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REVIEW ARTICLE

Prospects of common biomolecules as a coating substances for polymeric biomaterials

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Growing demand for ideal materials in biomedical field not only explores novel approaches, but also examines how existing resources can be modified to fit the requirements. This strategy resulted in the development of various surface modification techniques to improve the biocompatibility of materials already in use. Although various methods are available, the concept of using biological substances for improving the biocompatibility seems rational and effective because of the bio-friendly surface that they present which remains closer in mimicking the innate environment. Some common biomolecules like proteins, lipids, carbohydrates and peptides are extensively applied on material surface through innovative mechanisms. This review will help us to keep abreast of the different types of coating methodologies used and the importance of biological substances as a coating alternative for improving biocompatibility of the polymers. Further, it will also encourage the development of these techniques to modernise materials to make them a more putative choice for biomedical applications.

1. Introduction

Biomaterials are a resourceful choice of materials for an extensive range of applications in both the diagnostic and therapeutic fields. It has been defined by scientists in different ways based on its changing outlook [1-3]. In recent days, the advent of new technologies and ground breaking inventions has considerably improved its properties. Typically, it can be defined as materials which can virtually provide a natural environment to assist the rehabilitation of biological systems or even replace the entire system itself. Biomaterials have a strong reputation in the field of tissue engineering, clinical devices, drug delivery systems, medical implants, biosensors, cosmetics and food industries [4, 5]. Hence, the total market value of biomaterials-based industries are predicted to exceed $88.4 billion by 2017 from a current value of $58.1 billion. Further, every year USA spends 7-8% of total global healthcare outgoings exclusively for biomaterial related usages alone [6]. Meanwhile, in coming years the demand for promising biomaterials is anticipated to surge radically due to increasing numbers of diseased persons. So, biomaterials have a significant future in both medical and commercial fields.

Essentially, biomaterials can be classified into three groups based on their origin and applications as 1. Synthetic materials, 2. Naturally derived, 3. Semisynthetic or hybrid materials. Among the above, synthetic materials such as metals, ceramics, polymers and composites are most commonly used for various biomedical applications. The unique atomic structure of metals offers them better strength and admirable properties which made them us a reliable choice for widespread load bearing usages. However, the problem of corrosion associated with metals limits their utility. On the other hand, ceramics emerged as desirable biomaterials because of its captivating bioactive, bio-inert and biodegradable properties. They have been used in several applications in the dental field; though the poor mechanical characteristics associated with ceramics like brittleness and low strength, made them unsuitable for further exploitation. Afterwards, polymers gained greater attention than other materials because of their versatility and easy to tailor nature. Presently, polymers are reported to be the most promising materials among all the other types. The second category includes naturally available common biological substances like collagen, heparin, proteins, peptides, carbohydrates, bio-ceramics, etc., which have been utilized for both surface coating and material synthesis. Though materials completely made of natural substances possess fascinating biocompatible properties they fail in several aspects because of poor mechanical properties. In order to avoid that complication, natural materials are widely coupled with artificial synthetic substances which falls under the third category [5].

The longevity of an implant inside the human body is dependent on its ability to avoid creating any adverse

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reactions or damaging the surrounding environment which chiefly banks on the biocompatibility of materials used. But the biocompatibility of a material is greatly influenced by its physical, chemical, mechanical and biological characteristics [7]. If we analyse deeply, we can infer the existence of interconnections between all these essential properties and the durability of a biomaterial. In general, the physico-chemical surface properties, such as roughness, hardness, temperature, wettability, surface chemistry, surface reactivity (inert or active) and surface charge, play a crucial role in controlling cell affinity, adhesion, spreading and tissue regeneration. On the other hand, mechanical properties, such as elasticity, yield stress, ductility, toughness, deformation, fatigue, hardness, wear resistance, etc., will determine the ability of a material to withstand the dynamism of the internal environment. So, the presence of appropriate surface, mechanical and biological properties will ensure desired function and longevity of an implant [8-12]. Several strategies have been established to improve both surface and mechanical properties of biomaterials [13].

However, in this review we have focused only on strategies employed to improve surface properties of polymers, especially by means of biological methods. As mentioned, copious techniques were revealed by numerous researches and each has its own pros and cons. Nowadays, the concept of using biological substances is gaining great attention among scientists because of their non-toxic nature, biocompatibility, mimic property, easy availability and ability to hinder adverse reactions like inflammation, platelet adhesion etc., initiated by synthetic polymer surfaces. Furthermore, a surface coated with biological substances will greatly encourage cell adhesion, proliferation and differentiation which has great potential to be utilized for tissue regeneration and wound healing purposes. Hence, we have summarized several works carried out on utilizing common biological substances such as protein, carbohydrates, lipids, peptides and heparin as coating alternatives for several polymers. Meanwhile, we also briefly discuss the benefits and possible usages of biomaterials in the medical arena.

2. Polymers and host mediated complications

Polymers are highly recognised and one of the most fascinating types of biomaterial which have a broad spectrum of benefits. Basically, polymers are a large chain of molecules made of many repeating and covalently bonded subunits known as monomers. In general, the composition, structure and organization of constituent macromolecules influence the properties of a polymer. Several natural, synthetic and semi-synthetic polymers are employed as base elements for manufacturing cosmetics products; meanwhile in medical field polymers are exploited in surgical devices, implants, drug delivery systems, biosensors, bio-adhesives, ocular devices, dental and tissue engineering materials [12-20]. They also have abundant applications in food and packaging industries as well. The main advantage of polymers compared to metal and ceramics are their ease of manufacturability to produce anticipated shapes such as membranes, films, fibres, gels, sheets, capsules, etc. In addition, they can be easily tailored with desired properties in minimum cost. Currently, a wide variety of polymers are available in the market in the form of biodegradable, bio-adhesive and bio-responsive materials. Further, the advent of several modern explorations has paved the way for exploitation of polymers in the form of hydrogels, sponges, nanofibers, nanocomposites, nanoparticles, nano-capsules etc., [14]. Although polymers express versatile properties and admirable mechanics, they fail on numerous occasions because of their poor surface characteristics which activate undesired host-mediated reactions.

2.1 Host reactions initiated by naive polymer surface

All the materials, including polymers, which are intended to use in medical devices or prostheses experience a sequence of reactions when placed inside the human body. Irrespective of the location of the implant the tissue response remains the same and it includes the steps normally observed after injury, such as blood components activation, coagulation, inflammation, wound healing responses and foreign body reaction, which eventually leads to fibrous encapsulation of the prosthesis. However, the degree of host reaction is reported to vary among individuals and commences within 2-3 weeks after the appliance.

The process starts off with agitation in homeostatic conditions, triggered in response to the tissue damage followed by the implantation of a prosthesis or biomaterial. Successively, the defence system initiates cellular cascades to promote the process of wound healing. In general, the extent of subsequent reactions such as blood interaction, inflammation, provisional matrix formation, etc. depends on the magnitude of the injury caused by a biomaterial. Soon after the occurrence of injury, various components present in the blood rush into the leakage site which in due course causes thrombus formation and inflammation. Usually, when the implant comes in contact with blood its surface will be topped by the plasma proteins thereby creating an ambient environment for platelets to adhere; followed by aggregation and release of several thrombogenic factors. Furthermore, these released factors will activate series of coagulation pathways such as intrinsic, extrinsic, complement, fibrinolytic and the kinin-generating system which ultimately ends in thrombus formation (figure 1). In brief, the intrinsic and extrinsic are the most important one, especially the intrinsic coagulation system because its activation is triggered upon the encounter with foreign materials; hence it is also called as contact activation. On the other hand, the extrinsic pathway is driven by the tissue factors released from the site of wound. However, in both cases the activated protein factors will ultimately convert the inactive prothrombin to thrombin which in turn produce clot by activating fibrinogen into fibrous fibrin. Meanwhile, the 'factor XII a' activated through intrinsic pathway will also

involves in the conversion of pre-kallikrein into kallikrein, which subsequently yield bradykinin from high and low molecular weight kinins. Then, the bradykinin receptors (B1 & B2) will cause the vasodilation to restrict the blood flow in the damaged vessel and it also plays a crucial role in triggering inflammation process. And, the possible pathogens attack will be defended by the activation of complement system. The biomolecules triggered through this system will involves in lysis of microbes and damaged cells, moreover it will also release series of inflammatory proteins to kick start the inflammation. These reactions will take place within several seconds to a few minutes. Whilst the activation of coagulation pathway, platelets, inflammatory products and formation of fibrin immediately leads to development of the provisional matrix at the site of the prosthesis. The matrix which is formed after blood-mediated reactions (i.e) activation of factor XII or the release of tissue factors, is reported to control successive processes involved in wound healing by releasing mitogens, chemoattractants, cytokines and other growth promoting factors. However, the role of the matrix on the biomaterial surface has not yet been clearly determined; so far it is reported to play a key role in controlling cell adhesion and proliferation [15, 16].

Meanwhile, the accumulation of blood cells, platelets and clots at the site of injury cause inflammation. It is one of the essential processes of healing, since it serves as a scavenging agent to take away harmful microbes and remains present at the implant site. It also promotes reactions to replace injured tissue by regenerating parenchymal and fibroblast cells. Classically, the inflammation occurring at the site of prosthesis is categorized into two types based on the time of occurrence, as acute and chronic inflammation. Both the acute and chronic inflammation process are involved in the activation of several factors which consecutively form granulation tissues followed by proliferation of fibroblasts and endothelial cells. Depending on the degree of injury, the granulation tissue is probably seen at the site after 3-5 days following implantation and it is also considered to be the precursor of fibrous capsule formation. Typically, the final stage of the healing process is mainly composed of macrophage fusion, foreign body giant cell formation and fibrin encapsulation. As far as biomaterials are concerned, foreign body giant cell and fibrin formation are reported to be crucial parts since most of the rejections are thought to commence in this stage. In general, the adhered macrophages and foreign body giant cells on the biomaterial surface form a microenvironment and are also reported to release several degradation products like reactive oxygen species (ROS), degradative enzymes and acids. If the prosthesis lacks significant physico-chemical properties, its surface will gradually degraded by the action of the above substances. Consequently, it makes the material lose its mechanical strength which eventually ends in failure of the device or prosthesis [10, 17-20].

The above summary is just an overview of the possible host reactions initiated by all type of biomaterials including polymers and the complete process is clearly discussed in the articles [15-20]. Further, the intensity and occurrence of host reactions depend on the size, shape, chemical and physical properties of polymeric material. So, in order to avoid the above complications the implants or prostheses made of polymers are subjected to appropriate surface modification techniques which will be detailed consecutively.

3. Surface Modification of polymers

Basically for improving the biocompatibility of polymers, the following surface modification strategies have been proposed: Physico-chemical methods, 2. Mechanical methods and 3. Biological methods. The first method (i.e.) physico-chemical modification is mainly involved the use of harsh chemicals like acids, active gases or radiation for improving the surface characteristics. On the other hand, the mechanical modification is achieved through deploying roughening techniques, etching, etc. whilst, in biological method, common biomolecules like proteins, peptides, ligands, receptor, drugs, and lipids, etc., will be applied on material surface via one of the following approaches namely physical adsorption, self-crosslinking and chemical conjugation [21]. The avoidance of dangerous chemicals and the usage of eco-friendly substances has made the biological method as a more attractive choice for improving the biocompatibility of polymers. In addition, studies reported so far have delineated distinct advantages and also inferred to provide the polymer with better superficial properties. Hence, in the following sessions a detailed review of the works published on utilizing common biomolecules like proteins, peptides, lipids and carbohydrates for coating various synthetic polymers is presented.

3.1 Polymers coated with proteins

Proteins are the large, complex biological molecules formed by the combination of one or more chains of amino acid residues. Proteins are highly essential for the regulation of cell structure, function and regeneration of tissues and organs. They also play a vital role in catalysing metabolic reactions, responding to stimuli, replication of DNA and transporting molecules from one location to another [22]. There are about 500 amino acids are explored and few of them will combine together to make a protein [23]. The structure and function of each protein are decided by the sequence of amino acids [24]. Proteins are one of the eminent biological substances which have been widely utilized for surface treatment, especially to inhibit platelet adhesion and to promote tissue regeneration [25]. Basically, proteins present in the blood can be classified as plasma and extracellular matrix (ECM) proteins which includes albumin, fibrinogen, globulin and glycoproteins like collagen, elastin, fibronectin, vitronectin, laminin, etc. These biomolecules have high affinity towards synthetic surfaces like polymers. Based on the type of protein adsorbed i.e. serum or ECM protein, the blood and biocompatibility of the material vary drastically. As mentioned, protein adsorption is a complex series of biochemical and biophysical phenomena which varies

depending on the surface properties, affinity of particular proteins and certain *in vivo* factors. Most of the time, protein adsorption will lead to undesirable platelet activation, coagulation factors release, complement cascade initiation, inflammatory reactions and microbial adhesion. However, passivating of polymer surfaces have been performed to encourage the absorption of specific proteins which has the ability to promote regeneration process, mainly ECM related [26]. So, to achieve the above objective and to improve the biocompatibility of polymer, protein itself has been reported to be utilized as a coating material.

3.1.1 Protein immobilization for improving hemocompatibility

Steinberg et al. examined the ability of protein-coated polymer surfaces to influence the interaction and adhesion of human erythrocytes. Commonly used polymers such as polyurethane (PU), polystyrene (PS), segmented polystyrene (SPS), polyethylene terephthalate (PET) were coated with five different human serum proteins (albumin, fibrinogen, immunoglobulin G, fibronectin, and transferrin) in different concentrations ranging from 0.01 mg/mL to 50 mg/ml. Basically the polymer surfaces selected had a diverse wettability range, which helped to delineate the importance of surface properties in promoting desired protein adsorption or vice-versa. Interestingly, the erythrocytes displayed a distinct degree of adhesion and spreading on different protein-coated surfaces with least adhesion noted on fibrinogen-coated samples. However, the coated surfaces showed less erythrocyte adhesion when compared to its counterpart i.e pristine ones. This expressed the necessity of specific protein adsorption on solid surfaces to avert the damage to blood cells and to avoid the release of coagulation factors followed by cell damage. Hence, blood cell adhesion and spreading is concluded to be influenced by protein adsorption as well as the surface properties of the desired polymers [27]. In addition, the role of plasma proteins in influencing platelet adhesion was methodically displayed by Neumann and co-workers. In this work, the researchers coated plasma proteins on several synthetic polymers (sulfonated polystyrene, acetal resin, Teflon FEP, polystyrene) and glass surfaces (control), respectively. These materials have different degrees of wettability ranging from highly hydrophobic to hydrophilic surfaces, alike previous study. The polymer surfaces were initially made smooth and homogenous using special techniques. Further, the protein coating was carried out using different methods based on the surface energy of the material. For instance, the acetal resin and Teflon FEP were modified using a heat-pressing method; the polystyrene surface was prepared by centrifugal spinning and other materials were simply immersed in the solution of desired protein to be used as coating. The protein-coated polymers possessed different contact angles, displaying either an increase or decrease in wettability when compared to the pristine substrate. The polystyrene polymers showed increased hydrophobicity in all types of plasma protein coating whereas for acetal resin (56±9˚) and Teflon FEP (73±2˚) the contact angle decreased. Prior to protein coating, platelet adhesion was inferred to depend on the wettability of the polymer surface, with hydrophobic substrates expressing minimal platelet adhesion. But after coating, the fibrinogen-glazed surfaces encouraged platelet adhesion and aggregation irrespective of the material surface property whereas the lowest level of adhesion was reported on albumin-fixed samples [28].

Accordingly, in another study Chang et al. displayed adhesion pattern of protein-free pig platelets on bare polymers, glass surfaces (control) and well-defined protein coated polymer surfaces. The reactivity of platelets was normalized using appropriate techniques prior to the adhesion test. Regardless of underlying surfaces, the protein adsorption is thought to reduce platelet adhesion and aggregation. The proteins albumin and fibrinogen exhibited an identical pattern of platelet aversion on different substrates used, in contrast to previous study which may due to the difference in amount of protein coated. For bare polymers the adhesion was observed to be influenced by surface properties such as wettability and also the surface chemical structure meanwhile after coating it chiefly depend on the type of protein adsorbed on the polymer surface. Hence, it can be inferred that platelet adhesion and corresponding blood coagulation activation can be greatly influenced by both type of protein adhered and its quantity which eventually rest on the surface properties. So, appropriate physico-chemical properties are essential for both specific protein adsorption and designing of biocompatible materials [29].

3.1.2 Coating of ECM proteins to improve biocompatibility

ECM glycoproteins play a pivotal role in controlling cell behaviours like adhesion, migration, differentiation and proliferation in the tissue. Therefore, the functionalization of polymer surfaces to display commendable biocompatibility mostly targets ECM proteins, especially the fibronectin (FN) since it is reported to chiefly mediate cellular interaction with biomaterials. Recently, Bax et al. proposed covalent attachment of ECM protein to polytetrafluorethylene (PTFE) by subjecting it to plasma immersion ion implantation (PIII) technique. PTFE is one of the commonly used polymers for various medical applications; however because of its hydrophobic nature it often fails to support cell adhesion and proliferation. Hence to overcome the wettability problem and to prepare the surface for promoting regeneration they glazed it with tropoelastin, a type of cell adhesive protein and a major component of ECM. Elastin basically provides elasticity to muscles and it is chiefly present in skin, lung, cartilage etc. To improve the reactivity and enable covalent attachment of tropoelastin, the PTFE surfaces were modified by the PIII approach and it was incubated in tropoelastin solution along with untreated PTFE at 4˚C for 16 h. The ELISA test showed the presence of tropoelastin, and on both untreated and PIII-modified PTFE surfaces the amount of tropoelastin bound was

inferred to be similar. However after washing with SDS, more protein was retained on the PIII-treated surface when compared to the pristine one. Moreover, the presence of characteristic amide peaks in the ATR-FTIR spectrum confirmed the above conclusion and also portrayed the covalent attachment of protein to PIII-subjected PTFE surfaces. Interestingly even after 264 days, the stored tropoelastin-glazed PTFE surfaces maintained more than 70% of protein adhered when compared to freshly prepared surfaces which indicate the presence of strong covalent interaction. In the interim, the adhesion of human dermal fibroblasts (HDF) was reported to increase on protein-coated PTFE; while the spreading and adhesion increased with coating concentration of tropoelastin and the maximum adhesion of 92.3±1.9% was noted at a concentration of 2 mg/ml. Like protein retainment, the modified PTFE surface exhibited a significant amount of HDF adhesion even after 264 days. In addition the wettability of PTFE was improved after the coating process, the contact angle of 114.4±3˚ was noted on pristine substrate fell to a hydrophilic level however it mainly depended on the length of exposure to PIII treatment rather than tropoelastin concentration [30].

In another study, Chen et al. studied the effects of ECM protein (laminin, fibronectin, vitronectin, type I, type II and type IV collagen) coated polystyrene. The coating of biomolecules was carried out by freezing the polystyrene plates immersed in protein solution at -4˚C for the period of 12 h. Then, rat islet cells (RIN-5F) were cultured on control and coated surfaces in serum-free medium for a period of 8 h and the morphological changes were observed through phase-contrast microscopy. The cells were observed to significantly spread on laminin-coated PS whereas in the presence of a fibronectin they showed moderate spread; but on collagen-coated surface the cells were noted to be aggregated. However, protein coated surfaces such as cationic polyelectrolyte (poly (L-lysine), poly (allylamine)) and control PS demonstrated no apparent spread. Even in the absence of serum, laminin-coated PS showed increased adhesion when compared to other counterparts with the lowest adhesion on poly (allylamine)-glazed PS. Accordingly, the proliferation rate calculated at different time periods of 30 min, 24 h and 48 h also supported the above outcomes by showing enhanced growth on laminin surfaces. In addition, type IV collagen also induced cell proliferation to some extent whereas fibronectin promoted only adhesion not proliferation. But those coated with type I, type II, vitronectin and control surfaces illustrated same degree of effects [31].

In a different study, Zhang et al. reveals the role of coating method in influencing resultant biocompatibility of the polymers. Hence, they coated PET surface with fibronectin by using two different immobilization techniques, adsorption and conjugation. The conjugation process was initiated by incubating the PET surface in ethylenediamine solution for 40 min at 50°C and this was followed by glutaraldehyde-assisted immobilization of fibronectin. During adsorption, fibronectin was added to the glutaraldehyde-conjugated PET surface and it was allowed to react for a period of 24 h. The quantification analysis showed the presence of higher adsorption of fibronectin while using the conjugation technique compared with the adsorption method. In the conjugated approach, multiple layers of fibronectin were observed to form and a stable immobilization of 918±134 ng/cm^2 was achieved while in adsorption method fibronectin grafting reached saturation at a low level of 400 ng/cm^2 itself. The wettability of modified PET was affected by the type of method used and the resulting fibronectin concentration achieved; the contact angle of unmodified PET (74.1°±2.6°) was reduced to 56.7°±2.0° in the conjugation and 61.8°±2.1° in the adsorption technique, respectively. In addition to wettability, surface roughness also greatly differed for the coating technique used. In adsorption method, fibronectin was observed to be evenly distributed with Ra value of 11.2 nm but in conjugation the coating was closely packed with an average roughness of 119 nm. Micro-BC and ELISA tests showed the bioactivity was highly maintained in the adsorption method compared with the conjugation approach, irrespective of the amount of fibronectin immobilized. Accordingly, the baby hamster kidney 21 (BHK21) cells adhered and proliferated well on fibronectin glazed surfaces obtained through adsorption method. This study clearly shows the influence of the coating approach and chemicals used in changing the bio-activity, surface properties, adsorption range and ultimately the biocompatibility of desired polymer [32].

Zhu et al. modified the surface of biodegradable poly (L-lactide-co-caprolactone) (PLLC) with fibronectin and collagen respectively. Before starting the grafting process, PLLC was aminolysed to establish the adhesion of proposed proteins and it was cross-linked with glutaraldehyde to avoid aggregation. Then it was immobilized with fibronectin and collagen by incubating in the corresponding solution for a period of 24h at room temperature. However, before introducing the desired proteins the aminolysed PLLC was analysed to check for the presence of free amino groups which are essential for the binding of collagen and fibronectin. The existence of the desired function group (-NH_2) was clearly illustrated using fluorescence agents; further the required time for treatment was optimised using fluorescence intensity. After the coating, the presence of coating substances was confirmed by XPS, which shows variations in surface carbon, oxygen, nitrogen and silicone content of PLLC according to the type of immobilization. Meanwhile, the researchers tested the influence of type of coating method in affecting the water contact angle; for that collagen and fibronectin were attached by both normal physical adsorption and covalent bonding methods. Even though the protein-coated samples showed an excellent improvement in wettability compared with the control (83.47±1.1), when considering both methods the covalent bonding approach yielded much lower contact angles and the coating was also observed to be homogenous. Moreover the smooth muscle cells (SMC), fibroblast and epithelial cells (EC) derived from porcine esophagus were separately cultured on both coated and control PLLC. Initially,

all the surfaces exhibited better cell attachment and MTT activity; however after 12 days the control PLLC shows poor/no growth of SMC cells whereas both protein-coated polymers exhibited greater DNA content which illustrates free adhesion and proliferation. In contrast, for fibroblasts the unmodified PLLC was inferred to be more suitable and for endothelial cells the fibronectin-glazed PLLC surface was found to be more suitable [33].

In view of that, Qu et al. (2005) modified the surface of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) copolyester by plasma assisted fibronectin coating. The polyester films were manufactured by solution casting method and it is followed by the treatment of ammonia plasma (50W) for the period of 120 s. Then, substrates were coated by incubating both plasma treated (P-PHBHHx) and pristine copolyester in the solution of 10 mg/cm^2 (fibronectin) at 37˚C for the period of 1 h. Due to plasma treatment, the percentage of nitrogen (0.92 %) and oxygen (27.82 %) were increased on PHBHHx copolyester surface which shows the formation of new bonds whereas on fibronectin coated surfaces the nitrogen level (1.46 %) further increased which exhibits the presence of coating. Moreover, the surface modification also results in the addition of polar groups which significantly increased the wettability of pristine copolyester from 81.77˚ to 55.9˚ in Fn-PHBHHx (fibronectin coated) and 25.37˚ PFn-PHBHHx (plasma assisted fibronectin coated) substrates respectively. This trend was followed in surface energy improvement where the fibronectin glazed substrates illustrated greater energy which shows the ability of FN in modifying the hydrophobicity of desired polymers further it also confirmed the advantages of covalent bonding. Because of the enhanced surface properties Human umbilical vein endothelial cells (HUVECs) were observed to adhere and proliferate well on the modified surface especially on PFn-PHBHHx when compared to P-PHBHHx and Fn-PHBHHx. Conversely for Rabbit aorta smooth muscle cells (SMCs), the modified surfaces haven't exhibited any significant difference when compared to pristine which shows that modified fibronectin coated substrates are more suitable for HUVECs regeneration. Meanwhile the microscopic observations also indicated higher affinity of modified copolyester surfaces towards HUVECs by showing inappropriate morphology of cells on pristine copolyester. Interestingly after 5 days of incubation, the fibronectin concentration on modified surface was inferred to increase (119.6740.9 to 474.4728.3 ng/cm^2), it shows the ability of cultured HUVECs to synthesis ECM protein which required for differentiation. In a different work, Van et al. (1987) compared the ability of both plasma and ECM protein coated surfaces to improve the adhesion and proliferation of human endothelial cells (HEC). Initially, they observed the adhesion pattern of HEC on bare hydrophilic polymeric control TCPS (tissue culture polystyrene) and hydrophobic polymers such as PETP (polyethyleneterephthalate), FEP (fluoro-ethylene propylene copolymer). In contrast to TCPS, hydrophobic polymers PETP (Dacron) and FEP showed reduced or no adhesion of HEC cells. Then the same experiment was repeated after coating both polymer substrates with different proteins - albumin, high density lipoprotein, immunoglobulin G and fibronectin. Surprisingly, the fibronectin-coated hydrophobic PETP and FEP polymers promoted enhanced adhesion and proliferation of HEC cells, which was also confirmed by MTT assay. However, the hydrophobic polymers coated with albumin, high density lipoprotein and immunoglobulin G inhibited cell adhesion in a similar way to bare polymers. This clearly illustrates the ability of protein coating to boost the biocompatibility of even complicated hydrophobic polymers. In the interim, it also discriminates the nature of different proteins to aid regeneration and vice-versa [34].

The above studies clearly portrays that the biocompatibility of modified polymer surface is depend on the type of protein, coating approach employed and the corresponding physico-chemical properties. In many cases, the covalent bonding noted to improve the coating properties such as spreading, thickness, strength etc., but it often results in damaging the bioactivity of the protein which eventually affects the expected outcome. So, notable care should be taken while choosing the chemicals and coating method for better results.

3.1.3 Protein coating for preventing immune system attack

In addition to blood compatibility, the interface of polymers with neutrophils is also reported to play a vital role in successful functioning of desired implants, since it is thought to cause implant deterioration and peripheral tissue damage. Its adhesion to the biomaterial surface has been recorded as eliciting clinical complications during cardiopulmonary bypass, hemodialysis, oxygenators, vascular grafts and ventricular assist devices. In order to avoid this complication Yue et al. suggested the coating of human kininogen, a precursor form of globulin type of plasma protein, to a biomaterial surface. They coated kininogen on four differently charged polyurethane (PU) substrates namely non-charged (PU), cationic (NR_4^+), anionic (SO_3^-) and zwitterionic (GPC) respectively to study neutrophil adhesion pattern. The cationic polymer NR_4 was obtained by quaternizing PU using chain extender 3-trimethylamino-1,2-propanediol iodide, the anionic SO_3 was derived by replacing urethane hydrogen with propyl sulfonate through bimolecular nucleophilic substitution; whereas the GPC was synthesized by using chain extender L-a-glycerophosphorylcholine. Initially, the coating was performed by immersing the desired polymer surfaces in a solution of radiolabelled human kininogen for 6 h under optimum conditions. Then, the adsorption studies revealed the coating was influenced by the concentration of solution and the charges present on PU. The cationic PU displayed higher protein adsorption followed by neutral PU, anionic (SO_3^-) and zwitterionic polyurethane (GPC); meanwhile the presence of homogenous coating was observed through an optical microscope. Accordingly, the neutrophil adhesion was minimal on human kininogen-coated anionic PU and it highly differed

among different polymers used based on the concentration of kininogen solution in which the substrates were initially immersed. So, from the above observation it is inferred that adsorption of kininogen is dependent on the surface charge of the polymer. Moreover, the coated substrates, especially the anionic PU, can be selected to avoid the problem of native tissue injury and implant failure caused by neutrophil adhesion and activation [35].

3.1.4 Protein coating to promote antimicrobial property

Besides plasma and ECM proteins, other natural proteins were also extensively scrutinised using various synthetic polymers especially for antimicrobial applications. Shi et al. coated the surfaces of four polymers (PMMA, silicone, tecoflex-polyurethane (PU) and polystyrene) with bovine submaxillary gland mucin (BSM) which was initially purified by size exclusion chromatography (SEC) and successively characterized with Polyacrylamide gel electrophoresis (PAGE). Mucin is a group of large glycoproteins, one of the major components of the mucous layer which covers the luminal surface of epithelial organs. It has a unique structure consisting of a peptide backbone which densely packed with carbohydrate side chains. This makes them easily adhere to super hydrophobic surfaces; meanwhile its aqueous interaction with the outer environment will be facilitated by the carbohydrate chains. Firstly, the polymer substrates were glazed with BSM by incubating identical pieces in purified mucin solution on rotary shakers at room temperature for 24 h. PAGE analysis showed the presence of subsequent peptide and carbohydrate chains on the polymer surface after coating and the presence of BSM was further confirmed by amino acid assay. Further, the protein uptake assay showed increased adsorption of PMMA followed by PS, PU and silicone surfaces respectively. Interestingly, after mucin coating, the hydrophobic polymers were turned into super hydrophilic surfaces especially in the case of PMMA samples; the water contact angle was reduced to 5˚ from 75.0±1.2° observed before coating. The rejection of an implant due to bacterial infection is another critical scenario reported in the clinical arena. Few biomaterials encourage bacterial adhesion, and on hydrophobic surfaces the lipids and proteins present in the bacterial membrane are reported to establish strong bonds. Inspired by the result of contact angle studies, they used *Staphylococcus aureus* and CNS *S. epidermidis* bacterial strains to demonstrate the ability of coated surfaces to avoid adhesion. As expected, the formation of bacterial colonies was inferred to be at negligible levels on BSM-coated polymers when compared to their pristine hydrophobic counterparts. Moreover, the inhibition pattern is in accordance with the adsorption of BSM reported on the polymer surface. It clearly expresses the plausible application of mucin coating to improve surface energy and antibacterial properties of polymeric biomaterials [36]. Recently, efforts have been taken to use specific ligands for inducing anticipated protein absorption on the polymer surface under *in vivo* conditions rather than *in vitro* coating of protein to increase the biocompatibility [37].

Besides, proteins have also been used as a mediator for safe and selective of binding of various biomolecules. Recently, Yang et al. has proposed a novel method for biomolecules immobilization on non-fouling surfaces by utilizing rapid phase transition property of lysozyme. Through this approach they reported to avoid pre-activation of material surface and typical problems associated with current methods. In their initial studies, they have demonstrated selective binding of avidin on EG3 self-assembled monolayer (SAM) and they also replicated the same effect in other non-fouling coatings such as poly(ethylene glycol) (PEG), bovine serum albumin (BSA), and dextran, using a “superglue” (i.e) dissolved lysozyme in commonly used buffer solution. The inferred binding was highly stable even under extensive ultrasonic washing and selective towards lysozyme by avoiding other proteins like fibrinogen, this approach can be plausibly preferred for developing cost-effective biochips [38]. Accordingly, they also proposed the usage of phase-transited proteins as a universal surface modification tool by demonstrating the stable binding of synthesized lysozyme product on different substrates namely metals, oxides, semiconductors, and polymers. This coating observed to increase the hydrophilicity of underlying material and also expressed significant corrosion resistance (metals) [39]. Using this approach it is possible to achieve the binding of other desired biomolecules as well. Later they also reported the potential use of this phase transited lysozyme layer in accomplishing soft landing of cell-sized vesicles. Actually, the interaction of lipid vesicles with solid substrate is one of the long standing research in medical field. The lipid vesicles are divided as small (SUVs), large (LUVs) and gaint unilamellar vesicles (GUVs); extensive approaches have been proposed to achieve safe binding of SUVs and LUVs. But in GUVs, a series of hurdles were reported due to its less stability, which frequently results in quick deformation and rupture on solid surface. To rectify this issue, they have employed the coulombic force mediated interaction between anionic GUVs and positively charged lysozyme substrate by introducing a middle layer containing biomimetic lipid membrane. Through this, a steady capturing and safe landing of GUVs was demonstrated, meanwhile a controlled release was also achieved under mild heat stimuli [40]. These studies portrays the requirement of more researches need to be carried out for exploring the hidden benefits biomolecules.

3.2 Polymers coated with peptides

In addition to proteins, peptides were also utilized for several coating applications. Generally, the molecules small enough to be synthesized from the fundamental amino acids are called peptides. Amino acids that have been incorporated into peptides are termed residues [41]. Depending on the number of amino acids, peptides are categorized into dipeptides, tri-peptides, tetra-peptides, and so on [42]. A polypeptide is one type of peptide which is generally long, continuous and

unbranched. Peptides are distinct from proteins on the basis of size and a protein is obtained from the combination of one or more polypeptides [43]. And among various peptides, the RGD (arginine-glycine-aspartate) is extensively studied since it is the integral component of most of the ECM proteins.

3.2.1 Peptide coating to improve biocompatibility and to promote tissue regeneration

Recently, Wang and co-workers, studied the adhesion of endothelial cells taken from different parts of the human system on peptide-incorporated polymers. They isolated human umbilical vein endothelial cells (HUVECs), cord blood endothelial colony-forming cells (ECFCs), and human blood outgrowth endothelial cells (HBOECs). RGD peptide was incorporated during the functionalization of hydrophilic polymers H20, H20P15NHSRGD and H20P15NHSRGE respectively synthesized by combining different combinations of hexyl methacrylate, methyl methacrylate, poly (ethylene glycol) methacrylate (PEGMA) etc., as polymeric materials. Of the above three, H20 possessed the highest contact angle of 80.1±2.1˚ while the other two polymeric materials were found to be more hydrophilic, with contact angles of 55.3±2.5˚ and 54.2±1.7˚, respectively. The NMR spectrum revealed unbound mixing of used polymeric materials and the amino acid assay expressed the amount of RGD peptide adhered is more on H20P15NHSRG (5.7±0.5 nmol peptide/mg polymer). Then, the different endothelial cells were cultured separately on the polymer surfaces. The results showed increased adhesion of endothelial cells on RGD-linked polymer surface compared to polymers without adhesive peptides. The proliferation rate was highly influenced by surface properties, the presence of RGD peptide and the shear rates. They also suggested that ECFCs and HBOEC adhesion was higher when compared to HUVECs hence these adhesive peptide coated polymers can be exploited as a promising source for *in vivo* seeding and recruiting of cells for vascular regeneration [44].

In a different study, the surface modification of PDMS with cell adhesive peptides was demonstrated by Sheardown et al. for improving biocompatibility. Firstly, the PDMS surface was functionalized with PEO spacer and then it was immersed in PBS solution containing cell adhesive arginine-glycine-aspartate-serine (RGDS or YIGSR) for 12 h to obtain modified surfaces. The peptide incorporation gives rise to amide bonds further the presence of essential functional groups confirms covalent bonding facilitated by PEO spacer. As expected, the contact angle shows drastic drop after PEO spacer assisted peptide coating, the PDMS surface was turned from hydrophobic (100˚) to super hydrophilic (40˚). Accordingly, the elemental composition analysed through XPS depicted increase in N1s and C1s signal which demonstrates successful incorporation of PEO spacers followed by peptide coating. Though the cellular compatibility was tested through corneal epithelial cells, the cell adhesive peptide coated PDMS is proposed to use as a possible replacement to avoid non-specific protein adsorption [45]. Consequently, Sagnella et al. manufactured a novel heparin and RGD peptide-modified poly (vinyl amine)-based polymer composite. Initially, the heparin binding peptide was synthesized through a solid-phase peptide synthesis method and to facilitate the peptide attachment to PVA backbone, it was coupled with PEO, which resulted in the formation of peptide surfactant polymers HBP1–PEO, HBP2–PEO, xHBP1–PEO, xHBP2–PEO and RGD–PEO, respectively. Then the presence and amount of peptides bound to polymeric material was quantified through FTIR and 1H-NMR techniques. The successful immobilization resulted in a drastic decrease of the contact angle from 110°. While culturing with human pulmonary artery endothelial cells (HPAECs), the above polymers exhibited different adhesion and growing patterns. After a period of 48 h, the cells were found to be healthy on RGD-heparin peptide-coupled polymer and positive control (fibronectin glass surface). In case of HBP1, the proliferation was slow and on HBP2 the cells were observed to be almost lysed or not to have proliferated at all. At all times, the cells present on the surface with both heparin and RGD peptide molecules were inferred to possess a better proliferation rate, which depicted the synergetic ability of multiple peptide-immobilized surfaces to act a promising biomimetic material [46].

Vondele et al. synthesised RGD peptide-modified PLL-g-PEG copolymers for averting non-specific protein adsorption but still making the material suitable for the attachment and spreading of specific cells. The copolymer was synthesized by following a unique protocol and the RGD peptide incorporation was facilitated by PEG spacers. Interestingly, the non-specific protein adsorption increased with the concentration of RGD peptide coupled and the maximum adsorption was found on copolymers containing 58% RGD. The RGD coating can avoid the undesired protein adsorption, even after vigorous washing which portrays formation of strong covalent bonds. While the *in vitro* ability of the synthesised co-polymer in supporting the adhesion of fibroblasts was noted, in the presence of RGD protein the cell were freely adhered and spread. Moreover, adhered cells were also inferred to make a strong bond with the surface and in the presence of RGD enhanced mitochondrial activity was also observed [47]. In a work, Davis and co-workers illustrated the effects of peptide-coated silicone. Silicon-based polymers have been used in various biomedical applications like neural stimulation, implantable encapsulation, drug delivery and biosensors. But the major limitations of these polymers is lack of biocompatibility and poor tissue integration. In order to improve the biocompatibility they coated the surface with bio-peptides. The modification process started with cleaning of the silicone surface with ammonia and H_2O_2 which was followed by the establishment of a series of surface preparation procedures. Finally, the arginylglycylaspartic acid (RGD) was immobilized by incubating in peptide solution with period sonication. Because of the modification techniques, the changes in surface oxygen, nitrogen and carbon content were

delineated by XPS analysis. Further it confirmed the presence of aminosilane-linked RGD peptide film. Meanwhile, the AFM images showed formation of uniform coating with a peak to valley distance of 2.5 nm and retainment of peptide bioactivity even after extensive modification procedures. This RGD-modified silicone supported the adhesion, proliferation and spreading of fibroblast cells; the microscopic images taken after 2 days of incubation showed a three-fold increase in fibroblast population on modified silicon compared with the pristine surface. It is inferred that this was due to the existing receptor sequences of RGD which were noted in several cell adhesive proteins like fibronectin, vitronectin and fibrinogen. Hence RGD-immobilized silicone substrates can be used for cell adhesive and regeneration applications [48].

3.2.2 Peptide coating for bone regeneration

Bone tissue engineering is one of the active fields of research and numerous techniques were explored for achieving better *in vivo* outcomes. Among those, the use of protein growth factors to promote osteoregeneration is reported to be effective and promising approach. The bone morphogenetic proteins (BMP) is one of the preferred drugs for above application, which is currently under clinical trials [49]. However to get significant results, BMP is coupled with biomaterials to avoid the complication of instance burst and to increase local concentration. As a result, Smith et al. modified the surface of thermoreversible Nisopropylacrylamide (NiPAM) polymer to make it more biocompatible and also to enhance the specific adsorption of bone morphogenetic protein-2 (BMP-2). To achieve this requirement they coated the polymer surface with RGD protein with the help of amine-reactive N-acryloxysuccinimide (NASI) groups which yields successful conjugation by utilizing 8.5% of peptide present in incubation solution. The Multipotent C2C12 cells was used to scrutinize the ability of RGD modified surface to induce osteoblastic differentiation. Interestingly, the cell adhesion, spreading and proliferation was reliably higher on RGD coupled NiPAM polymer meanwhile the Coulter counter shows 2.5 to 5 fold increase in proliferation rate . Further, the cell behaviour was inferred to depend on RGD peptide concentration and significant osteoblastic ability was indicated by the presence of higher level of ALP (biomarker) in cultured cells mainly due to BMP-2 protein exposure. In a different work, Kantlehner et al. (2000) proposed the utilization of highly active and cell adhesive αvβ3, αvβ5-integrin-selective peptide c(RGDfK) molecules for eliminating the inertness of medical implants, which frequently results in rejection or failure. They illustrated the importance of proposed biomolecule by coating it on PMMA polymer substrates. Initially the peptide was modified to have acrylamide group so that it can form covalent bonding with PMMA upon coating. In the interim, the in vitro studies exhibited the ability of coated PMMA to attract human osteoblasts cells at a minimum distance of 3.5 nm further the cells on coated surface inferred to have zero percentage of apoptosis even after incubation over a period of 22 d and proliferate by a factor of 10. Accordingly, while implanting the peptide coated PMMA grafts in animal models (rabbit), it promotes faster regeneration of bone tissue than the pristine one [50].

3.2.3 Coating of antimicrobial peptides (AMPs)

In addition to biocompatibility issues, several clinical studies showed that there is a considerable amount of rejection of implants due to microbial infection. A promising biomaterial should possess commendable antimicrobial properties in addition to better biocompatibility. To achieve this requirement, Steven et al. used E14LKK (synthetic peptide) to yield a poly (ethylene) surface with improved antimicrobial activity. Initially the PE surfaces were oxidized and it was grafted with NH2-PEG-NH2 or NH2-PEG-COOH using 1-ethyl-3-(3-aminopropyl)-carbodiimide. NH2-PEG-NH2 was utilized to confirm the grafting and NH2-PEG-COOH was used for peptide immobilization, which involves the incubation of films in E14LKK solution for a period of 24 h at 48˚C with constant agitation. The contact angle of PE (101˚) was decreased to 61˚ following oxidation which was further decreased after peptide immobilization. In addition, the dye adsorption assay confirmed the presence of coating by indicating the increase in acidic and basic groups present on the PE surface. Because of oxidation, O_2 was noted to be increased; in contrast the carbon content had a stable fall and the presence of nitrogen depicted successful surface incorporation of E14LKK peptide. Finally, the antimicrobial activity was assessed using *Escherichia coli* (ATCC 25922) cells; as expected the bacterial growth was reduced to 5.9 $(0.3)^a$ log (cfu/mL) in peptide-glazed surfaces from 9.1 $(0.1)^a$ log (cfu/mL) when compared with controls and it had 3-4 log difference. More interestingly the total number of colonies formed was less than 30 on E14LKK-PE, hence the actual range is reported to be lower [51]. In another study, Gao et al. developed a coating consisting of covalently grafted hydrophilic polymer chains conjugated with an augmented series of antimicrobial peptides. The polymer or the implants coated with the AMPs reported insignificant platelet activation and adhesion. The coated polymers demonstrated excellent activity against microbes; meanwhile the coating had no toxicity towards normal cells. So, they suggested that antimicrobial peptides can be considered as a promising agent to avoid the rejection of implants due to biocompatibility and microbial infections [52].

3.3 Polymers coated with carbohydrates

Any group of organic compounds such as sugars, starches, celluloses, gums which serves as a major energy source in the diet of animals is called carbohydrates. In other words it can be defined as the compounds which are produced by photosynthetic plants and contain only carbon, hydrogen, and oxygen, in the ratio of 1:2:1. The carbohydrates are divided into four chemical groups namely monosaccharides,

disaccharides, oligosaccharides, and polysaccharides. In general, the monosaccharides and disaccharides, are smaller (lower molecular weight) carbohydrates which are commonly referred to as sugars and have different derivatives, each with a vital role to play [53]. Carbohydrates perform numerous roles in living organisms, for instance the polysaccharides serve as the storage house of energy (e.g., starch and glycogen) and as structural components of cells (e.g., cellulose in plants and chitin in arthropods). Furthermore, 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of genetic molecules such as RNA while the related deoxyribose is one of the DNA components [54]. Different types of carbohydrates and their derivatives play some key roles in the immune system, fertilization, preventing pathogenesis and blood clotting. Among the various types of carbohydrate, polysaccharides (chiefly heparin and chitosan) are highly utilized for surface coating for improving blood compatibility, antimicrobial activity and also for drug delivery systems.

3.3.1 Heparin immobilization

Heparin is widely recognised utilized for its excellent anticoagulation properties. Basically, it is a sulfated glycosaminoglycan (polysaccharide) compound which is naturally found in the liver and other tissues to inhibit blood coagulation [55]. Heparin usually stored within the secretory granules of mast cells and released into the vasculature during the time of tissue injury. Apart from inhibiting blood coagulation it also reported to act as a wall at the site of injury to avoid the entry of bacteria and other foreign materials [56]. Hence it is highly utilized in the clinical field for various applications like injectable anticoagulant, blood thinner and natural clot lysis agent [57]. Moreover, heparin has been extensively exploited as coating material for different polymeric implants as well [58]. Various types of techniques have been deployed to immobilize heparin onto commonly used polymers such as ePTFE, PET, polyurethanes, poly (lactic acid), polypyrole and polysulfone [59]. The coating techniques are ranges from simple physical adsorption to covalent side-on or end-on. In the interim, different concentrations of heparin on the surface is found to have consecutive effect on blood and vascular cells, including ECs [60] and SMCs [61].

3.3.1.1 Coating to improve hemocompatibility

Michanetzis et al. immobilized the surface of four commonly used polymers in clinical field with heparin to improve the blood compatibility and also to find the influence of coating procedure on the same. Heparin was coated on polydimethylsiloxane (silicone rubber), polyvinylchloride, polyethylene and polypropylene through direct and indirect method respectively. In a brief, the direct approach involves in three step modification process which starts with pre-surface treatment followed by surface grafting and immobilization by incubating in heparin sodium solution for 48 h. Whilst, in indirect method the coating was performed with the help of coupling agent (glutaraldehyde) and basically the direct method uses functionalization whereas the indirect employ coupling agent to form heparin coating. However, in both cases heparin was attached to respective polymer surfaces through covalent bond. The percentage of heparin immobilized was higher in case of indirect method however in both cases only few amount of total heparin utilized is infer to be coated. Further, the coating process didn't affect the mechanical properties of the polymers, since the coated surfaces exhibited no significant difference in tensile strength. Accordingly, the non-activated partial thromboplastin time was increased on all heparin coated surface compared to control and it wasn't depend on the type of coating approach established. The same trend was noted in platelet adhesion assay, where the heparinized surfaces illustrate better anti-adhesive properties. Moreover, AFM analysis displayed the presence of homogenise coating on samples glazed though indirect method whereas in direct method the heparin was inferred to present in certain places or less uniform. Though the coating method was not observed to cause any significant changes in hemocompatible property, the presence of uniform coating is always reported to be more favourable [62]. Accordingly the effects heparin immobilisation on surface grafted polyurethane (PUS) was displayed by Kang et al. Initially, functional group-grafted polymer was developed by subjecting PU to oxygen plasma treatment further it was followed by the grafting of acryloylbenzotriazole (AB). Finally the heparin was coupled with carboxyl and amine group containing PUS respectively to form PU-C-Hep and PU-N-Hep. The thrombus formation was increased with incubation time and obviously the AB coupled PU showed larger amount of clot when compared to both heparin coated substrates. Successively, the heparin surfaces exhibited higher APTT time (37, 38±1 s) when compared to pristine PU (31±1 s) further it was also followed in case of plasma recalcification because of the adhesion of antithrombin III factor on heparin coated PU. Though both heparin coated PU noted to have similar hemocompatibility, the PU-N-Hep (1.4±0.08 μg cm^2) noted to have more amount of heparin (2±0.13 μg cm^2) than PU-C-Hep and its bioactivity was also retained significantly on former one. Interestingly, the functional group grafted PUS (PU-AB, PU-COOH and PU-NH2) supported more platelet adhesion in contrast to coated surface meanwhile the increased amount of serotonin found on functional group grafted depicts activation of platelets. In addition, peripheral blood mononuclear cells (PBMCs) was minimally adhered on coated PU after 2 and 5 h of incubation. Moreover, the better surface properties of coated PU reduced cytokine synthesis and aggregation of cells which portrays excellent biocompatibility of modified PU, since the activation of PBMC is considered to be a vital step in foreign body reaction. And among various PUS, the PU-N-Hep displayed better blood-compatible as well as biocompatible characteristic [63]. In a sequel (Kang et al. 2001), the work reported on using PEO spacers to immobilize heparin on PU expressed similar result (i.e) in all cases the heparin coated surface showed superior compatibility [64].

In another work, Anderson and co-workers performed a comparative study on the hemocompatibility effects of a bare polyethylene (PE) surface, hydrophilic polymer-coated and heparin-coated polyethylene surfaces, respectively. The hydrophilic polymer composite Hydrolene (polyvinylpyrrolidone and polyacrylamide) was covalently attached and considered as a negative control while the pristine substrate was also a control. Meanwhile heparin was covalently bound with PE with the help of photoheparin reagent which was followed by the examination of its blood interaction and protein adsorption properties. Among several modified PE, the hydrolene coated PE showed less fibrinogen adhesion than both heparin and unmodified surfaces incubated in tris-saline; however when exposed to platelet poor plasma they exhibited a lower magnitude of adsorption. Even in this case, the hydrophilic polymer-embedded surface showed 25% lesser affinity and, surprisingly, no significant difference was noted between control and heparin-glazed surfaces. Successively, the fibrinogen-adsorbed surfaces were subjected to antibody binding tests and the untouched PE exhibited 4.5 times greater binding of undesired protein than heparin and hydrolene-glazed PE. However, the heparin and hydrophilic polymer-coated PE demonstrated less adhesion of antibodies than the percentage of fibrinogen present. This observation is significantly reflected in platelet adhesion tests, where the number of platelets adhered was more on pristine PE due to its defective nature of encouraging non-specific protein adhesion. On hydrolene and heparin-immobilized PE the platelets were found in a dendritic state with few spread; in contrast the native PE encouraged universal spreading almost throughout its surface. As per the observations made in *in vitro* blood compatibility tests, both heparin and hydrolene-glazed surfaces of PE are able to prevent the adhesion of platelets and undesired fibrinogen by a factor of 100 (approximately). Hence, this modified PE was found to possess an excellent reputation in temporary blood contact devices [65]. Further, Park et al. studied the effects of immobilized heparin on a segmented polyurethane surface. Here, heparin was sealed onto segmented polyurethane surfaces using hydrophilic poly ethylene oxide spacers of different chain lengths. It was observed to play a significant role in maintaining the bioactivity of bound heparin and was depended on the chain length of the PEO spacer. The heparin-immobilized PU showed an excellent ability to avoid both protein and platelet adhesion; however the spacer did not influence its blood compatibility. More interestingly, the heparin bound using the PEO spacer demonstrated improved properties in all characterization tests when compared to those using a C6 alkyl spacer and Biomer controls. These results were also sustained under *ex vivo* conditions where all heparinized surfaces showed a commendable increase in occlusion time and inhibited thrombosis in whole blood [66].

3.3.1.2 Coating to promote vascular regeneration

Hoshi et al. modified POS by establishing several surface preparation techniques, followed by immersion in heparin solution which resulted in the formation of covalent bonds between functional groups of both materials. The POS-heparin complex was attached to the surface of ePTFE through chemical crosslinking. The presence of nitrogen and sulphur indicated by the XPS spectrum showed the formation of coating, while on pristine samples these changes were not recorded. In addition, the formation of purple colour after the addition of toluidine blue stain showed the luminal presence of heparin on modified grafts. It was also used to estimate the adsorption quantity and stability of the immobilized coating layer; there was no significant improvement in complex content by increasing incubation time. The heparin surface density after incubation for 14 days and 28 days was 35.5 and 37.4 ng/mm^2, respectively, compared with 47.2 ng/mm^2 for freshly prepared samples. Interestingly, after coating POS-heparin complex the contact angle of pristine ePTFE dropped from 98.43˚ to 20.18˚. The bioactivity with modified ePTFE grafts was assessed by incubating with whole blood and the presence of anticoagulant POC-coupled heparin layers exhibited significant anticoagulation activity over a long period. At the end of 28 days the glazed surface had 15.6% of clot formation which was only a quarter percentage noted on the pristine surface (70.6%). Even using 100% platelet poor plasma the POC-heparin-ePTFE substrates showed commendable delay compared with their counterparts. Subsequently, the same result was also shown in the case of platelet adhesion. SEM images showed better inhibition activity and an LDH assay revealed 8 X 10^7 ± 5.8 X 10^6 per cm^2 platelets were adsorbed on ePTFE grafts while on POC-Heparin modified grafts only 1.5 X 10^6± 4.7 X 10^5 per cm^2 platelets were adsorbed. Apart from blood compatibility, surface-modified grafts also showed improved adhesion and proliferation of HUVECs while hindering the growth of HBOECs which is highly essential for synthetic vascular grafts. In addition, the endothelial cells were noted to secrete nitric oxide on POC-heparin-ePTFE substrates which shows a promising signature for the regeneration of diseased vessels [67].

3.3.2 Surface grafting of chitosan

3.3.2.1 Coating to improve hemocompatibility and antimicrobial property

Chitosan is also widely utilized as a coating material for several polymeric implants and medical devices for the purpose of reducing clot formation and to incorporate antibacterial property. Recently, Asadinezhad et al. demonstrated the antibacterial activity of Medical-grade PVC coated with different layers of polysaccharides (chitosan and pectin). Equal sized PVC sheets were incubated in deionized water for 10 min at 30˚C to remove surface impurities and to form strong adhesion. The PVC was irradiated by plasma rays of 200 W for 15 sec. After preparing the plasma radiated surface, the PVC was immersed in chitosan solution for a period of 24 h

followed by dipping it in pectin solution. The coating procedures were repeated several times to obtain multiple layers of chitosan and pectin on the PVC surface. The changes in wettability induced by coating were demonstrated using pristine, plasma treated, PAA-grafted, chitosan-coated and chitosan/pectin complex-coated PVC. Among all the types, the chitosan/pectin complex-glazed PVC showed higher wettability (31.0˚) and surface energy, respectively. Meanwhile, the presence of appropriate functional groups was analysed using ATR-FTIR spectrum whereas the increase in the concentration of carbon, oxygen and chlorine content demonstrated by XPS graph confirmed chitosan/pectin coating. Apart from wettability, the surface roughness was also enhanced on modified PVC surfaces due to plasma treatment which formed several ablations and the SEM images also depicted enhanced adhesion of chitosan in the presence of pectin. The significant improvement in surface energy, wettability, roughness and the presence of active chitosan/pectin complex influenced the antibacterial property of PVC substrates. When incubated with Gram positive *S. Aureus* bacteria the modified PVC showed 30% inhibition while in the case of Gram negative *E. coli* the glazed substrates showed minimal adhesion and proliferation [68]. In another work, Yang et al. improved the biocompatibility of segmented polyurethane (SPU) by reducing non-specific protein adsorption and bacterial growth with the help of four step surface modifications performed on its surfaces which results in immobilization of a chitosan/poly (vinyl alcohol) (PVA) hydrogel layer. The highly lubricated coating was achieved by pre-designed modification steps such as oxidation, functionality modification, carbodiimide reaction and finally hydrogel crosslinking. The contact angle studies revealed that the hydrogel coating created a highly hydrophilic surface and atomic force microscopy analyses confirmed that by displaying the slippery nature of the coated surface. Meanwhile, the hydrogel coating also significantly reduced protein absorption on SPU polymers. Further, they demonstrated improved antibacterial properties of hydrogel-coated films and it was reported to be incorporated by chitosan. So, they concluded that the chitosan-coupled hydrogel coating can be employed as a plausible agent to minimize the complications related to SPU-based urethral catheters. The formation of a chitosan/PVA layer was confirmed by FTIR test and, as expected, the hydrogel coating formed a super hydrophilic surface by decreasing the contact angle from 80.78±1.98˚ to 24.58±1.58˚. The AFM images clearly illustrated the formation of highly lubricated surface produced by the activity of glazed chitosan and PVA. Since the coating resulted in formation of a hydrogel layer, the modified SPU surface was shown to absorb a significant amount of water. Even a small amount of chitosan/PVA mixture is more than enough to produce a high water binding surface when compared with commercially available LubriLAST. Because of the formation of a slippery surface, protein (albumin) adsorption was notably reduced ($p=0.007$) when compared with both pristine and LubriLAST-coated substrates ($p=0.043$). In addition, the modified SPU substrates highly encouraged the adhesion of fibroblast cells and the cytotoxicity was notably reduced against its counterparts. Apart from improved cytocompatibility, coated SPU surfaces showed better inhibition against cultured *S. aureus, P. aeruginosa,* and *E. coli* bacterial strains. The chitosan/PVA enriched surfaces showed antiseptic zones of 39.0 mm, 28.0 mm and 23.8 mm diameter in the presence of the above respective bacterial cells while the pristine one showed none [69].

Conversely, Sagnella et al. developed chitosan-based surfactant polymers which can be utilized as a coating alternative for various biomaterials to improve their blood compatibility. The surfactant consists of a chitosan backbone, PEG and hexanal side chains to reject protein adsorption. They coated PE surfaces with chitosan surfactant and the blood compatibility studies displayed an 85-96% reduction in platelet adhesion while the plasma recalcification time also reduced significantly. Moreover, the chitosan surfactant-coated polymer is also reported to acquire suitable properties highly essential for the development of plausible cardiovascular implants [70]. Later, Zhua et al. studied the effects of chitosan/heparin (CS/Hp) complex in reducing platelet adhesion on vascular grafts made of expanded polytetrafluoroethylene (ePTFE). Initially the ePTFE grafts were sonicated in an aqueous solution of acetic acid and chitosan/azide mixture, then the grafts were dried in a desiccator. To strengthen chitosan adhesion, the ePTFE grafts were irradiated with UV rays and immediately after radiation treatment the substrates were immersed in heparin solution to obtain CS/Hp coating. The presence of hydrocarbons and ether on the surface was revealed by ESCA spectra which confirmed the presence of the CS/Hp complex while the UV-spectrometer measured the adsorption as 10.25 mg/cm^2. Further, the *in vitro* hemocompatibility assay performed and analysed using SEM showed no sign of blood cell adhesion or damage. This trend was also reflected in a platelet adhesion assay where the modified ePTFE grafts showed less platelet adhesion whereas on the pristine sample both adhesion and aggregation were observed. Furthermore, modified ePTFE grafts placed in a dog animal model demonstrated the formation of granulation tissue which was inferred due to endothelial regeneration induced by the presence of chitosan and heparin complex. This depicts excellent biocompatibility of polysaccharide coated ePTFE grafts under both *in vitro* and *in vivo* conditions [71].

3.3.2.2 Coating to promote hard tissue regeneration

Moreover, Li et al. demonstrated layer by layer coating of chitosan and hyaluronic acid on polyethylene terephthalate (PET) to promote it for the application of osteo-regeneration. Equally sized PET sheets were initially immersed in alcohol solution for 4 h to remove surface impurities, then it was subjected to several surface preparation protocols. Finally the PET samples were immersed in chitosan solution which was followed by subsequent dipping in hyaluronic acid; the above procedure was repeated several times to obtain ten bilayers of

chitosan and hyaluronic acid coating. After incubating at room temperature for 48 h, the samples were subjected to FTIR analysis and this clearly confirmed the presence of layer by layer coating by showing characteristic peaks of glazed materials. The load bearing capacity of the coated PET was significantly improved (102.8 ± 17.6 N) which is highly essential for hard tissue regeneration materials. Further, the improvement in the biocompatibility properties was confirmed by culturing mouse osteoblastic cells (MC3T3) on both coated and untouched PET sheets. The proliferation extent was measured using MTT assay after an incubation period of 7 days and this clearly showed the optical density on coated PET (0.211 ± 0.018) was significantly higher than on pristine PET (0.160 ± 0.022), which directly relates with enhanced proliferation in the presence of layer by layer coating. In addition, SEM micrographs revealed better adhesion and spreading while the cells cultured on coated PET were observed to secrete more ECM constituents. To check the reproducibility of the observed *in vitro* osteoregeneration ability of coated PET under *in vivo* conditions, the grafts were implanted in rabbit animal models. In contrast to control, the chitosan and hyaluronic acid-coated PET promoted the formation of new bone within a period of 8 weeks. It clearly showed improved biocompatibility and promising osteo-integration of prepared PET surfaces, hence it could possibly be used for bone regeneration applications to eliminate the problem of poor load bearing and inflammation observed in pristine surfaces [72].

3.3.3 Coating of other polysaccharides

In work, Osterberg et al. performed a comparative study on the ability of different types of cellulose polysaccharide and PEG coating on decreasing fibrinogen adsorption of polystyrene polymer. Fibrinogen is one of the types of plasma proteins whose adsorption on biomaterial surfaces plays a vital role in promoting platelet adhesion and proliferation. Hence, to avoid this complication, PEG has been preferred in most cases. However, cellulose-based polysaccharides are expected to perform better than PEG because of their enhanced bioactivity and better adhesion on polymer surface. They coated polystyrene surfaces with ethyl (hydroxyethyl) cellulose 37 & 65 (EHEC), hydroxyethyl cellulose (HEC), methyl (hydroxyethyl) cellulose (MHEC), methyl-(hydroxypropyl) cellulose (MHPC) hydroxypropyl cellulose (HPC) or methyl cellulose (MC) and dextran respectively. The immobilization was performed using both physical adsorption and covalent bonding. In adsorption method, the polymer samples were simply incubated in corresponding polysaccharide solution while to make covalent bonding PS surface was functionalized with appropriate chemicals. The successful coating of polysaccharides were confirmed through electrophoresis; further among different polysaccharides used, HPC and EHEC were adsorbed more because of their hydrophobic nature similar to the polystyrene surface. Accordingly, it was confirmed by XPS and the percentage of polysaccharides immobilized in adsorption method was determined as 21.5% (MHEC), 23.6% (HPC) and 37%, 27.0% (EHEC 37 & 65). Though in covalent bonding all type of cellulose utilized were found to adhere more. Interestingly, the fibrinogen absorption was noted to follow different patterns, based on the type of polysaccharide and coating method used. For instance, the dextran coating performed via physical adsorption encouraged more fibrinogen adhesion in contrast to covalent bonding. The ELBA measurement exposed significant ability of EHEC, HPC and MHPC coated PS surfaces in preventing fibrinogen adhesion similar to PEG coated surface. From this we can clearly infer that the absorption of non-specific proteins is influenced by thickness of cellulose coating hence minimal adhesion was noted on covalently bound surfaces. So, the cellulose-based polysaccharides can be preferred as a plausible alternative for synthetic agents. Moreover, they also effective in forming covalent attachment which may retain the biocompatibility of polymers even under *in vivo* conditions [73].

On the other hand, Sally et al. demonstrated the protein-rejection ability of polysaccharide coating formed by covalently attached successive chains of carboxymethyldextrans (CMDs) on aminated fluoropolymer surfaces. The polysaccharide glazed surfaces were subjected to chicken egg lysozyme, human serum albumin (HSA), bovine colostrum lactoferrin and g-globulin (IgG) respectively. The presence of coating was confirmed by XPS analysis. Though CMD-coated polymer expressed protein adsorption, the amount was significantly decreased when compared to an untouched surface and it was also influenced by the type of protein used. Meanwhile, the increase in surface energy was clearly determined from the drastic decrease in the contact angle of hydrophobic polystyrene to 20˚. They also showed the ability of this polysaccharide-coated polystyrene in preparing non-fouling biomaterials by allowing specific protein adsorption [74].

3.4 Polymers coated with lipids

Lipids are a group of naturally befalling molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, triglycerides, phospholipids, etc., [75]. In other words, any group of organic compounds that are greasy to touch, insoluble in water and soluble in alcohol or ether is termed as lipids. It perform various vital biological functions such as storing energy, signalling and acting as structural components of cell membrane. Classically, the biological lipids originate entirely from two distinct types of biochemical subunits or building-blocks - namely ketoacyl and isoprene groups. Using this, lipids may be divided into eight categories - namely fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides (derived from condensation of ketoacyl subunits), sterol lipids and prenol lipids (derived from

condensation of isoprene subunits) [76]. Lipids have wide applications in cosmetic, food and nanotechnology fields [77]. Apart from this, lipids are also used to coat the polymer surface to increase the biocompatibility.

Korematsu et al. proposed surface grafting of segmented polyurethane (SPU) with a phospholipid analogous vinyl monomer, 2-(methacryloyloxy) ethyl phosphorylcholine (MPC) for improving the surface properties and blood compatibility of SPU. Firstly, SPU was synthesized by a typical two-step addition polymerization reaction, and before grafting the surface with the desired phospholipid the segmented polyurethane was subjected to hydroxylation by using peroxodisulfate. Further, based on the incubation time in MPC solution the polymers were grouped into HPIPSPU-P-2, HPIPSPU-P-4, HPIPSPU-P-18 and HPIPSPU-P-24, respectively. The characteristic absorption bands of NH, CH_2 and aromatic linkage showed the presence of casted MPC film; further the changes in carbon, nitrogen and oxygen content on modified SPU inferred through XPS analysis confirmed the presence of grafting material. The wettability was observed to increase with increasing incubation time in MPC solution, with HPIPSPU possessing the highest contact angle of 102.3±2.3˚ and HPIPSPU-P-24 exhibiting the lowest contact angle of 26.7˚. Accordingly, the enhancement in blood interaction properties was analysed by incubating the grafted substrates in PRP for a period of 60 min along with controls, uncoated SPU and medical grade BioSpan. As with the contact angle studies, the degree of platelet adhesion also varies depending on the graft time with MPC, which shows the importance of finding the optimal concentration to gain significant results. However, all the modified SPU substrates demonstrated minimal platelet adhesion when compared to both controls; further the presence of round-shaped platelets expressed by SEM micrographs portrays the absence of adhered platelet activation [78]. In another work, Morimoto et al. modified the surface of SPU to rectify the problem of biofouling by coating the surface with the phospholipid 2-methacryloyloxyethyl phosphorylcholine (MPC). The coating was carried out using semi-interpenetrating polymer network (semi-IPN) and visible light irradiating under argon atmosphere. The preparation and immobilization procedures made the SPU surface more porous by creating holes with the thickness of 10mm. Meanwhile, the presence of characteristic phosphorus group inferred through XPS spectrum confirms successful surface adhesion of MPC. In spite of pores formation after coating, the mechanical strength of SPU substrates (14.57±2.5) were reported to retained, since coated SPC (15.87±1.2) shows no significant changes in young's modulus. Further its mechanical strength sustained, even after subjecting the PBS immersed films to rigorous stress loading mainly because of the presence of semi-IPN network. More number of platelets were adhered on pristine SPS while exposing to PRP mainly due to surface properties. However on MPC glazed substrates the adhesion was significantly reduced even compared to PMEH poly (30 unit mol% MPC-co-EHMA) coated SPU, since it allowed 10 folds increased adhesion [79].

In a study, Ishihara et al. studied the hemocompatibility of a polymer containing a phospholipid polar group, poly (2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA)) with human whole blood. When human whole blood without an anticoagulant was placed in contact with polymers, blood cell adhesion and aggregation on the polymer without the MPC moiety was extensive, and considerable fibrin deposition was observed. This phenomenon was suppressed with an increase in the polymer MPC composition. Thus, the MPC moiety in the copolymer plays an important role in the non-thrombogenic behaviour of the copolymer. These results were also confirmed by the measurement of the whole blood coagulation time on the polymer surface which was determined by the Lee-White method. The adsorption of phospholipids and proteins from human plasma on poly (MPC-co-BMA) was investigated to clarify the mechanism of non-thrombogenicity observed with the polymer. The amount of phospholipid was increased whereas adsorbed proteins were decreased with an increase in the MPC composition. From these results, we conclude that the phospholipids adsorbed on poly (MPC-co-BMA) play the most important role in the non-thrombogenicity of the MPC copolymer [80]. Moreover, Hall et al. scrutinised the biocompatibility properties of phosphatidylcholine, a major phospholipid found on the outer cell membrane of human erythrocytes. They studied the blood coagulation effect of different substrates which have been used in prosthetic devices such as polyethylene terephthalate, expanded polytetrafluoroethylene and phosphatidylcholine-coated polymers. Ultimately, the coagulation effect analysed by material thrombelastography revealed improved blood compatibility of lipid-sealed surfaces when compared to bare polymer [81].

4. Conclusion

The field of medicine and healthcare has been rapidly upgrading its outlook in recent decades. Presently, the advent of new techniques and modern tactics, promises better healthcare than in ancient days. Biomaterials is one of the renowned divisions in medical science, since it is known to have the ability to mimic the properties and functions of body organs. Though, there are various techniques emerging on a regular basis to improve the quality of biomaterials, problems associated with its use is not yet eliminated completely. Biocompatibility is the major problem associated with all of its types (from metals to the materials under current attention). Whenever a biomaterial is exposed to the blood stream it will elicit a series of reactions, so the favourable material is a one which has the ability to avert such reactions. Despite, there are different types of biomaterials exist, polymers have gained more attention because of their reliability and uniqueness. However, it is also not an exemption for the common biocompatibility problems associated with other biomaterials.

As detailed in section 1 and 2.1, the problems associated with a material utterly depend on its surface properties and its way

of interaction with the biological environment. So, polymers will be subjected to appropriate modification techniques to improve superficial characteristics before further exploitation. Among several methods, coating of surfaces with common biological substances such as proteins, lipids, carbohydrates and peptides is found to be more reliable because of their non-toxic and eco-friendly nature. From the studies discussed, it can be inferred that the physicochemical properties of polymers and the type of coating approach preferred are the two vital factors involves in a successful coating of biological substances. Nevertheless, the optimum range of physico-chemical properties differ significantly based on the polymer of interest, however, to establish thick and strong coating of biomolecules the methods intended to form covalent bonding is observed to be more effective than physical adsorption. Therefore, to achieve this, the polymer surface can be either functionalized to acquire appropriate bonding groups or pre-grafted with other adhesive materials like PEO spacers to form strong covalent bonding. Besides, the table 1 outlines, excellent ability of biological substances in delaying and inhibiting several frontline problems associated with polymers. The coating mainly reduced protein adsorption, platelet adhesion and aggregation, release of thrombogenic factors, activation of coagulation pathway and thrombus formation. In addition, they also boosted the regeneration process by encouraging fibroblast adhesion and endothelial cell proliferation. Apart from this, a promising polymeric material should also possess significant ability to defend it from the attacks of microbes. As per the studies mentioned in this article, coating of certain proteins, peptides and carbohydrates are found to improve the antimicrobial properties of the polymers as well.

These, essential biocompatible properties are highly anticipated in temporary blood contacting devices like catheters, hemodialyser, implantable defibrillators, pacemakers, etc. On the other hand, the observed cellular compatibility can be applied for promoting tissue regeneration and wound healing process by employing common biomolecules as a coating agent for polymeric stents, vessel grafts, vascular prosthesis and scaffolds (as illustrated in figure 2). Therefore, the selection of specific biological substance is purely depends on the polymer and its intended application. For instance, PS coated with plasma proteins is reported to have better hemocompatibility; while grafting with BSM it illustrated excellent antimicrobial property and whereas coating with ECM proteins it acquired the ability to promote the proliferation of endothelial cells.

In the interim, the works reported in this article have scrutinized most of the commonly utilized polymers in medical field. Hence it can be concluded that, the coating of biological substances may be applied for any polymeric materials, however care should be taken while choosing chemicals and reagents involve in the coating process. Because in few studies, the extensive use of these agents is noted to affect the bioactivity of the biological substances employed; which eventually reduced the anticipated outcome. In future, more investigations need to be carried by extending coating of biological substances to modern generation materials like nanocomposites, nanofibers, nanoparticles, etc., to make them more competitive and reliable for various biomedical applications.

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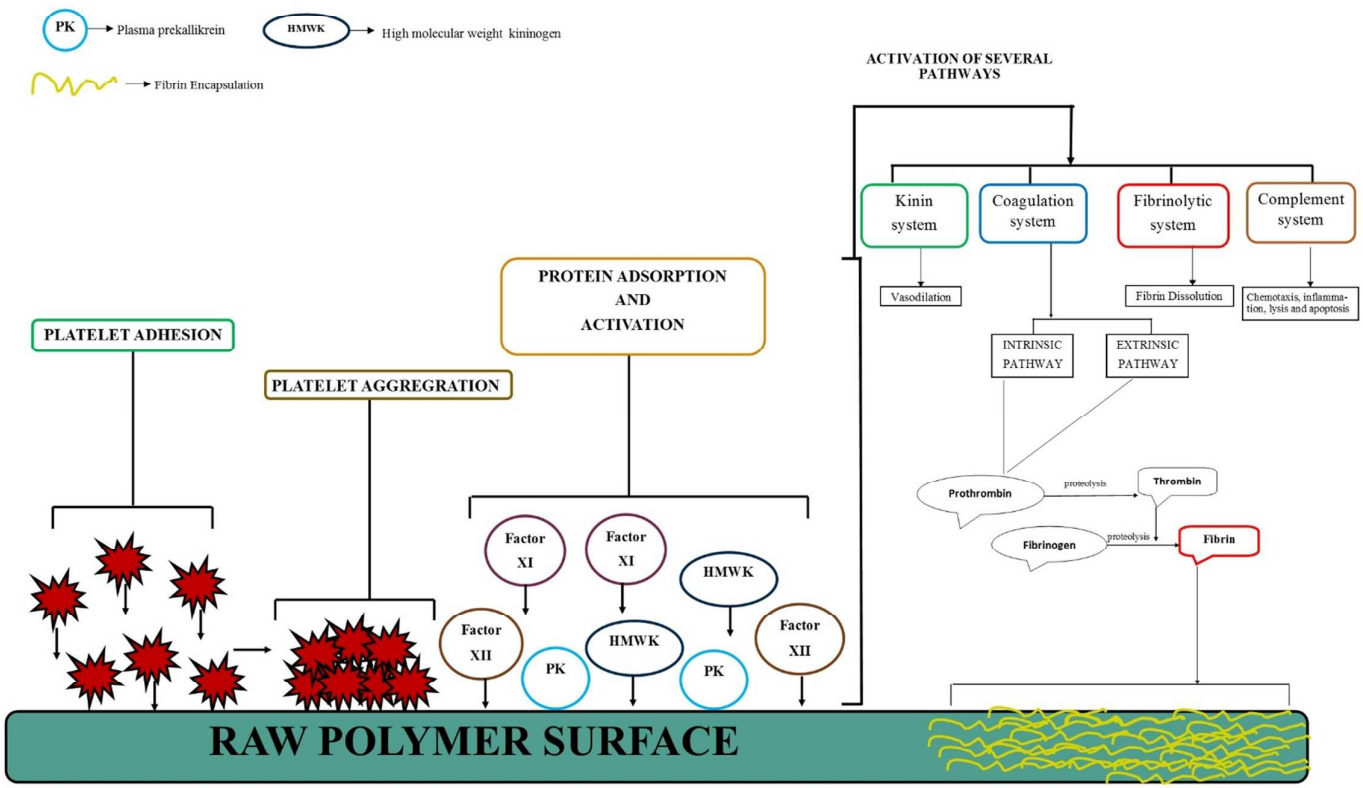

Figure 1: Key factors involved in activation of coagulation pathways

Figure 2: Possible biomedical applications of polymers coated with biological substances