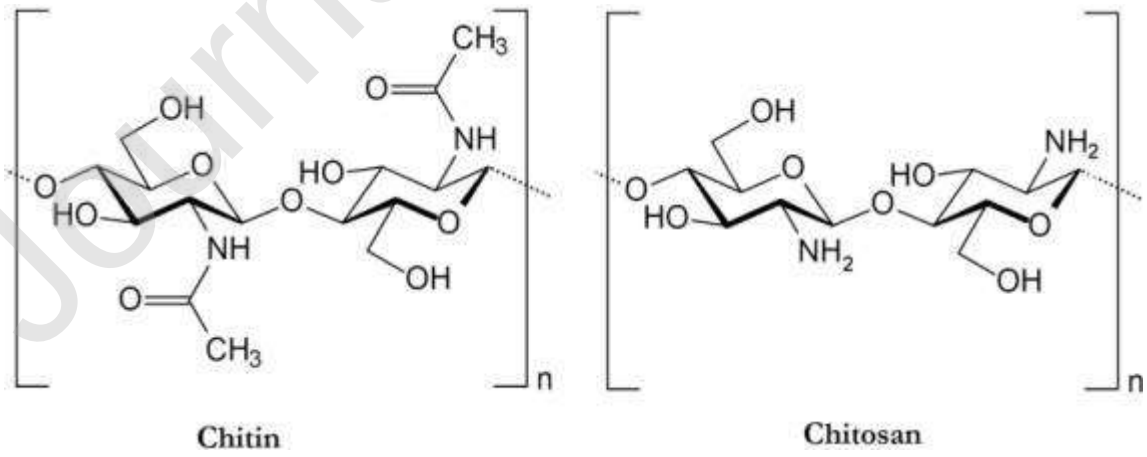

Figure 1. Structure of a) Chitin and b) chitosan (Younes & Rinaudo, 2015).

2.1. Electrophoretic Deposition (EPD)

EPD is a colloidal technique that uses the electrophoresis mechanism for the movement of charged particles suspended in solution under an electric field, and to deposit them in an ordered manner on a substrate to develop thick and/or thin films, coatings and free-standing bodies. The two-electrode set up for the EPD is schematically represented in Fig. 2 (Besra & Liu, 2007; Boccaccini et al., 2010). EPD is achieved via the motion of charged particles in a liquid dispersion towards the working electrode. The mechanism involves two steps. In the first step, an electric field is applied between the two electrodes and charged particles suspended in a suitable liquid move towards the oppositely charged electrodes (electrophoresis). During the second step, the particles accumulate at the deposition electrode and create a relatively compact and homogeneous deposition. After the deposition, a heat treatment step is normally needed to further densify the deposit and to eliminate the porosity (Corni, Ryan, & Boccaccini, 2008). Such heat treatment of polymers improves their mechanical properties and tribological properties (Aly, 2015).

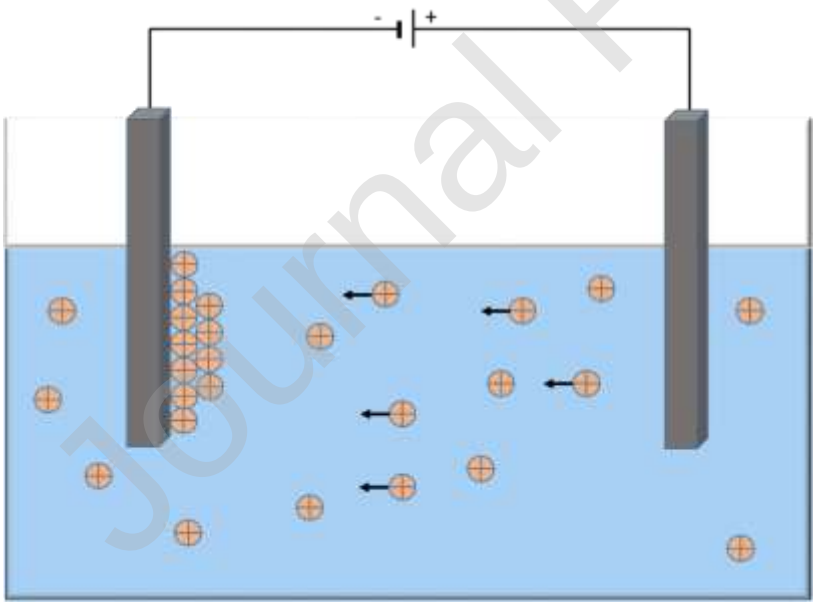

Figure 2. Two electrode cell setups for EPD

Highly versatile applications, simple apparatus, short processing time, facile modification, desirable dense packing of particles on the substrate, high quality and variety of microstructure produced (from micro to nanometer level), easy production of geometrical techniques, simple control of thickness and morphology have contributed to the popularization of the EPD over the decades (Amrollahi, Krasinski, Vaidyanathan, Tayebi, & Vashaee, 2016; Tahmasbi Rad, Solati-Hashjin, Osman, & Faghihi, 2014). EPD has been widely utilized for deposition of mostly CS-based composite coatings over the substrates for imparting the bio functionalities such as biocompatibility and antibacterial activity to both metallic and polymeric substrates (Boccaccini et al., 2010). Table 1 shows different kinds of surface modification on CS coatings and the specific property variations according to the nature of modifications.

Table 1. Different types of chitosan coatings prepared by EPD

Coating composition	**Substrate**	**Nature of the surface modification**	**Highlighted property**	**Ref**
CS	316L Stainless Steel (SS)	Not modified	Contact angle steel/CS coated steel 55° ± 3°/67°-74° ± 2°	(Gebhardt et al., 2012)
CS/GO	SS	Nanocomposite	50 % decreased bacterial (*E.coli*) viability on CS-GO surface	(Metze et al., 2015)
CS/doped glass composite	316 L SS	Original 45S5 bioglass doped with Zr and Sr^{+}	While Zn enhanced glass reactivity to SBF, Sr maintained the same level	(Miola, Verné, Ciraldo, Cordero-Arias, & Boccaccini, 2015)
CS/BG/GS	316L SS	Multifunctional composite	Coating released 40 % of its gentamicin payload within five days. 13 mm zone of inhibition against *S.aureus* for two days.	(Fatemehsadat Pishbin et al., 2014)
CS/BG/N-Ag	316L SS	Composite, the formation of CS-Ag complex	<2.5 ppm concentration of Ag^{+} showed antibacterial activity against *S. aureus* up to 10 days	(F Pishbin et al., 2013)
BG/CS (MW: 200 kDa ; DA:85%)	316L SS	Composite, <1 µm bioglass (2 g/L) in 0.5 g/l CS at 10 $V.cm^{-2}$	Current density in artificial saliva decreased by 52% in the CS composite.	(Mehdipour & Afshar, 2012)

HAp/CS/Coll	Austenitic SS	Nanostructured coatings with variable thickness	Higher yield strength and better adhesion for HAp/CS composite than HAp coating	(S. A. Mali, 2012)
CS/Au	Ti	Deposition at -0.4 V (*v/s* SCE with in Ti sheets) for 600 s	Non-toxic bio-nano composite coatings with enhanced electrochemical behaviour Antibacterial activity against *S. aureus*	(Farghali, Fekry, Ahmed, & Elhakim, 2015)
CS/C-HAp coatings	Ti-6Al-4V	$CaCO_3$ after EPD on Ti, treated in phosphate buffer solution and such carbonated HAp modified with CS	Biocompatible coating with better cell morphology, attachment and proliferation	(Tang, Tian, Guo, Zhu, & Guo, 2014)
CNT/C-CS/M-HAp	Ti-6Al-4V	Mineral-HAp/CMC composite, later mixed with CNT	Coatings with improved mechanical, better cell adhesion and viability of HOS MG63 human osteoblast cells	(Gopi, Nithiya, Shinyjoy, Rajeswari, & Kavitha, 2014)
HAp/CS	Ti-6Al-7Nb	HAp/CS coatings on two-phase (alpha+beta) substrate below 10 V; the homogeneous presence of needle-like HAp in CS	770-800 nm thick homogeneous, crack-free, well adherent coatings were obtained	(Moskalewicz et al., 2015)
CaP/CS/CNT	AZ91D	Composite coating and immersion in PBS	Addition of CNT resulted in an increased loading amount of gentamicin and increased cell viability (SaOS-2 osteoblast-like cells)	(J Zhang, Wen, Zhao, Li, & Dai, 2016)
CS/Ag	NiTi	CS and $AgNO_3$ mixed solution deposition at 6 $V.cm^{-2}$ for 10 min	Coatings exhibiting enhanced antibacterial activity against *E. coli*	(P. Li et al., 2013)
CS/nBG	Ti	CS/BG composites up to 20 wt% deposited by cathodic EPD	10-11 weeks with antibacterial properties against *Streptococcus* mutants and drug-eluting capability	(Patel et al., 2012)
HAp/CS	SS and Pt Foils	Nanocomposite coating thicknesses up to 50 µm	enhanced the corrosion resistance of SS substrate	(Xin Pang & Zhitomirsky, 2005)
HAp/CS	Ti and Pt Foils	Composite coating thickness up to 50µm	Dense, adherent and uniform coatings with better corrosion protection	(PANG & ZHITOMIRSKY, 2005)
CS	ITO-glass/ Au patterned Si wafer	Nanoscale deposition (0.05 mA/cm^2 for several minutes) of quantum dots and proteins	Coating with the ability to reversibly swell in response to hydration	(L.-Q. Wu et al., 2005)

HAp/CS	316L SS	Nanocomposite (40.9–89.8 wt.%) HAp coating by cathodic EPD	Adherent uniform coatings with thickness up to 60 µm, better corrosion resistance	(Xin Pang & Zhitomirsky, 2007)
CS/HAp composite	SS or Pt	Zirconium Hydroxide/CS, Aluminum Hydroxide/CS, Iron oxide/CS, Au/CS coatings	Better corrosion resistance when deposited on conducting substrate	(I Zhitomirsky & Hashambhoy, 2007)
CaP/CS	Ti	Electrolytic deposition of composite	good bone marrow stromal cell attachment	(Jiawei Wang, van Apeldoorn, & de Groot, 2006)
CS/HAp	Pt, SS and graphite foils and wires	Cathodic deposition produced 50 µm thick HAp/CS coatings	Coatings provided higher corrosion resistance	(Igor Zhitomirsky & Pang, 2007)
HAp/CS/Heparin	304 SS, Pt and Graphite	Multilayer, functionally graded materials coating with thicknesses in the 2-100 µm range	Deposits with thickness 2-100 µm were obtained with probable use as biomedical coatings with higher blood compatibility	(Sun, Pang, & Zhitomirsky, 2009)
HAp-$CaSiO_3$-CS	Pt foil, Graphite, 316L SS	Functionally graded HAp-$CaSiO_3$-CS monolayer or multilayer coatings	Monolayer and Laminate layers were achieved with better corrosion protection	(Xin Pang, Casagrande, & Zhitomirsky, 2009)
HAp-silica-CS	Ti, Graphite and 316L SS foils and wires	Coatings on conducting substrates of thicknesses up to 100 µm at room temperature	thick and adherent bio-composite coatings	(Grandfield & Zhitomirsky, 2008)
BG-HAp-CS	SS, Pt foils, Ti wires and platinized Si wafers	Cathodic deposition of cationic chitosan-based on the pH increase and anionic alginate deposition based on pH decrease.	room temperature fabrication of bioactive composite useful in biomedical applications	(D. Zhitomirsky, Roether, Boccaccini, & Zhitomirsky, 2009)
CS-MW-CNT	Graphite and NiTi	cathodic EPD to generate monolayer nanocomposite coating	Coating of 0.2-5µm thickness with higher corrosion resistance to NiTi SMA	(Grandfield, Sun, FitzPatrick, Cheong, & Zhitomirsky, 2009)
CS reinforced-HAp/W	Ti	Coating Ti surface with CS thin layer followed by three alternate coatings of ceramic and CS to obtain a homogenous coating	*In-vitro* bioactivity, enhanced mechanical properties (9.23±0.94 GPa Young's modulus), increased adhesive strength	(Sharma, Soni, & Bellare, 2009)
Octa-CaP micro fibre/CS	Ti	micro-porous structured coatings with OCP fibres of	Enhanced osteoconductivity and better osteoblast	(Lu, Leng, & Zhang, 2008)

		20–30 μm length and 0.1–1 μm width	attachment and proliferation	
HAp-Ag-CS	Pt foil, graphite, 316L SS foil	Dense (0-20 μm thickness) uniform coatings on the substrates of complex shapes	Biocompatible antimicrobial coatings with controlled Ag^{+} release, higher corrosion resistance of SS substrate	(X Pang & Zhitomirsky, 2008)
CS/nano-HAp	316 L Austenitic SS	SS processed by controlled phase reversion and vein-type interconnected structure of HAp fibrils are formed	Bioactive coatings with superior adhesion strength due to nano-HAp electro crystallization	(S. Mali, Misra, Somani, & Karjalainen, 2009)
CS/Gel	Ti	Blend solution coating forms a macroporous coating	Higher shear bond strength and osteoblastic cells response	(Jiang et al., 2010)
CS/Silk fibroin	Ti	Low-temperature EPD at 4 °C forms gelatinous coating with pore size 100 to 300 μm	Better cellular affinity towards MG63 osteoblast cells for coating with increased shear and tensile bond strength	(Z. Zhang et al., 2011)
CaP/CS	MAO AZ91D Mg alloy	nano-HAp/CS/AA aqueous solution of 60% and 80% and conversion in PBS	Homogeneous and bioactive coatings with better adhesion strength	(C. Wu, Wen, Dai, Lu, & Yang, 2010)

Various parameters regulate the efficiency of the EPD process and thus the final coating performances. This involves the EPD parameters like voltage, current *etc.* in addition to physical and chemical characteristics of the solution. Modification of 316L SS surface with pure CS (MW 80 kDa, DA: 80%) by Gebhardt *et al.* (Gebhardt et al., 2012) illustrated the influence of applied electric field on the deposition efficiency. The EPD was carried out at room temperature for 7 min at a constant voltage of 2.5 V with different electric fields, *i.e.* 2.5, 5 and 10 V/cm, and by varying the electrode distance. A dilute CS (0.33 wt.%) solution in a mixture of distilled water and acetic acid (AA) was used as an electrolyte with a pH between 3.50-3.58. When the applied voltage was higher, the breakdown potential decreased due to the larger formation of bubbles. This caused defects in the CS coating and lowered the barrier properties when tested in simulated body fluid (SBF). The thickness of the coating was also observed to be increasing with applied voltage as follows, 1–2 μm (E = 2.5 V/cm), 3–4

μm (E = 5 V/cm) and 5–6 μm (E = 10 V/cm). Therefore, by properly adjusting the EPD voltage, homogeneous, transparent and adherent coatings of CS can be obtained with better corrosion behaviour compared to bare 316L AISI SS. The particular stainless steel was selected due to its biocompatibility in orthopaedic applications, and the CS coated films were also observed to enhance the bioactivity. In addition, CS composites are found to be with better performance when compared to neat CS coatings (Metze et al., 2015). Compared to bare SS, CS/GO coated surface provides 50 % decreased cell viability towards *E.Coli*. According to Sharma *et al.* (Sharma, Soni, & Bellare, 2008), the current density and coating duration regulates the behaviour of HAp-W/CS composite coatings on Ti. Higher current density and longer coating durations resulted in better adhesion and bioactivity of metallic implants. Homogeneous, thick and adhesive coatings were obtained with enhanced mechanical properties when the deposition was carried out at a current density of 7 mA/cm^2 compared to deposits at 5 and 9 mA/cm^2.

Nature of electric current (alternate or direct) is another important parameter influencing the surface properties of the CS-based materials. Miola *et al.* (Miola et al., 2015), modified the AISI 316L SS substrate with CS containing doped BG. The BG was modified by the introduction of Sr and/ or Zn oxide (6 mol %) instead of CaO. While Sr was added for its ability to stimulate the bone formation, Zn controlled its role in metabolism, anti-bacterial and anti-inflammatory effect. The authors demonstrated the influence of both DC-EPD and AC-EPD on the deposition. DC-EPD was carried out at 20-80V with deposition times between 30 s to 2 min whereas, for the AC-EPD, the deposition time was varied between 1 and 2 min in the same range of voltage and at a frequency range of 4-10 kHz with a duty cycle of 70-80 %. The distance between the electrodes was kept constant (10 mm) at all times. The modified bioactive glass with Zn and Sr resulted in different bioactive behaviour. While the presence of ZnO allowed silica gel formation and its enrichment in Ca and P after the SBF

immersion and inhibited the formation and precipitation of HAp, SrO triggered the formation of crystalline HAp. The concentration of Zn was varied to maintain the antibacterial activity and anti-inflammatory effect without inhibiting the bioactivity, whereas the bioactivity and biological effect of Sr helped in developing good coatings for orthopaedic applications. The difference between AC and DC deposition was also pronounced as the formerly allowed coating without bubbles or cracks, and the latter caused bubble formation. This is due to the electrolysis of water happening during DC deposition. Finally, the authors concluded that the AC-EPD resulted in a compact, bubble-free and crack-less coating.

Pishbin *et al.* (Fatemehsadat Pishbin et al., 2014) conducted a series of investigations to test the influence of EPD deposition on coating performance. In one report, they generated 45S5 BG (particles size 1.6-26.7 µm), CS (DA: 85%) and GS (anti-bacterial agent) containing composite coatings by EPD. CS (0.5 mg/mL) in 1 vol.% AA in water solutions was prepared with pH 3. The deposition was carried out in pure CS solution, CS/BG solution, and CS/BG/GS solution at a single voltage of 10 V, and for time periods of 800, 400, and 400 seconds respectively. The coatings enhanced the HAp formation in SBF, confirming bioactivity, and released the GS. The releasing kinetics study demonstrated the bacterial growth inhibition for the first two days and supported the proliferation for up to 10 days. The authors also investigated the influence of n-Ag on the antibacterial property of BG (9.8 µm) /CS/n-Ag coating, by depositing on 316L SS by EPD in a single step (F Pishbin et al., 2013). Deposition of CS (0.5 mg/ml) solutions in 1 vol% AA/DI water was carried out at 21±2 °C with a constant distance (1.5 cm) between the electrodes. CS, CS/BG, CS/n-Ag coatings were prepared at a voltage of 10 V for 800, 400, 300 s and CS/BG/n-Ag coating at 15 V for 400 s. In addition, Ag release kinetics from the coatings was studied, and the coating was further evaluated for antibacterial activity against *S. aureus*. At higher applied EPD voltages, the polymer moves towards the negative electrode, the amines get deprotonated at high pH, and

silver-chitosan complexes are formed. This causes the reduction of Ag^{+} to Ag nanoparticles, and thus CS acts as a nucleating agent for Ag-nanoparticles, at the same time preventing the agglomeration. This clearly indicates the influence of EPD voltages on regulating the antibacterial activity, especially when silver nanoparticles are embedded. The proposed method of *in situ* formations and encapsulation of silver particles maintains the fouling resistance of the final composites, in addition to the proliferation of MG-63 osteoblast-like cells during culture for seven days. However, higher silver particles (342 μg) exhibited cytotoxicity.

Influence of pH on the efficiency of the EPD process is further explained with the deposition of a ternary CS composite. Batmanghelich *et al.* (Batmanghelich & Ghorbani, 2013), studied the effect of pH and CNT concentration on corrosion behaviour of CS/HAp/MWCNT (CS with MW: 80 kDa; DA:85 %) composite coatings. HAp, ($Ca_{10}(PO_4)_6(OH)_2$), particles with grain size 100 and 200 nm were synthesized by a wet chemical precipitation method. MW-CNT with average diameter 60-130 nm and length 30 μm were synthesized by CVD. The deposition was carried out at a constant voltage of 20 V/cm at different pH values of 3.3, 4.4 and 5.1. Coatings obtained at higher pH exhibited better corrosion protection in SBF, and thus influences the osteoconduction behaviour. Increased MW-CNT content increased corrosion resistance value compared to CS/HA.

In another study by Simchi *et al.* (Simchi, Pishbin, & Boccaccini, 2009), bioactive coatings of CS on 316L SS metallic substrate were developed, and the film growth kinetics were studied as a function of pH. The deposition rate increased with increasing pH from 2.9 to 4.1, since the protonation/deprotonation behaviour of CS is pH-dependent. The pH varies with the acetic acid concentration, and an increased concentration decreases the degree of ionization and net charge density. A porous film was obtained, due to hydrogen entrapment.

Also, the film growth rate varies according to the pH change during the range of 0.02–0.08 µm/s.

Other than the pH, experimental conditions like electrophoretic bath and sonication time also affect the EPD efficiency. Molaei *et al.* (Molaei, Amadeh, Yari, & Reza Afshar, 2016), performed subsequent studies on various composite coatings to test the efficiency of CS surface functionalization. The CS/HNT ($Al_2Si_2O_5(OH)_4.2H_2O$) composite was deposited on a Ti substrate by preparing an electrolytic bath of 0.5g CS/L in 1% AA solution. For the composite, the bath was prepared as a suspension of 0.6 g/L HNT with 30 vol.% pure water or 30 vol.% CS solution. The EPD was performed at 30 V for 5 min, and the enhanced HAp formation ability and corrosion resistance were observed for CS/HNT. The coating efficiency in terms of corrosion resistance depended on isoelectric point of HNT (pH 3), and so the composite deposits were produced at pH 2.5 to 3. By varying the pH of the suspension and time of deposition, CS (MW:80 kDa, DA:85%), BG and HAp composite was also deposited on Ti substrate by EPD (20 and 30 V, 20±2 °C) (Molaei, Yari, & Afshar, 2015). The performance depended on the pH and time of deposition as smooth BG/HAp/CS coatings were obtained at pH 4.5 and with less voltage. The suspension with 70% vol. Ethanol was the most stable with uniform distributions of HA and BG, and homogeneous porosity. Electrochemical testing in SBF revealed the formation of polymeric-ceramic deposition, which led to increased corrosion resistance.

Surface functionalization and EPD deposition of the CS-based materials are tuned for their applications in the biomedical field. Ordikhani *et al.* (Farideh Ordikhani, Dehghani, & Simchi, 2015) applied the CS-Laponite (LP) nanocomposite coatings on Ti implants with good antibiotic drug eluding capacity in drug delivery. CS (DA:85%; MW: 190-310 kDa) at 0.5 g/L concentration was made in 1% glacial AA and the amount of LP was varied (0.05, 0.1, 0.25, 0.5 and 1 g/l). EPD was done in 25 ml of suspension at 10 V for 19 min. Studies on

the drug release kinetics and *in-vitro* material interactions confirmed the positive effect of LP on the drug encapsulation efficiency. The initial burst release of Vancomycin was observed, followed by a reduced release rate. The prepared CS/LP coatings exhibited non-cytotoxic behaviour with high stability with enhanced cell proliferation. The subsequent studies of the same research group further explained the significance of drug/polymer weight ratio on the drug release mechanism (F Ordikhani, Tamjid, & Simchi, 2014). The CS solution in glacial AA at pH 3±0.1 with variable drug/polymer weight ratio (0.5-4) was coated on Ti substrate at 10 V/cm electric field for 15 min (Molaei et al., 2015). The three-stage release mechanism was achieved with burst release at the early stages, followed by almost no release for about 90 hours and gradual release of antibiotic during the last stage. The coating exhibited no adverse effect on MG-63 osteoblast-like cell with higher corrosion resistance and bioactivity. The antibacterial activity was examined against *S. aureus* bacteria which was specifically due to the presence of glycopeptide. Ordikhani *et al.* also studied about CS/BG (0.5 g/L BG) composite coatings (F Ordikhani & Simchi, 2014) as bioactive drug-eluting agents. The EPD was carried out at 10 and 15 V/cm for CS and CS/BG composite coatings for 10 min. Vancomycin (2 g/L) was added to CS/BG suspension in order to impart the antibacterial activity to the coating. *In-vitro* bioactivity was also evaluated in the SBF solution. The inclusion of BG particles improved the apatite forming ability. Biocompatible coatings of 55±6 µm thickness were obtained with bactericidal and antibacterial capability.

Nature of electrolyte used for coating is another significant parameter influencing the coating efficiency. Yan *et al.* observed the formation of a dense, porous and antibacterial CS/Ag/HAp coating on anodized Ti (Yan et al., 2015) when the electrolyte containing 0.043 $molL^{-1}$ $Ca(NO_3)_2$, 0.025 $molL^{-1}$ $NH_4H_2PO_4$, 0.036 gL^{-1} CS and 0.1$mmolL^{-1}$ $AgNO_3$ at pH 4.3. The deposition was conducted in a three-electrode cell at a current density of 0.85 $mA.cm^{-2}$, for 35 min at 50 °C. The dense coating of 6.2 ± 0.7 µm thickness exhibited antibacterial

activity when tested using Gram-positive and Gram-negative strains. The synergistic effect of silver and CS also showed nontoxicity to MC3T3-E1 cells, substantiating the biocompatibility. Favourable cell adhesion and spreading properties were also reported for the coating. Another CS composite (medium MW, DA:85-90%), containing C-MWCNT and Zn substituted HAp was coated on Ti using a particular dispersion of CS (0.5 g/L) in 5/95 vol% water/ethanol with 0.1% AA, CNT (0.5 g/L) and HAP (5 g/L) by Zhong *et al.* (Zhong, Qin, & Ma, 2015b). The deposition was carried out at 30 V for 60 seconds, and the coated implant showed improved HAp formation in SBF and better corrosion properties compared to the HAp coating. Homogeneous morphology, favourable apatite formation ability, adhesive strength and better corrosion resistance were emerged out for the substituted HAp coatings. CS and SA (1:28 ; P:SA for a two wt% aqueous solution at 30 °C) solutions obtained by dissolving 75 mg of each material in 100 mL of DI and 100 mL of 1% (V/V) AA respectively were the subject of study for another research group (Z. Wang et al., 2014). Such Alg/CS LBL coatings on Ti substrates was achieved by using the substrate as an anode at a constant voltage 20 V for 20 min. The thicknesses of the deposited CS layer and Alg layer were respectively found to be 20 and 10 µm. It is worthy to note that the anodic deposition of anionic Alg decreases with pH at the anode surface, whereas the cationic CS deposition at the cathode surface enhances with increased pH. Better cell morphology and coating efficiency were revealed on Alg/CS/Alg coatings as compared to Alg/CS coatings.

Pulsed EPD process using specific electrolyte also gave interesting results such as higher corrosion resistance and inductivity of AZ91D metal substrate (Jia et al., 2016). In this way, the HAp/CS/$RuCl_3$ composite coatings were made by using the electrolyte mixture of 0.042 $molL^{-1}$ Ca $(NO_3)_2$, 0.025 $molL^{-1}$ $NH_4H_2PO_4$ and one $molL^{-1}$ $NaNO_3$ with pH 5.0. The additive electrolytes were prepared by the addition of 0.125 g of CS (DA: 85%) in 12.5 mL of 1% AA with different amounts of $RuCl_3$. The pulsed EPD was monitored at variable

applied voltages of 90, 100, 110 and 120 V for 30 min. At 110 V, with a Ca/P molar ratio of 1.660, uniform and dense coating were obtained. In fact, the rod-like HAp nanoparticles with $RuCl_3$ enhance the bioactive corrosion resistance and inductivity as a function of $RuCl_3$ concentration. The coating was capable of inducing a new Ca-deficient apatite layer when immersed in Hank's solution for seven days. This reveals the effect of the chemical composition of the coating material and its interfacial interactions on the coating efficiency.

Addition of various nanomaterials, especially the modified/functionalized nanomaterials, always enhance the interfacial interactions existing in CS composites. Zhang *et al*. (Jie Zhang, Dai, Wei, & Wen, 2012) studied the bonding strength of CaP/CS composite coatings on micro-arc oxidized AZ91D Mg alloy as a function of various EPD parameters, and best corrosion resistance was achieved at optimum conditions of deposition. The bonding properties were not influenced by both CS and HAp content, but the EPD parameters such as the electrophoretic voltage (40-110 V) and AA content during deposition influenced the mechanical bonding strength. Ahmed *et al*. (Ahmed, Fadl-allah, El-Bagoury, & El-Rab, 2014), modified NiTi surface by CS (0.50 g CS in 98 mL water and gradually adding 2.0 mL glacial AA) containing n-Au coating at pH near 3. Au solution prepared by dissolving $HAuCl_4.4H_2O$ in 0.1M HNO_3 was added to CS gel to form a dispersion. The EPD was carried out in a three-electrode cell at a potential of -0.4 V (v/s. Ag/AgCl within NiTi electrode) for a time period of 600 s. The electrode was then dipped into glutaraldehyde for 1 min. The inhibitory effect of CS was due to the interaction of poly-cationic amines with negatively charged ions at the bacterial cell surface while the n-Au binds to the functional group of proteins resulting in protein deacetylation and denaturation. n-Au penetrates to the cell wall causing inactivation of their enzymes, generating hydrogen peroxide leading to cell death. Thus, a stable, dense and passive coating was obtained with high corrosion resistance at

different immersion times and at all pH values, demonstrating dual mechanisms of action for antibacterial activity from n-Au and CS cationic effect.

Wang *et al.* (J Wang, de Boer, & de Groot, 2004), again explained the ionic interactions between the CS and the filler CaP particles and discussed its influence on EPD. Incorporation of CS influenced the formation and crystallization of CaP, which might be due to two reasons, the positively charged CS molecules bind to negatively charged PO_4^{3-} ions, thus inhibiting CaP formation. The other possibility might be the binding of CS to the substrate surface inhibiting CaP deposition. Higher CS concentration decreased the coating thickness and roughness, and the hybrid coating exhibited higher dissolution rates in simulated fluids. However, the adhesive strength for the original and hybrid coatings was almost the same. These composite coatings were proved to be more favourable for the goat bone marrow stromal cell attachment than pure CaP coatings. Selective patterning process was also practised on CS deposition on Au surfaces by Wu *et al.* (L.-Q. Wu et al., 2003). Here the reactive amine groups of CS provided a spatially resolved templated CS surface suitable for subsequent coupling reaction on which other biomacromolecules can be assembled. The authors were also deposited positively charged CS molecules (under mildly acidic conditions) onto electrode surfaces using voltage bias *via.* EPD (L.-Q. Wu et al., 2002). Thickness in the order of microns was achieved when CS is deposited on a negative electrode from an acidic solution. The coating thickness varies with the applied voltage, CS concentration and the time for the deposition.

In short, the EPD technique mainly addresses deposition of CS on conductive substrates, and the surface interactions and EPD parameters play a vital role in the coating efficiency. CS coatings containing other polymers (Alg, Gel, polydopamine, *etc*.), metallic nano-particles (n-Ag, n-Au), ceramic particles (HAp), BG and antibiotic drugs (GS, vancomycin, *etc.*) are produced to impart multifunctionality to the implant surface. In terms

of orthopaedic implants, the main effort for CS coatings is to integrate BG or HAp particles or by adding drugs or carbonaceous materials to improve the bioactivity and cell attachment ability. The optimization of the EPD process parameters such as applied voltage, time taken for deposition, and distance between electrodes is critical to fabricate effective CS coatings. Moreover, the suspension characteristics have a crucial role in the success of EPD based coatings. The factors affecting the stability of the CS-based suspensions include size, shape, viscosity, conductivity, pH, the concentration of the suspended particles, etc. need to be more clearly studied for the successful commercial applications.

Limitations: EPD is a versatile technique for coating applications; however, it is not highly recommended for industrial applications. This is mainly because of the expensive and hazardous solvents required in EPD processes. However, the number of researches, as mentioned earlier, used different solvents, including water to apply effectively in EPD technique, and thus addresses the limitations.

2.2. Layer-by-Layer (LBL) Deposition

LBL adsorption of polymers onto the solid substrates has become a powerful tool for the assembly of thin films with the controlled composition (Hammond, 2004; Sukhishvili, 2005). The LBL approach is based on the alternate adsorption of materials containing complementary charged or functional groups to form integrated ultrathin films, as shown in Figure 3 (Hammond, 2004). The LBL technique has many advantages over other methods of multilayer fabrication as no complicated instruments are needed for this process, thus making it easy and inexpensive. It also allows the incorporation of various building blocks/charged polymers/molecules into the multilayer with desirable structure and properties. Moreover, the LBL deposition is independent of the shape and size of the substrate (Xi Zhang, Chen, & Zhang, 2007).

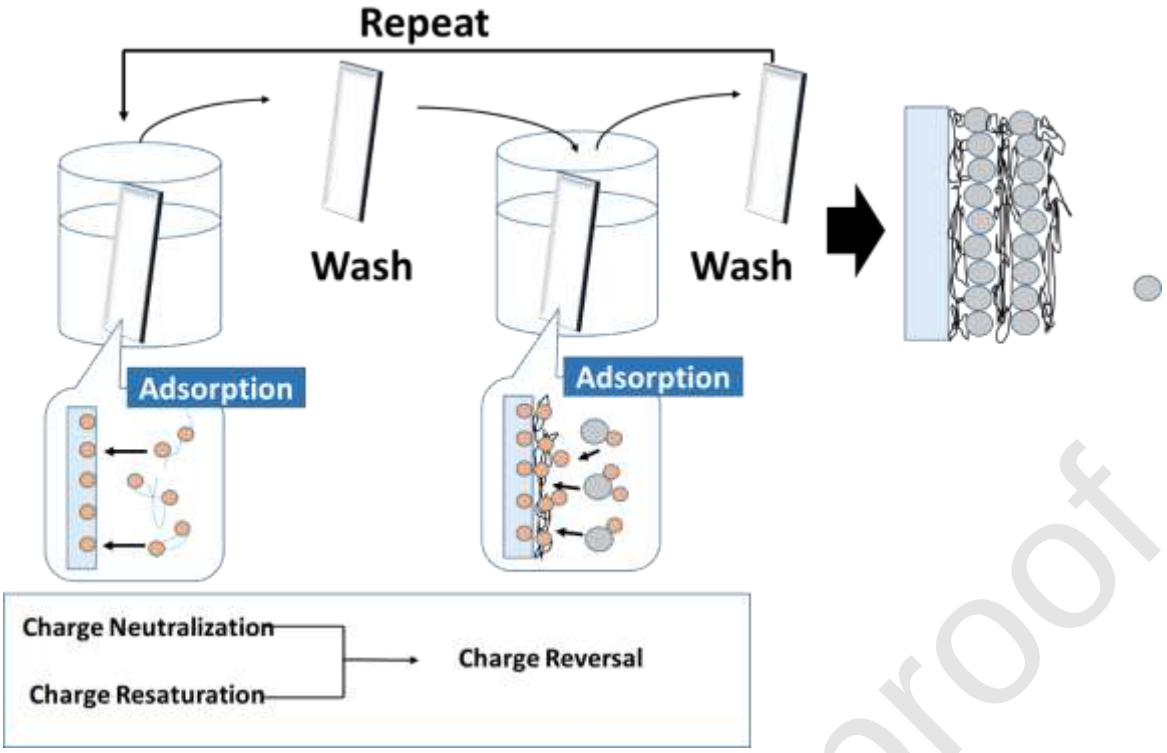

Figure 3: Schematic representation of LBL assembly by electrostatic interaction (Ariga, Hill, & Ji, 2007)

A lot of work has been carried out regarding the biofunctionalization of implant surfaces (metallic, polymeric or glass substrates) by LBL deposition (Table 2). The biomaterials (mostly polymers), especially the water-soluble proteins, have charged sites on their surface, thus can be deposited easily *via.* LBL technique (Chicatun et al., 2019). Joint reconstruction by osteochondral tissue engineering is achieved by CS/Coll bilayered hydrogel. The specific number of layers generated through LBL assembly helps to develop proper interactions with the extracellular matrices in bones and cartilages. The deposits consist of charged organic and inorganic molecules. Charged inorganic substances have also been used for LBL assembly (Ariga et al., 2007). CS, a cationic polysaccharide, is thus suitable for LBL deposition.

Table 2. Publications describing LBL deposition of chitosan

Coating composition	Substrate	Nature of Surface Modification	Results	Ref

Poly-β-cyclodextrin/ CS	Ti	Electrostatic interactions	Coating act as a drug-eluting system and to treat infections (validated with gentamicin)	(Pérez-Anes et al., 2015)
CS/Gel and CS/HAp	Ti-6Al-4V	Polyelectrolyte multilayer coating	Improved implant anchorage for osseointegration	(Zankovych et al., 2013)
Dopamine modified HA/CS	Ti-24Nb-2Zr	Multilayer dip coating at pH 5	D-HA/CS multilayer with enhanced osteoblast proliferation	(Xinming Zhang, Li, Yuan, Cui, & Yang, 2013)
CS/Casein phosphopeptides	Co-Cr-Mo alloy	Covalently bonded to the substrate	Increase in proliferation and differentiation of MC3T3-E1 osteoblast-like cells	(Qin, Dong, Mu, Zhang, & Dong, 2015)
CS/Chondroitin sulfate	Glass	Biomimetic coating of polyelectrolyte multilayer with calcification.	The multilayer traps Ca^{2+} and PO_4^{3-} ions, which may further lead to favourable sites of nucleation, initiating precipitation of CaP.	(Leite, Sher, & Mano, 2014)
CS/Heparin	Aminolyzed PET films	Ionic interaction	Antiadhesive antibacterial multilayer coatings	(Fu, Ji, Yuan, & Shen, 2005)
CS/Chondroitin Sulphate	PET	electrostatic adsorption of oppositely charged polymers	Linearly grown CS and chondroitin sulphate layers promote adhesion of endothelial cells	(Y. Liu, He, & Gao, 2005)
CS/Gel	Ti coated borofloat glass	polyelectrolyte-mediated electrostatic adsorption	Improved bone-bonding ability, better cell-substrate interaction, flattened cell morphology and better cell-to-cell and cell-to-substrate interaction	(Cai, Rechtenbach, Hao, Bossert, & Jandt, 2005)
HAp/CS	Mica	polyelectrolyte films deposition on oppositely charged surfaces	Uniform, homogeneous and smooth coatings with nice miscibility and lower roughness	(Feng, Zeng, Yang, Wang, & Cai, 2005)
Heparin/CS-Ag^+ complex	Aminolyzed PET membrane	amino groups of CS chelate Ag^+ and nano-Ag with coordination interaction	Antibacterial coatings with good anticoagulation activity and low cell toxicity	(Fu, Ji, Fan, & Shen, 2006)

Heparin/CS	316L SS	Self-assembled coating	Promote re-endothelialization and intimal healing. Safe and stable coating during sterilization and storage	(Meng et al., 2009)
Alg/CS	Poly(ethyleneimine)	Tubular structure formation through electrostatic forces	Stable and smooth coatings with good biodegradability	(Yang, He, Duan, Cui, & Li, 2007)
CS/mAb	PLLA coated stent	electrostatic adsorption of oppositely charged polyelectrolytes and proteins	Drug-eluting CS/mAb with better hemocompatibility and cytocompatibility *in-vitro*	(Luo et al., 2011)
CS/HA	NiTi	sequential adsorption of weak polyelectrolytes	Biocompatible coatings with better haemocompatibility	(Thierry, Winnik, Merhi, Silver, & Tabrizian, 2003)

The Layer-by-Layer (LBL) approach was utilized by Song *et al.* (W. Song et al., 2015) to modify the Ti substrate using sodium hyaluronate (polyanion) and CS/si RNA (polycation) nanoparticles. Hybrid structured multilayered smooth coatings were obtained on the substrate, and such coatings promoted osteogenic differentiation of MG63 osteoblast-like cells. The nanoparticles were uniformly distributed in the coatings, and such distribution influenced the topography and roughness of the CS. During LBL deposition, the surface roughness and topography vary, which leads to layer-layer adsorption. As the deposited layers increases, the siRNA cumulated load enhances, forming a stable coating for one week time. The films containing osteogenic siRNA enhances the osteogenic differentiation in MG63 cells as well. Antibacterial coatings of minocycline-loaded CS/Alg on Ti were also made following LBL self-assembly by Lu *et al.* (Lv et al., 2014). Multilayer coatings were achieved by effectively modifying the substrate surface and by improving the substrate-coat interaction. Ti substrate was hydroxylated first and then treated with 3-aminopropyltriethoxysilane to obtain amino-functionalized surface, and as the second step, the CS was covalently conjugated to it. Such coatings are tested for minocycline-releasing kinetics and found inhibiting biofilm formation

by *S. aureus*. LBL deposition can thus develop long-lasting antibacterial coatings as the multi-layers provides enhanced surface charge and hydrophilicity.

While smooth coatings of dopamine-modified HAp/CS multilayer on Ti-24Nb-2Zr alloy with improved wettability and hydrophilic properties enhanced osteoblast proliferation (Xinming Zhang et al., 2013), CS/Casein phosphopeptide composite coatings on Co-Cr-Mo alloy with dendritic topography and hydrophilicity increased the expression of osteoprotegerin (OPG) mRNA and the ratio of OPG/receptor activator of nuclear factor-κB ligand mRNA (Qin et al., 2015). Both these materials are potentially applicable in many orthopaedic implants. Depending on the number of layers during LBL assembly, the surface topography and roughness can be tuned to adapt the coatings for different functional applications. Leite *et al.* (Leite et al., 2014) modified glass substrates utilizing an LBL self-assembly method and produced CS/Chondroitin sulphate multilayers using polyelectrolyte biopolymers. A nanostructured multilayer was synthesized with improved calcification by sequentially passing Ca^{2+} and PO_4^{3-} for a sufficient number of cycles over the previously formed multilayer. This was beneficial for the nucleation and growth of CaP precipitates, and good precipitation was observed in 10 bilayers coating when compared to the five bilayers. The study evidence, the advantage of the LBL method in terms of the multi layer's ability to trap ions. The whole process was according to biomimetic approach, and bone tissue engineering has emerged as a targeting application.

Functionalizing the substrate will enhance the substrate-coating interaction through ionic as well as covalent interactions. A good demonstration was done by Fu *et al.* (Fu et al., 2005), in which antibacterial and antiadhesive coatings of CS/heparin were made on aminolyzed PET films by LBL assembly method. According to the *in vitro* antibacterial tests, there was a 7 % reduction in the number of viable bacteria after seven h contact with PET films, whereas, for the coated films, the reduction was 46-68%. Even after 24 h, only 3-8%

of the cells were viable on the coated PET. The authors also studied the significance of pH on the coating efficiency and noticed a 68%, 58% and 46%, respective decrease in the number of viable bacteria at pH 3.8, 2.9 and 6.0. In another research work by Liu *et al*. (Y. Liu et al., 2005), the PET substrate was hydrolysed and later, coated with CS and Chondroitin sulphate by LBL assembly. When the charged PET film was dipped in CS solution, charge interaction initiates the CS deposition, and the PET surface charges get reversed from negative to positive. This deposition continues until multilayer deposition is formed on the substrate surface. The PET films incorporated with biomacromolecules exhibit a stronger ability to promote adhesion of human endothelial cells and to maintain the endothelial function, and biocompatibility.

Polyelectrolyte mediated electrostatic adsorption for the CS/Gel was practised by Cai and co-workers (Cai et al., 2005) to develop LBL coated Ti surfaces. The deposition of the multilayer was initialized by deposition of a PEI layer (positively charged). Full coverage by the coating was achieved after the subsequent deposition of PEI/(Gel/CS)$_2$. The multilayer-coated Ti substrates showed altered biocompatibility. *In vitro* investigations confirmed that cells (Osteosarcoma; ACC439) which adhered to the polymeric LBL-modified surface showed better cell-to-cell and cell-to-substrate interaction than on control unmodified surfaces. Osteoblast proliferation and cell viability were higher for the CS/Gel modified Ti substrate after 1- and 7-days culture. Self-assembled polyelectrolyte films containing CS/ HAp were developed on Mica substrates using an LBL approach by Feng *et al*. (Feng et al., 2005). Two strategies were used for developing the coating. The first strategy consisted of four steps, namely immersion in CS solution, rinsing with water, immersion in HAp solution and rinsing with water. The second strategy contained six steps; modification of the surface with $NiCl_2$, rinsing with water twice, immersion in CS solution, rinsing with water twice and finally immersion in HAp solution. It was observed that the film morphology depended on

the fabrication steps. Clustered morphology was achieved by the first strategy, while a smooth homogeneous film was achieved *via*. second strategy. It was concluded that the second strategy was advantageous for achieving smooth and homogeneous coatings.

Adsorption of CS towards porous substrate is another possible way to enhance the efficiency of the coating. Yang *et al.* (Yang et al., 2007) demonstrated the alternate adsorption of Alg/CS (negatively charged Alg and positively charged CS) nanotubes on to the inner pores of polycarbonate substrate to develop biodegradable and viable coatings. The number of deposited layers can vary the thickness of nanotube walls, and such tubular coating achieved good biodegradability and less toxicity. The minimum number of layers for stable and smooth coating was observed to be 4, and the most stable nanotubes were $(ALG/CHI)_8$ or $(ALG/CHI)_{16}$ nanotubes with well defined, smooth structure. Normal dimension and thickness of the nanotubes were respectively determined as 400 nm and 40 nm. Electrostatic LBL deposition was also addressed in fabricating multifunctional orthopaedic implants resolving the major clinical problems of peri-implantitis (biofilm formation) and osseointegration (insufficient bone formation) around the implant (Govindharajulu et al., 2017). Chitosan-based bilayers were developed with an Osseo-inductive recombinant elastin-like biopolymer containing peptide groups, on Ti surface. The electrostatic interaction between the chitosan (positively charged) and peptide biopolymer (negatively charged) causes effective bilayer formation with superior biomineralization and fast bone growth. The cytocompatible bi-layers with mouse pre-osteoblast cells showed a 50-fold decrease in the number of *S. gordonii* viable bacteria compared to the normal coatings. However, the lack of rigidity of the developed chitosan/biopolymer system did not influence the osteoblast cell differentiation. Polyelectrolyte multilayer coatings of chitosan/heparin on alkali-treated Ti surfaces were reported for osteoblast differentiation *in vitro* and to promote proliferation, and adhesion (Shu, Ou, Wang, Zou, & Li, 2011). LBL method is also applied to develop effective

coatings against osteomyelitis (implants failure due to bacterial growth). This is achieved by developing hydrophilic multilayer coatings with chitosan/hyaluronic acid on Ti alloy surface with excellent antibacterial properties (Valverde et al., 2019). In short, the LBL technique is very simple, easy, and based on the basic chemistry of the materials used in the coating. There is no need for any expensive equipment, and that makes this technique the most popular.

Limitations: The main advantage of the LBL method is the possibility to control the film growth in nanometer. Since the film thickness is related to the bonding between successive layers, the conformation, charge density and pH of the polyelectrolytes in solution, play a significant role in regulating the coating efficiency. Other than the linear growth, the supralinear and unstable adsorption-desorption regime also exist in LBL assembly. While linear growth displays fuzzy structuration with less interpretation to alternate chains, the supralinear growth generates thick films of more than one μm thickness (Michel, Toniazzo, Ruch, & Ball, 2012). If the film-solution contact time is not enough for the ions to diffuse the interface, the film growth will be affected. However, the modifications of the substrate surfaces, strengthening the substrate-coating interface etc. were the areas of intense research as mentioned above.

2.3. Dip Coating

Dip coating of a substrate is a simple and old way of deposition, especially for smaller substrates, forming thin layer deposits, which can be further compacted by thermal treatment (Scriven, 1988). Dip coating is an inexpensive way to deposit thin layers from chemical solutions with a relatively fair control over layer thickness (Grosso, 2011). The kinetics of dip coating is based on a steady-state flow condition, and the coating thickness is normally determined by the competition between the viscous force, the capillary (surface tension) force, and gravity, as well as, by the substrate withdrawal speed (Scriven, 1988). Table 3 lists the properties of various dip-coated films of CS composites used in biomedical implant materials.

Table 3. Dip coating of substrate for implant material (publications)

Coating composition	Substrate	Nature of the surface modification	Results	Ref
CS	Ti	functionalization of Ti with CS *via.* a silanation	A biocompatible and antibacterial adhesive coating with excellent scratch resistance	(Renoud, Toury, Benayoun, Attik, & Grosgogeat, 2012)
CS	Ti	Ti surface is covalently bonded to quaternised chitosan(hydroxypropyltrimethyl ammonium chloride chitosan	Coating with antibacterial properties promote the osseointegration of the implant	(Peng, Ao, Wang, Guo, & Tang, 2015)
$CaCO_3$/MW-CNT/CS	Ti-6Al-4V	electroless deposition	Compact and uniform coatings Improved corrosion resistance	(Ahmed, Fekry, & Farghali, 2013)
CA/HAp/CS	304L SS	Sequential dip-coating ensure dense packing	Improved electrochemical resistance of SS plates, Formation of the apatite layer	(Zhong, Qin, & Ma, 2015a)
CS	PE	functionalization of PE surface by covalent bonding of CS coating	Improved mechanical properties, surface hardness, Young's Modulus and better friction resistance	(Stoleru (Paslaru), Tsekov, Kotsilkova, Ivanov, & Vasile, 2015)
CS/PVP	PET	PET pretreatment by polyetherimide and polyacrylic acid and crosslinking	Coatings with anti-adhesion and bactericidal effects on S. aureus	(B. Wang, Wang, Li, Ren, & Ji, 2012)
CS	Mg-1Ca	Multilayer dip coating	Smooth and compact coatings containing six layers of CS with improved corrosion resistance	(Gu et al., 2009)
CS/γ-PGA	Au coated Si wafer	alternate immersion in CS and γ-PGA at pH five and deposition on ultra-thin CS coated substrate	Cytocompatible multilayered structure	(Antunes et al., 2011)
CS/n-Au	Quartz slides	multilayer Au nanoparticle assembly through electrostatic interactions on CS functionalized quartz	Aggregation of n-Au on CS layer onto quartz slide substrate	(H. Huang & Yang, 2003)

The greatest challenge in developing a substrate coating is always maintaining good interaction between the substrate surface and the coating material. Addressing this challenge, Renoud *et al.* (Renoud et al., 2012) functionalized the Ti surface with CS by silanation reaction followed *by* dip-coating technique, as shown in figure 4. The bioactive CS coating was adhesive to the substrate and showed better scratch resistance. The CS coating also exhibited an antibacterial effect against *Actinomyces naeslundii* with good cytocompatibility towards N1H3T3 fibroblasts, proving the efficiency of covalent functionalization on maintaining coat-substrate interaction.

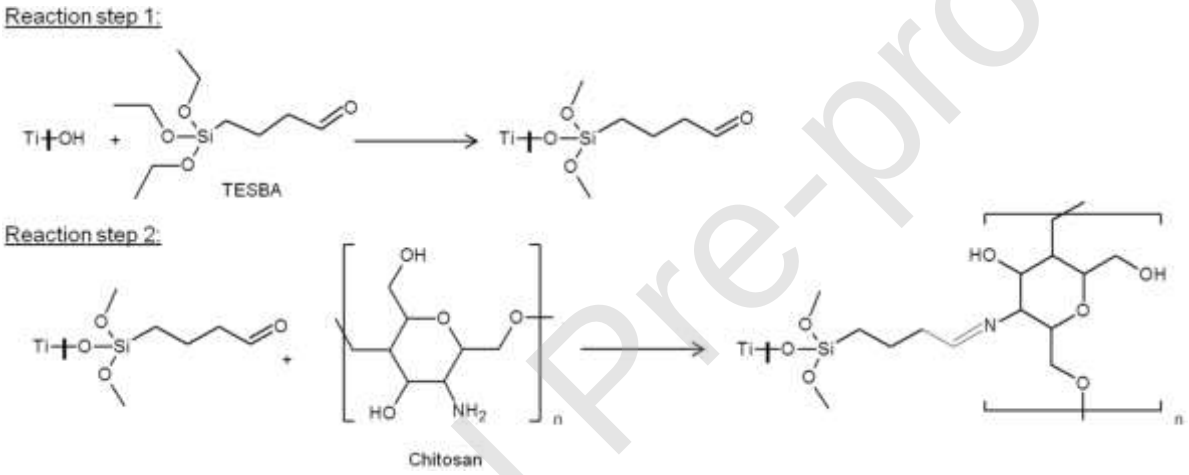

Figure 4. Reactions involved during silanation, allowing covalent bonding of CS with Titanium (Renoud et al., 2012).

Similarly, Peng *et al.* (Peng et al., 2015) also practised covalent bonding interaction between the coat-substrate interface by covalently bonding the Ti implant surface with quaternized CS. About 10^5 colony-forming units/10 µl of *S. epidermidis* is used to contaminate the medullary cavities of rat femora, and Ti rods were simultaneously implanted. Implant-associated infection was observed for the control, whereas no infection was noticed with Ti-coated implants. Thus good antibacterial properties were achieved against *S. aureus* and *S.*

epidermidis, in addition to bone osseointegration. The quaternized CS prevents the biofilm formations and implant-related infections, as confirmed from *in-vivo* studies.

Stoleru *et al*. (Stoleru (Paslaru) et al., 2015) explored the mechanical properties such as tear resistance and frictional performance for the CS coated PE surface and obtained a good correlation between the mechanical properties and coat-substrate interaction. In fact, the covalent immobilization of CS happens on the corona treated PE substrate and helps in achieving notable mechanical strength and gas impermeability, thus useful in medical and food packaging application.

Wang *et al.* (B. Wang et al., 2012) modified the PET substrate by polyetherimide and polyacrylic acid crosslinking pre-treatment and further dip-coated with CS/PVP. The coating was of 3.34 ± 0.48 μm thickness and even after 24 h of immersion in PBS, no abscission happened for the coating. The pre-treatment enhanced coating stability and strong adsorption with the composite. The coating exhibited antibacterial properties against *S. aureus* and *E. coli* and good biocompatibility *in-vitro*. As the PVP content in the composite enhanced, the antiadhesion activity of the coatings increased and 60–80% of *E. coli* were destroyed after eighth shaking culture**.** Antunes *et al.* (Antunes et al., 2011) utilized the polycationic activity of CS by complexing it with γ-PGA polyanion to produce the polyelectrolyte multilayer film on Au coated Si wafer by LBL coupled with dip coating. Six layers of deposition were formed, with the electrostatic interaction between the poly ions and surface electrical charge reversal. The composite coating showed no cytotoxicity against NIH3T3 cells as well, indicating the tissue engineering possibility.

Chen *et al.* (S. Chen, Wan, Zhang, Yang, & Li, 2020) combined the fluoride pre-treatment method and dip coating to develop zoledronate coating on AZ31 Mg alloy. Both fluoride treatment and zoledronate immobilization improve the corrosion resistance of the developed coatings. The chitosan-based coatings showed an initial burst and sustainable drug

release of 50–60% (of the initial 0.07–0.08 mg/mL) in 7 days. Even after 14 days of immersion, half of the drugs were hydrogen-bonded to chitosan. This facilitated the efficient clinical applications of chitosan coatings in orthopaedic applications. Thus, dip coating is a simple technique for carrying out polymeric deposition on a substrate. The coating thickness depends on many factors, like the viscosity of solvent and substrate withdrawal speed. Dip coating has been utilized over the years for depositing many polymers to impart bioactivity and antibacterial activities.

Limitations: The thickness of the films deposited depends on the nature of the solution such as viscosity, surface tension and gravitational threshold. Therefore, the thickness and uniformity are much sensitive to the conditions of the coating method, and faster withdrawal makes the film thicker. Volatile solutes and rapid drying processes are often practised for mitigating these limitations. In addition, various strategies such as evaporation induced self-assembly, capillary induced convection coating etc. are also practised to fully exploit the advantages of dip coating(Grosso, 2011).

2.4 Solution casting

Casting is another simple method of CS deposition on the substrate surface. For this, a polymer solution is prepared using a suitable solvent with different polymer weight percentages. Afterwards, this solution is cast over the substrate to produce a thin film deposition. This is usually followed by heat treatment or aging of the substrate at a particular temperature for a longer period of time to increase the bonding strength between coating and substrate. This simple coating method has been used to develop polymer coatings on a variety of substrates, *i.e.* metals, polymers, glass, *etc.* Table 4 displays various kinds of CS coatings, the nature of substrates, type of surface modification and the different applications.

Table 4. CS-based coating terms produced on implant materials by casting

Coating composition	Substrate	Mode of surface modification	Results	Ref
CS/Ag decorated CaP microspheres	Ti	Bonding by alkoxysilane reaction	reduces bacterial viability up to 90% in both anaerobic and aerobic pathogenic microorganisms	(Jennings et al., 2015)
CS-PVA	Ti	Polymer composite casting	Smooth, crack-free coating with sufficient mechanical properties for orthopaedic implants	(Mishra & Kannan, 2014)
Ciproflaxin loaded CS	Ti	Ionic interaction n situ release the antibiotic	Coatings do not affect MG63 osteoblast like-cell proliferation, adhesion and gene expression.	(Mattioli-Belmonte et al., 2014)
CS	Ti-6Al-4V .	alginate- and pectin-crosslinked chitosan	Coating promoted faster cell proliferation, higher ALP expression and greater collagen retention and calcium deposition.	(Lin & Chen, 2013)
CS/VEGF	Ti	CS chemically bonded to Ti *via*. silane- glutaraldehyde linker molecules	2-fold enhancement of the alkaline phosphatase activity and a 10-fold increase in calcium deposition	(Leedy, Jennings, Haggard, & Bumgardner, 2014)
Ciproflaxin loaded CS	Ti	ionic gelation by host-guest complexation equilibrium	Nanoparticle-based non-toxic biocompatible and antibacterial coating	(De Gigli o et al., 2012)
CS	Ti	CS covalently grafted to sulfuric acid pretreated Ti surface	Coating controlled implant-related infections and improved osteogenic properties	(Ghimire, Luo, Tang, Sun, & Deng, 2014)
CS	Ti	Ti sulfuric acid treatment and CS immobilization	Enhanced osteoblast adhesion while decreasing the bacterial attachment	(Foss, Ghimire, Tang, Sun, &

				Deng, 2015)
BMP2 encapsulated CS	Ti	Bonding between the aldehyde groups of GA-Ti and the amine groups of functionalized CS	Coating with favourable cell adhesion (Bone marrow stem cells), proliferation. differentiation and calcium mineralization	(Han et al., 2014)
CS-oxychitin	Ti-6Al-6V	CS on plasma-sprayed Ti with hydroxyapatite and with bioactive glass	Promote colonization by cell osteogenesis and osteointegration	(Muzzarelli et al., 2001)
CS	Ti	CS deposition on piranha treated substrate	High-quality coatings without affecting the bulk properties	(Martin, Schulz, Bumgardner, & Schneider, 2008)
CS	Ti	CS covalently bonded to Ti coupons *via.* silane-glutaraldehyde reaction	Smooth and crack free CS coatings with the ability to attach to Ti substrate after five weeks of dissolution, enhanced osteoblast response against osteosarcoma cells	(Yuan, Chesnutt, Wright, Haggard, & Bumgardner, 2008)
CS	Ti	CS coatings with 20% tetracycline or 0.02% chlorhexidine digluconate bonded to Ti by silane reaction	Coatings released 89% of tetracycline in 7 days and 100% chlorhexidine in 2 day	(Norowski, Courtney, Babu, Haggard, & Bumgardner, 2011)

CS	Ti	Covalent bonding by silanation	*In-vivo* studies on rabbit indicate better osteogenesis	(Bumgardner et al., 2007)

Various surface modification techniques are practised to develop CS-based coatings through casting method. According to Lin *et al.* (Lin & Chen, 2013), modification of Ti-6Al-4V substrate surface by alginate and pectin-crosslinked CS through the formation covalent linkages promoted faster cell proliferation, higher ALP expression, greater collagen retention and Ca deposition. The Alg-pectin crosslinking modified the CS drug release efficacy and also enhanced initial cell proliferation. This points towards the efficacy of CS modification in enhancing the characteristic properties in addition to ensuring the good substrate attachment. Leedy *et al.* (Leedy et al., 2014) also followed the similar route of the investigation by which improved surface characteristics of the CS-based coating enhanced the vascular endothelial growth factor and supported the osteoblastic cell formation. The CS coatings on Ti substrate was made by reaction through silane-glutaraldehyde linker molecules. SaOS-2 osteosarcoma cells on the coated Ti showed a two-fold increase in ALP expression and a 10-fold increase in Ca deposition compared to uncoated Ti. Enhanced vascular endothelial growth factor enhances the osseointegration, which helps in implant applications for those patients with less bone healing potential.

In another study, Ghimire *et al.* (Ghimire et al., 2014) developed Ti substrate coating by immobilizing CS on its surface *via.* a simple casting method. Sulphuric acid was used to treat the substrate surface, and later CS is cast on it to develop the CS-immobilized Ti (SA-CS-Ti) coating. This increased the antibacterial susceptibility of adherent bacteria, staphylococci, and the cell adhesion on SA-CS-Ti supported better cell proliferation and ALP activity compared to untreated Ti. The SA-CS-Ti surface showed better osteogenic property

and was antibacterial in nature as well. This helped in addressing the general complications in orthopaedic implants such as poor osseointegration and infections related to implants. The authors have established the role of CS increasing antibiotic susceptibility, and in limiting the invasion/internalization of bacteria into the osteoblast, which was first reported in that time. The same group (Ghimire, Foss, Sun, & Deng, 2016) has also studied the interaction between osteoblastic cells, *S. aureus* and CS modified Ti implant surfaces in a post-operative co-culture system. The coated surface resulted in a significant reduction of *S. aureus* adhesion on the surface and reduced the possible biomaterial related infections (bacteria: cell ratio of 0.001:1; 30 min after infection). The antifouling property was also remarkable as reduced bacteria proliferation ($p < 0.05$) was achieved for the modified surfaces. Moreover, the CS modified coating showed higher sensitivity to *S. aureus* ($p < 0.05$) against cefazolin (1 mg/L treatment) and gentamicin (10 mg/L and 100 mg/L treatment). Foss and coworkers also reported similar way of CS immobilization on Ti substrate (Foss et al., 2015) to combat implant-related infections. The results demonstrated that the presence of CS on the substrate has a positive effect on osteoblast behaviour, initial cell attachment, adhesion, and spreading. The coating prevented implant-related infections by demonstrated antibacterial behaviour against *S. aureus*.

Immobilization of functionalized CS on functionalized Ti substrate had a pronounced effect on regulating the osteoinductivity (Han et al., 2014). The CS was functionalized by Bone morphogenic protein-2, and this material was immobilized onto functionalized Ti substrate *via*. a simple casting. The Ti modification included alkali treatment, silanization with 3-aminopropyltriethoxysilane and aldehydation with glutaraldehyde. Highly efficient coat-substrate adhesion was maintained by the chemical reactions between the aldehyde groups of GA and the amine groups of CS. The coated Ti surface promoted bone marrow stem cells (BMSC) adhesion, proliferation, differentiation, and Ca mineralization. *In-vivo* studies were

carried out on Japanese big ear white rabbit and demonstrated the superior osteoinductivity of the modified surface. Martin *et al.* (Martin et al., 2008) improved the CS bonding onto Ti using four surface treatments, namely passivation, piranha treatment and two-step silane deposition. Toluene solvent was used for the silane deposition, and with increased deposition time, a 10-fold increase in adhesion strength for the CS coating was observed. High quality (with better adhesion due to addition of silane linker molecule; bond strength of 19.50 ± 1.63 MPa) hydrophobic CS coatings (contact angle 98.0 ± 3.6°) were produced with hardness, 0.19 ± 0.08 GPa and elastic modulus 4.90 ± 1.82 GPa. Yuan et al. also practised the silane reaction to explore the influence of coating performance on the CS coating bonding strength (Yuan et al., 2008). The group achieved 136 µm thick smooth and crack-free coatings. According to the results, the different DA, i.e. 80.6%, 81.6% and 92.3%, does not have much effect on the bonding strength. Average bond strength of the coatings ranged between 2.2 to 3.8 MPa. The coatings showed little dissolution over five weeks. The CS coatings also showed the enhanced osteoblast response, attachment and proliferation, for osteosarcoma cells.

Since the long-term success of a biomedical implant strongly depends on the cell adhesion, the osteoblast stiffness alterations with surface chemical composition upon adhesion on implants are of high research interest. The influence of CS-glass coatings on osteoblasts mechanical response in terms of the level of adhesion, spreading, and cytoskeleton reorganization was the subject of study by Moutzouri et al. (Moutzouri & Athanassiou, 2014). In addition to adhesive strength, the antibiotic uptake of CS coated substrate was investigated by preparing Daptomycin- and Vancomycin-loaded CS films. CS film showed the antibacterial activity against *S. aureus*, irrespective of CS characteristics like DA (61%, 71% and 81%) and drug loading and elution process. Better osteoblast response, rapid cell spreading, higher stiffness, proliferation, and biodegradability are achieved, with a maximum at 80% DA CS coatings (Smith, Bumgardner, Courtney, Smeltzer, & Haggard, 2010). Drug-

eluting CS coatings containing antimicrobial drugs, chlorohexidine digluconate and tetracycline were also solutions cast on Ti substrate (Norowski et al., 2011) by silane reaction. The antibacterial activity was tested against *Actinobacillus actinomycetemcomitans* and *Staphylococcus epidermidis*. *In vivo* studies were carried out on Sprague Dawley rats for seven days, and the coatings were found to be cytocompatible. Respective releasing rates for tetracycline and chlorhexidine were 89% off in 7 days and 100% in 2 days. While tetracycline inhibited up to 95%-99.9% pathogens for seven days (nontoxic to human cells), chlorhexidine showed 56%-99.5% inhibition for 1-2 days (toxic to cells). Hamilton et al. (Hamilton et al., 2006) carried out a study to correlate fibroblast attachment and proliferation of Normal Adult human dermal fibroblast (NAHDF) cells with known CS characteristics like DA (76.1, 80.6, 87.4, and 92.3%), MW, residual protein and ash content. Enhanced cell attachment was observed in CS films. No clear relationship between CS characteristic and cell attachment and proliferation was established.

Limitations: Though being simple in approach, the casting method suffers from disadvantages like poor bonding strength between coat and substrate. Thus, heat treatment is also performed in some studies in order to improve bonding strength. Moreover, the solution casting method is not suitable for gel polymer electrolytes and the solutions involving solvents of high boiling point.

2.5 Spin Coating

Spin coating is a method to produce a thin, uniform polymer film on a plane substrate. During the spin coating process, an excess amount of dilute solution is placed on the substrate, and then the substrate is rotated at a desired rotation rate. The liquid flows radially, owing to centrifugal force (Hall, Underhill, & Torkelson, 1998). Theoretical models of Bornside, Scriven and Macosko predict the thickness of films based on the spin speed, nature of polymer solution and the solvent. Thickness values (10 nm to 33 µm) within 10 % over the

experimental conditions are predicted using the modelling. Moreover, the high volatility of solvent and the initial polymer solution concentration under predicts the thickness, whereas the lower values over predict. The centrifugal and viscous forces, solute diffusion and solute concentration, affect the coating mechanics as well (Lawrence, 1988). According to this model, the film thickness depends on the diffusion boundary layer and the spin speed. Figure 5 schematically presents the spin coating process.

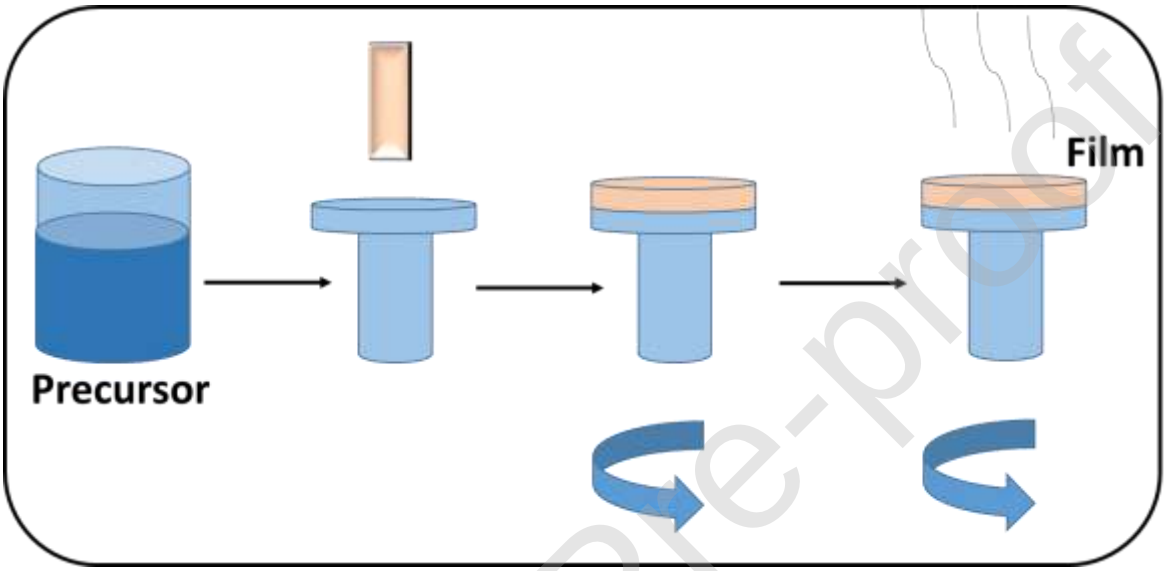

Figure 5. Schematic representation of the spin coating process

Table 5. Publications regarding the CS and CS-based spin coatings of implant materials

Coating composition	Substrate	Nature of the surface modification	Results	Ref
CS/Gel	Ti	Icariin loaded onto TiO_2 nanotubes and then sealed with CS/Gel multilayer coatings	Coating facilitates the adhesion, spreading and proliferation of osteoblasts	(Yanli Zhang et al., 2016)
CS	Ti	Selenium electrodeposition and CS spin coating	TNT-Se-CS substrates promoted biological functions of healthy osteoblasts and inhibited the growth of cancerous osteoblasts with good antibacterial property	(X. Chen et al., 2013)
HAp/CS	316L SS	Deposition of silicon modified HAp composite	Coating with enhanced bioactivity and bone-like HAp formation in SBF	(Sutha et al., 2013)
CS	PET-DLA copolyester substrate	Highly concentrated acetic acid enhance the interaction	Thin uniform coating with decreased surface tension	(Niemczyk, Kaczorowski, & El Fray, 2015)

Zhang *et al.* (Yanli Zhang et al., 2016) fabricated iccarin loaded TiO_2 nanotubes and further sealed with CS/Gel composite to develop a coating on Ti substrates by an electrochemical anodization method followed by spin coating. Iccarin was loaded onto nanotube arrays by physical adsorption, and multilayers of CS/Gel composite are spin-coated on the nanotube specimen. The biocompatible coatings facilitated initial adhesion, spreading and proliferation of osteoblasts. It also regulated the iccarin release profile and avoided the disadvantages of large diameter arrays. Through gradual degradation of multilayers, iccarin stimulated the osteoblast bioactivity and promoted osseointegration. Chen and coworkers also applied the combined procedure of electrodeposition and spin coating to develop selenium-deposited and CS-coated TiO_2 nanotubes(X. Chen et al., 2013). The authors particularly chose selenium due to its anticancer activity as the final application targeted was developing an antibacterial and anticancer implantable device. Such selenium-deposited CS coated TiO_2 nanotube substrates showed a better cellular response and antibacterial behaviour towards osteoblast cells isolated from neonatal rats. TiO_2 nanotubes-Se-CS substrates also enhanced the biological functions of healthy osteoblasts and repressed the growth of cancerous osteoblasts.

In another method of the two-step coating process, silicon substituted HAp nanoparticles with Ca/P ratio of 1.58–1.70 are developed for coating the 316L SS (Sutha et al., 2013). As the amount of Si increased from 0 to 1.6 wt.%, the particle morphology varied from spherical to rod shape. Spin coating of CS blended silicon-HAp produced smooth and uniform coating over the steel substrate without any cracks or pores. The increased Si concentration results in better corrosion resistance of the substrate with increased apatite formation ability and better bioactivity. At one wt. % of silicon-HAp/CS-coated SS implant, the corrosion resistance was the maximum and so as the long term biostability. In addition to

the apatite formation, antibacterial activity and bioactivity are also good at this concentration, and the composite is useful in implant application.

In addition to the numerous functionalization techniques, to enhance the coat-substrate interactions, CS solvent concentration was also varied to optimize its significance on the coating efficiency (Niemczyk et al., 2015). CS was dissolved in high concentration AA (50 wt.%), which increased the amino group protonation in CS and enhanced the coat-substrate interaction. The coated PET-DLA copolyester substrate was also modified by surface oxidation, to generate surface functional groups and thus the interfacial interactions. Finally, uniform thin coatings with 5 µm thickness were produced on the substrate by spin coating. Thus, it is concluded that the spin coating method is advantageous for developing thin films of organic/inorganic compounds on substrates for biomedical applications, as the variable coating thickness can easily be achieved by changing spin speed. It is also a low cost and fast operating process.

Limitations: Though the spin coating possesses many advantages such as fewer materials loss compared to vapour-phase deposition, simple and easy processing, and low cost in less deposition time, the method possesses many disadvantages as well. During spin-coating, there is a higher chance of contamination such as humidity, solvent, oxygen etc. It is hard to control the homogeneity of deposition and also to create multilayers, especially more than a 2-layer deposition. In addition, it is extremely difficult to generate thin films of less than 10 nm thickness. However, depending on the nature of the application and the coating films, this method is widely adopted for the polymer solution coating.

2.6 Electrospinning

Electrospinning is a fibre generating technique in which the polymer solution is ejected through a syringe pump by applying a high voltage. The fibres are directed to a collector,

fixed at a specific distance from the syringe tip. Figure 6 schematically shows the electrospinning process.

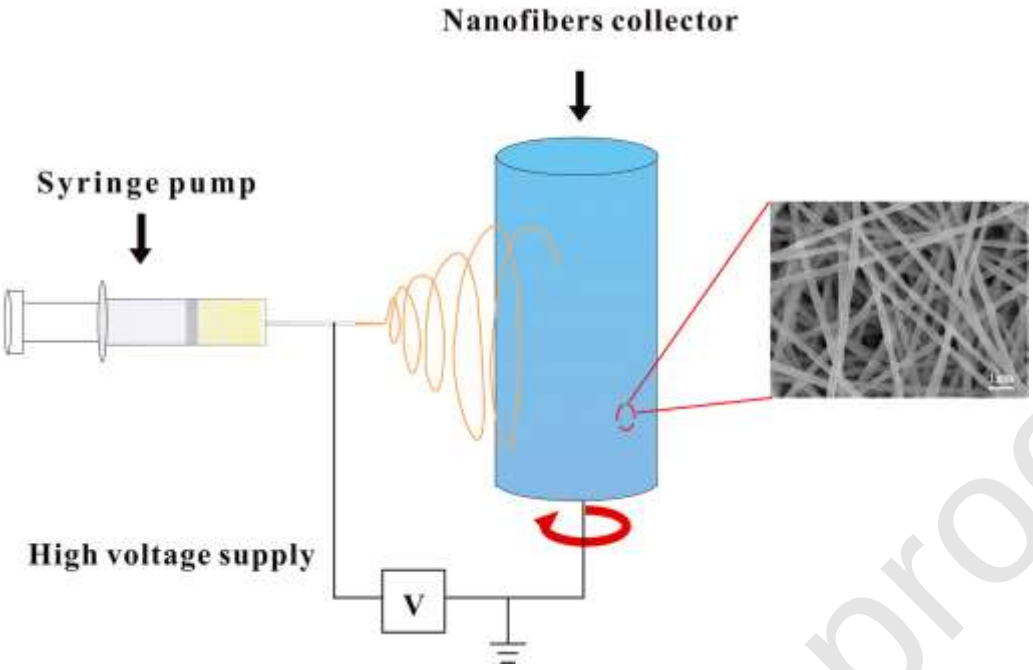

Figure 6. Schematic representation of the electrospinning process(Q. Liu et al., 2020).

Electrospinning is a widely employed process for fibre deposition and also in biomedical applications (Zadeh et al., 2019). Using this technique, Zing *et al.* developed a CA/HAp/CS coating on 304L SS plates with improved corrosion resistance and bioactivity (Zhong et al., 2015a). More specifically, the organic-inorganic coating was made by two steps; including the electrospinning of cellulose acetate on the substrate and further deposition of CS/HAp by dip coating. The coating enhanced the electrochemical resistance of the substrate, and long-term implant applications are targeted by this method. Also, the in vitro biomineralization formation ability of the coatings was evidenced by the formation of bone-like apatite layer, when immersed in SBF.

CS is a widely investigated material for electrospun fibre formation, especially in tissue engineering (Oprea, Ficai, & Andronescu, 2019). According to Zhang and co-workers (Yanzhong Zhang et al., 2008), a model HAp/CS biomimetic nanocomposite nanofibers with

30 wt% spindle-shaped HAp were realized by combining an in situ coprecipitation synthesis with electrospinning. About ten wt.% of ultrahigh molecular weight poly (ethylene oxide) was used as a fibre-forming additive, and the obtained nanofibers demonstrated significant bone formation when biological *in vitro* cell culture with human fetal osteoblast cells was done for 15 days. Stability of electrospun fibres by decreasing degradation during longer immersion times compared to the dip-coating method was also reported (Abdal-hay, Barakat, & Lim, 2013). The *in-vitro* cytocompatibility studies with MC3T3-E1 osteoprogenitor cells demonstrated excellent proliferation, cell adhesion and differentiation, thus making the Mg-based implant materials with an electrospun layer applicable in orthopaedic and cardiovascular devices. Boschetto *et al.* addressed electrospinning as an effective method to develop CS/PEO/BG nanocomposite to coat Ti, and the substrates were tested *in-vitro* against *S. epidermidis* (Boschetto et al., 2020). The nanofiber coated Ti alloy showed reduced bacterial growth, in addition to the mineralization upon exposure to osteoblasts. Thus, enhanced antibacterial and osteoconductive properties lead to orthopaedic applications.

CS/calcium silicate composite was coated on electrospun PLA mat, which formed HAp layers upon soaking in human mesenchymal stem cells culture. Compared to pure PLA, the CS coated materially enhanced cell proliferation and their osteogenesis properties. Moreover, increased Collagen I and fibronectin secretion level were also achieved for the coatings and are applicable in bone tissue engineering (Su et al., 2017).

Limitations: The efficiency of the electrospinning process depends on different parameters such as applied voltage, solution flow rate, the diameter of the needle, needle-collector distance etc. Nature of the polymer solution such as solvent evaporation rate, concentration, and viscosity in addition to the environmental humidity and temperature also affect the efficiency of fibre formation. Therefore, very careful optimization is needed for fibre generation, especially when aimed for biomedical applications and tissue engineering.

Conclusion and prospects

In this review, the relevant fabrication methods of CS coatings on various substrates and correlation with the surface interactions for orthopaedic applications were discussed. As a natural cationic polysaccharide, CS coatings provide biocompatibility, excellent adhesion, and anti-corrosion properties for various techniques such as EPD, LBL, solution casting, dip coating, and spin coating for a number of implant materials including metals, BG, SMA, as well as the combination with other polymers. In addition to the improved corrosion resistance, the CS- coatings were demonstrated to offer mechanical maintainability, convenient degradability, and enhanced osseointegration. Better cell adhesion, improved mineralization, spreading, proliferation and bioactivity were also exhibited with CS-based coatings. The extensive literature reveals that a lot of efforts need to be made to improve the better performance of CS coatings as well as the fabrication techniques for their successful integration of industrial applications. Maintaining stability of CS suspensions (EPD), strengthening the substrate-coating interface (LBL), improvement of bonding strength between coating and substrate (solution casting), managing the consistency of deposition (spin coating) etc. are the crucial areas for intense research yet to be explored. It is also essential to pay more attention to the physicochemical properties of CS suspensions and orthopaedic implant mechanism. Significant efforts must be made as new coating strategies with chitosan and other similar biopolymers to improve the bioactivity and better adhesion. In terms of orthopaedic devices with CS coatings, resilient integration and device-focused infection could be more properly addressed. In addition, an ultimate coating combination must be established with controlled release kinetics and negligible toxicity to host surfaces. However, the CS-based coatings are promising candidates for favouring effective orthopaedic applications; further advancements with long-term protection and the development of "custom made" CS coatings are still progressing.

Acknowledgement

This publication was made possible by Erasmus Mundus Programme Lot 5 (Asia), IMPAKT (International Mobility Programme for Academic and Knowledge Transfer) Project and supported by Qatar university Internal Grant No. IRCC-2020-013. The findings achieved herein are solely the responsibility of the authors.

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