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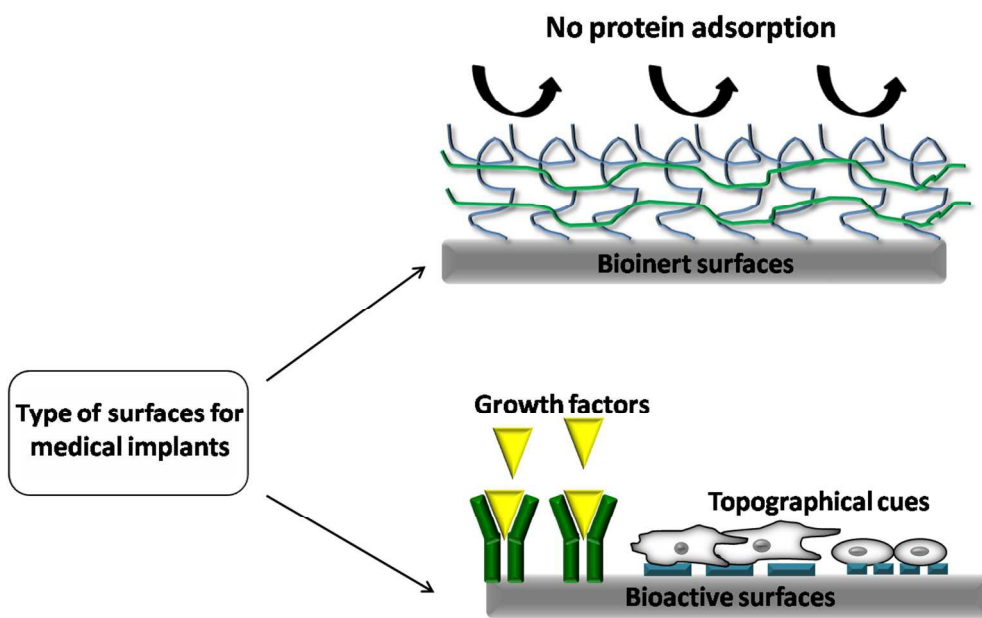


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REVIEW

Tailoring biomaterial surface properties to modulate host-implant interactions: implication in cardiovascular and bone therapy

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Host body response to a foreign medical device plays a critical role in defining its fate post implantation. It is thus important to control host-material interactions by designing innovative implant surfaces. In the recent years, biochemical and topographical features have been explored as main target to produce this new type of bioinert or bioresponsive implants. The review discusses specific biofunctional materials and strategies to achieve a precise control over implant surface properties and presents possible solutions to develop next generation of implants, particularly in the fields of bone and cardiovascular therapy.

1. Introduction

In recent years, biomaterial-based medical implants have gained much acceptance across the medical community because of their ability to modulate local inflammatory responses, replace or restore the integrity of damaged tissues that are no longer functional and attenuate adverse tissue reactions¹. However, once they are implanted in the body a layer of plasma proteins such as fibrinogen, albumin and fibronectin can coat their surfaces that leads to adverse host responses such as coagulation, complement activation as well as the recruitment of innate immune cells. Acute inflammation is then generally followed by chronic immune responses² that subsequently progress into a foreign body reaction. In this last stage macrophages can undergo fusion to form foreign body giant cells^{3,4} and be responsible for the fibrosis encapsulation of the medical device. In most of the cases, this last step impose a major impediment for successful *in vivo* performance of the implant⁵. For example, in the case of cardiac pacemakers, formation of a dense capsule can attenuate the propagation of the electrical signal while in intravascular stents, fibrosis can cause a re-narrowing of the stented area⁶. Hence, it is imperative that we develop strategies to tailor the material surface properties before implanting them so as induce favourable host-material interactions. For instance, to design vascular grafts and heart valves we opt for biomaterials that can efficiently resist protein adhesions and eliminate the chance of thrombosis and immunogenic responses⁷. However, this paradigm is not applicable to all applications as immune

response and the consequent remodelling can sometimes be important in defining the success of certain implants and for its integration with the host tissue⁸. This concept can be applied for example to bone grafts, in which the process of healing is regulated by close interactions among cells of the immune system, osteoclasts and osteoblasts⁹. Recently, macrophages have been considered as target to regulate bone morphogenesis due to their ability to polarize into pro-inflammatory and anti-inflammatory phenotypes and affect different stages of bone healing⁸. With more such evidences, biomaterials-based research is now focused on exploring immune modulation mechanisms to invent osteoinductive materials.

Furthermore, a precise understanding of the host response to changes in surface properties of biomedical implant is essential for fabricating a successful device for cardiac and bone applications¹⁰. In fact, surface chemistry and topography represent some of the important factors that can be effectively manipulated to control immunological responses such as cytokines expressions, monocyte differentiation, macrophage and dendritic cell activation and protein adsorption^{11, 12}. Chemical and topographical characteristics can be varied using several strategies to generate specific pattern or coatings on the biomaterial surface. Such coatings can also contain drugs or specific immunomodulatory proteins capable of exerting active control over the behaviour of local immune cells^{13, 14}. Our objective in this review is to delineate currently available techniques and strategies for controlling the surface properties of biomedical implants. Specifically, we will discuss the principles behind the preparation of both inert and immunomodulatory surfaces and the criteria governing their design. Further attention will be shed on how these strategies can be harnessed for fabricating new biocompatible medical implants for cardiac and bone applications. In addition, we will highlight the persisting impediments with these technologies and potential solutions for their successful applications in the field of cardiovascular and bone diseases.

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2. Bioinert surfaces: general considerations

Interactions between host proteins and implant surfaces are linked with various important biological reactions and cascading effects of host immune system. Non-specific binding of unwanted proteins on the implant surface, also known as biofouling, can be one such type interaction that leads to graft rejection by host system. This early stage can lead to a foreign body reactions causing a possible implant failure. This process can be avoided by designing bioinert or biomimetic surfaces that can prevent protein adsorption and the accompanying immune responses. A possible strategy to achieve this goal is to modify the surface properties of implantable devices using biocompatible polymer coatings capable of limiting protein attachments¹⁵. These polymer layers should be mechanically and chemically robust to sustain surface deposition on the implant, withstand sterilization and implantation procedures and most importantly retain their stability *in vivo*. Such a polymer coating can help achieve better control over several critical parameters such as the surface free energy, chemical composition, wettability and steric repulsion.

Each of these key factors can be tweaked to control the protein adsorption by synthesizing polymers with suitable chemical, physical and interfacial free energy properties. Interfacial free energy provides a measure of the driving force for the adsorption of proteins and cells on to a solid surface influencing the host response to a biomaterial¹⁶. Thickness of the coating along with the temperature, pressure and friction of the surrounding biological fluids are some of the key factors that determine the free energy at the interface. Polymeric coatings with low interfacial energies to water and blood represents the best choice in order to reduce protein adsorption on implants. In addition, the chemical composition of the polymer used along with its wettability also play a significant role in determining the degree of protein entrapment¹⁷.

Generally, hydrophobic coatings have higher affinity for proteins with respect to hydrophilic ones as the adsorption generally occur through hydrophobic interactions¹⁸. The presence of hydrophilic groups such as hydroxyl -OH, amine -NH₂ or carboxyl units -COOH can change the level of water on the surface of materials. This layer of water molecules can indirectly provide a low interfacial free energy with the biological fluids and reduce the tendency of proteins to adhere to the implant. Moreover, it has been recently demonstrated that the arrangement of water molecules at the polymer interface changes according to the polymer coating and can impact the degree of protein adsorption¹⁹. Another important component is also the ability of the polymer coating to provide a certain degree of steric repulsion to limit protein adsorption. Poly(ethyleneglycol) (PEG) is one such standard choice for polymer coating²⁰. PEG chains length and conformation combined with its high degree of hydration and flexibility can create a barrier to protein adsorption²¹.

Considering the above mentioned fundamental aspects, the following section will discuss in detail the currently available polymer coatings and strategies to engineer bio-inert surfaces.

Furthermore, specific attention will be given to surface coatings for cardiovascular implants, that aims to enhance compatibility with the blood.

2.1 Strategies to design bio-inert polymeric coatings

Bioinert coatings can be applied on a variety of materials including metallic, ceramic and polymeric surfaces. However, not all types of surfaces contain active and functional groups for chemical conjugation. This is the case of polymer surfaces that generally has to undergo a step of chemical pre-treatment or irradiation to be further functionalized²². Physical techniques can also be utilized for activating the surface for coupling bioinert polymers. This includes UV and gamma irradiation, electron beam, corona discharge, ion beam, laser and plasma treatment.

UV irradiation represents a fast and low cost process that allows for photo-grafting polymeric chains on the surface of biomedical implants. The reaction is generally carried out in the presence of a biocompatible photoinitiator that can break down into radicals responsible for the surface activation. Upon formation of free radicals from the photoinitiator, vinyl monomers in the surrounding solutions will act to propagate the radical reactions leading to the formation of grafted polymer chains on the surface of the implants²³. Nevertheless, one of the persisting concerns with the use of UV is that the process may not only alter the surface of the implant but also its bulk properties. In fact, if the material becomes too stiff after UV exposure, the risk of thrombosis in the case of cardiovascular implants such as synthetic vascular graft can dramatically increase²⁴. This problem can be overcome by using short wavelength radiation in the extreme ultraviolet region (EUV) that would result in the modification of a very thin layer (<100 μm) of the medical device. Thus, allowing for surface modification without inducing changes to the bulk.

An alternate strategy to introduce reactive sites on the material surfaces would be to employ plasma treatment. In this approach, the biomaterial surface is exposed to high energy radiation consisting of atoms, molecules, ions and radicals generated by gaseous mixture (CO₂, O₂, N₂, NH₃, and H₂), vacuum arc or a laser source^{25, 26}. This technique can specifically modify hydrophilicity, roughness as well as the chemical composition without changing the bulk properties of medical devices.

Once the required functionality has been introduced on the implant surface following the possible strategies above described, multiple methodologies can be pursued to apply the desired polymeric coating. The type of coating can be chosen considering several parameters including the anchoring site, polymer topology and steric effect that can influence its stability. Self assembly, non covalent physisorption and covalent binding of polymer brushes represent some of the well-established strategies to generate polymeric bioinert coatings. A brief description of these techniques will be provided in the following subsections.

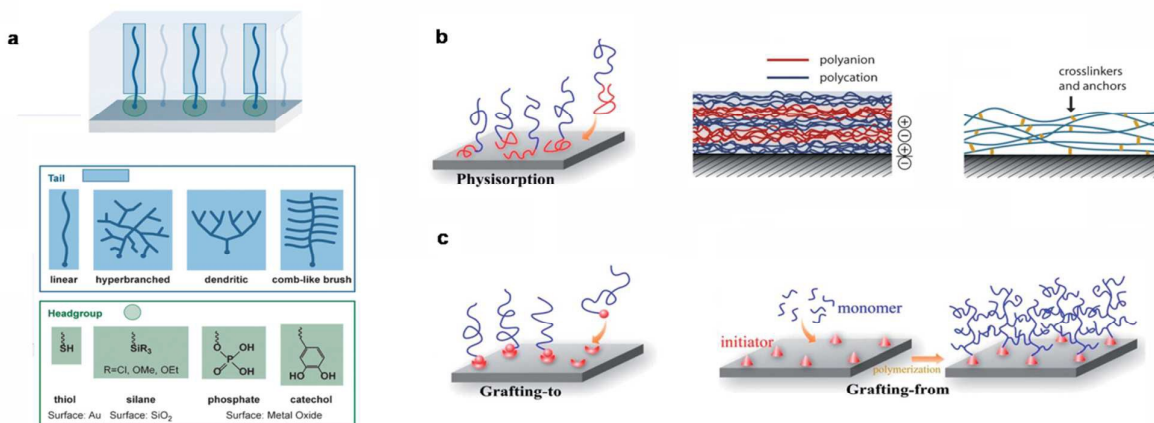


Figure 1: Overview of available techniques for bio-inert coatings. a) Schematic of a SAM containing examples of typical head and tail domains, b) Illustration of physisorption, layer-by-layer coatings through electrostatic interaction and presence of internal crosslinking among layers to increase stability, c) grafting-to and grafting-from strategies. Reprinted with permission from 28 and 35. Copyright 2014 John Wiley and Sons and Copyright 2013 Royal Society of Chemistry.

2.1.1 Self-assembly monolayers (SAMs) and physisorptions of polymeric multilayers

Self-assembly monolayers (SAMs) can be considered as molecular assemblies that are generally created by the spontaneous adsorption of organic molecules on a solid surface such as metal implants²⁷. In general, molecules used to obtain SAM consist of three parts: a head anchor group (such as thiol, silane, phosphate and catechol), an alkyl chain and functionalized end regions²⁸. The head anchor has to provide a stable attachment of the coating to the surface and usually the ones having multifunctional groups represent a better option to achieve an increased stability of the coating²⁹ (Fig 1a).

These monolayers generate homogeneous structures with high stability due to strong inter-chain interactions that can affect surface wettability and friction. However, one of the disadvantage of SAM technique is that it is not possible to obtain dense coating on the surface of bioimplant due to steric effect that can limit the corresponding "anti-biofouling" activity.

Another possibility is represented by physical adsorption on chemically inert polymeric surfaces. Such anti-fouling coatings are usually made of amphiphilic block copolymers like poly(ethylene glycol)-b-poly(propyleneoxide) PEG-PPO, poly(L-lysine)-b-poly(ethylene glycol) PLL-PEG, that are adsorbed through hydrophobic or electrostatic interactions respectively^{30, 31}. In contrast to a single layer, multilayers of physically adsorbed polymers offer a better control over the thickness of the coating and pose as a useful strategy to obtain a higher surface coverage. Moreover the presence of possible hydrophilic, hydrophobic or covalent crosslinking during multilayer formation can increase the general stability of the coating^{32, 33} (Fig 1b).

2.1.2 Polymeric brush coatings

Apart from physical interactions, covalent bonding of polymer brushes offers a unique platform to exert desired surface properties on implants. Polymer brush coating present a series of advantages including the presence of multifunctional groups in the branched structure, that permit a further modification

with biological molecules and also exhibits controlled swelling and wettability³⁴. Furthermore, parameters such as molecular weight and crosslinking density can be controlled over the process of grafting, guaranteeing a defined 3D architecture. Specifically, densely grafted polymeric brush can act as barrier to limit undesired interactions between the surface of the implants and the immunological components. "Grafting to" and "grafting from" are the two distinct approaches used for the preparation of polymer brush coatings^{34, 35} (Fig 1c).

The former involves a reaction of pre-functionalized polymers with a surface that contains the necessary complementary groups. This approach cannot produce high grafting density as the presence of previously included polymeric chains can hamper the introduction of new ones³⁶. Due to this limitation, the latter "grafting from" strategy embodies the most attractive strategy for the preparation of brush coatings. Particularly, it involves the immobilization of specific initiators on the surface of biomaterials that starts the process of monomer polymerization. "Grafting from" can allow for a higher graft density that is mainly regulated by the initiator density on the material surface. Nevertheless, low initiator efficiency and variable diffusion of the monomer to the active polymerization site are some of the persisting drawback of this technique^{37, 38}. These limitations usually results in the formation of brushed coating with a broader chain length distribution respect to SAM. "Grafting from" can be applied on both organic and inorganic surfaces and this process of brush polymer formation can be obtained following several methods such as atom transfer radical polymerization (ATRP), initiator transfer terminator polymerization (INIFERTER) and reversible addition fragmentation chain transfer (RAFT)³⁹⁻⁴¹. Different type of polymer brushes can be produced using these techniques including neutral, zwitterionic, homo and block-copolymer^{44, 45}. All the proposed approaches so far can be chosen according to the material surface and the type of biomedical implant designed for a particular application. Moving further, state-of-the-art techniques for modulating cardiovascular implant surfaces to prevent undesired host responses such as thrombogenesis and inflammatory responses will be discussed.

2.2 Bioinert surfaces for cardiovascular implants

One of the main obstacles that we still need to overcome while developing cardiovascular implants is their compatibility when exposed to blood⁴². Specifically, it is desirable to obtain surface properties that can avoid thrombogenesis, the major concern for vascular grafts, stents and heart valves. This can be achieved by preventing platelet adhesion and activation, hence reducing the chance of thrombosis. As discussed above, a possible approach to achieve this goal can be the formation of anti-fouling polymer coatings on the surface of medical devices. Interesting examples of this include hydrophilic neutral coatings that can impart protein resistance to a surface with the help of PEG⁴³ and oligo ethylene glycol (OEG)⁴⁴ polymers. These polymers have been easily grafted on Nitinol stents, Dacron, expanded polytetrafluoroethylene (ePTFE) and polyurethane (PU) heart valves⁴⁵. A detailed analysis of chemical strategies using PEG and its derivatives as polymer brush coating has been reported elsewhere⁴⁶. Apart from PEG and OEG that exhibit excellent antifouling properties to a wide variety of proteins, other synthetic polymers generally defined as poly(2-oxazoline)s (POXs) such as poly(2-methyl-2-oxazoline) (PMeOX), poly(2-ethyl-2-oxazoline) (PEtOX), and polypropyloxazoline are promising starting material for the preparation of amphiphilic coatings with similar properties of PEG^{47, 48}. At the same time zwitterionic polymer brush coatings are another emerging class of anti-fouling coatings that possess positive and negative charges in equal number. Because of their presence, these coatings are highly hydrated inducing extremely limited protein adsorption.

An interesting example is represented by phosphorylcholine (PC)-based polymer brushes such as 2-methacryloyloxyethyl phosphorylcholine (MPC)^{49, 50} that mimic the property of PC, a phospholipid found in the cell membrane, that is capable of preventing proteins and platelet adhesion. MPC coatings have been used to cover ePTFE vascular grafts and stents surfaces to increase their blood biocompatibility⁵¹. Sulfobetaine (SB)^{52, 53} and carboxybetaine (CB) derivatives⁵⁴ are other emerging classes of zwitterionic polymer brushes that are potentially useful as coating to prevent platelet adhesion. Apart from polymer coatings, an alternate way of addressing this problem could be covering the surface of implants with passive coatings made of less inflammatory proteins such as haemoglobin or albumin. However, this approach is not much reliable as pre-absorbed proteins can undergo passive exchange and displacement with the ones present in plasma such as fibrinogen⁵⁵. These examples highlight the importance of passive coatings, trying to avoid or minimize the contact with the biological components of blood. However, the presence of bioactive functional groups in their external layer can pave the way for further modifications including entrapment of drugs and small biological cues. This concept has been widely investigated in the cardiovascular field in order to come up with interesting and smart designs that can play a direct role in controlling the host response.

2.3 Bioactive surfaces for cardiovascular implants

Bioactive coating can be a highly useful technology for cardiovascular applications, particularly to reduce adverse biological reactions such as thrombosis and inflammation. The innovative solutions proposed for these two important aspects will be briefly described in the following two sections.

2.3.1 Antithrombotic surfaces for cardiovascular implants

To restrict thrombosis, two different bioactive coatings can be utilized including anticoagulant and fibrinolytic interfaces. In the first case, surfaces of biomedical implants should be modified in order to include biological molecule capable of minimize thrombin formation. Thrombin is the key enzyme involved in blood coagulation and in this sense immobilizing heparin on the surface of implants could represent a solution. Heparin is a natural polysaccharide capable of binding antithrombin (AT-III) and forming a complex that is responsible for the inhibition of thrombin⁵⁶. Heparin can be immobilized using physical adsorption through ionic bonding. In this case, heparin can be absorbed via electrostatic interaction in the presence of cationic agents such as benzalkonium chloride or tridodecylmethylammonium chloride (TDMAC)⁵⁷. In a one year long study, vascular grafts made of heparin-ePTFE were found to provide resistance to thrombosis and significantly reduced early graft failure⁵⁸. Additionally, the stability of heparin coatings can be enhanced using covalent grafting strategies that can be realized through cross-linking reactions involving one of the several functional groups present in heparin chain such as hydroxyls, carboxylic, or amines functionalities. However, it is essential that after grafting the biological activity of heparin is preserved. Polymer brushes made of poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA)⁵⁹ and diblock polymer POEGMA-b-poly(N-hydroxysuccinimidyl methacrylate) (PNHSMa)⁶⁰ were used to covalently link heparin, combining in the same system antifouling properties and the inhibition of thrombin. Table 1 summarizes a brief list of recent examples of heparin based coatings.

Despite its importance as coating for blood-contacting devices, heparin immobilization have a few side effects including osteopenia and thrombocytopenia. Moreover, the ability of heparin to bind various bioactive molecules such as growth factors and plasma proteins could determine inflammatory responses caused by heparin itself or the polymer coating used⁶¹. For this reason other inhibitors has been explored as potential candidates to hinder thrombin activity or its production. Hirudin, a potent natural inhibitor of thrombin, has been covalently grafted to several materials in cardiovascular tissue engineering application. As for heparin, chemical conjugation can alter the bioactivity of hirudin and selective attachment using the ϵ -amino groups of Lys instead of the N-terminus were found to preserve the inhibitor activity against thrombin⁶². Hashi *et al.* has recently used PEG as a spacer to covalently link the C-terminus of r-hirudin to a biodegradable small-diameter microfibrillar vascular graft fabricated using electrospun poly(L-lactide) (PLLA)⁶³. The hirudin coating caused a reduction in adherent and activated platelets on the vascular graft and increased patency six months after implantation into rat carotid arteries.

Covalent conjugation of others thrombin inhibitors has been tested. For example Yang *et al.* have tried to conjugate bivalirudin on plasma polymerized allylamine coated 316L stainless steel. The presence of bivalirudin was able to prolong clotting time, limiting adhesion and activation of platelets and fibrinogen. Moreover, *in vivo* studies indicated that thrombus formation was reduced by a rapid growth of intact endothelia on the bivalirudin-coated surface⁶⁴. Another possible strategy to create bioactive coating with anticoagulant properties is to bind on the surface nitric oxide (NO). In fact NO can limit platelet adhesion and thrombus formation on the surfaces of cardiovascular implants⁶⁵. Several NO-donor compounds can be chosen modulating the rate and mechanism of NO release. Covalent grafting to pendant groups is the most common strategy to include NO-releasing compounds. A particular

class of NO donors are diazeniumdiolates that can be linked to different polymer such as polyvinyl chloride (PVC), PU or ePTFE vascular grafts^{66, 67}. In addition to limiting platelet adhesion, NO-coatings can guarantee a reduction in smooth muscle cell proliferation and promote endothelial cell proliferation that can be extremely important in defining long-term patency of vascular grafts⁶⁶.

As mentioned above, an alternate way to improve blood biocompatibility of medical implants for cardiovascular applications is to produce fibrinolytic surfaces. In this case, the coating should be able to local activate the endogenous fibrinolytic system reducing the formation of thrombus. One of the main strategies to achieve this goal has been reported by the introduction of lysing groups on the surface that present high affinity for plasminogen a key factor in the fibrinolytic system⁶⁸. Chen *et al.* have proposed PU modification with polymer brush made of lysine-poly(2-hydroxyethyl methacrylate) Lys-(PHEMA) which is able to bind selectively plasminogen capable of causing rapid clot lysis on the surface⁶⁹. Similarly, in another study, poly(OEGMA-co-HEMA) copolymers grafted on polyurethane surface were further functionalized with lysine groups showing selective binding of plasminogen and low adsorption of fibrinogen and other plasmatic proteins⁷⁰.

2.3.2 Bioactive surfaces to control immune response in cardiovascular implants

Bioactive coatings for cardiovascular implants surfaces should be also designed taking in consideration the immune response. In fact, the interaction between the immune and coagulation systems can profoundly affect the fate of implanted biomaterials⁷¹. An important aspect to consider is the suppression of complement activation and its fixation on the surface of medical devices⁷². To obtain this control over the immune responses, bioactive coatings should be able to suppress the immune response once implanted *in vivo* along with their antithrombotic properties. This can be achieved by grafting in the polymeric coatings bioactive molecules such as growth factors or specific receptor and antibodies. For example factor H a protein present in serum can inhibit complement activation and has been used as a strategy to control the complement inflammatory response⁷³.

In an interesting study, Andersson *et al.* created a hybrid surface in which the anti-coagulation properties were combined with a specific complement-inhibitory effect due to the presence of factor H⁷⁴. In specific Pluronic™, a class of triblock copolymers consisting of a block of polypropylene oxide (PPO) surrounded on each side by polyethylene oxide (PEO) blocks was chemical conjugated with factor H and tested with human serum showing promising results in terms of reducing complement inflammatory cascade. Alternatively, to chemical strategies that can be costly another possible solution is to bind on the surface peptides that show high affinity for factor H. In another study, Lambris *et al.* have identified a specific peptide (5C6) capable of specifically capture factor H without altering its functional activity⁷⁵. At the same time, it is even possible to consider the possibility to bind on the surface receptor or biological proteins to reduce cell inflammatory adhesion. CD47 is a ubiquitously expressed trans-membrane protein that functions as a biological marker of self- recognition and showing the ability to C-

Table 1. Innovative recent heparin based coatings for cardiovascular implants.

Substrate/ Coating	Coating strategy	<i>In vitro/in vivo</i> studies	Ref.
Duraflor™ heparin and sirolimus	Layer-by-layer	APTT assay, drug release studies (drug eluting stent)	76
Amino-silanized titanium /Heparin and collagen Type- IV	Layer by layer	platelet adhesion test, APTT assay	77
NiTi shape memory alloy/Heparin	UV- pretreatment and physical adsorption	hemolytic tests, dynamic clotting time experiments, platelet binding tests	78
316L stainless steel/heparin	Chemical conjugation using dopamine and HMA	APTT assay, HUVECs & HASCs proliferation studies/ subcutaneous implantation in white rabbits	78
Nanofibers/ Heparin	Chemical conjugation using diamino- poly(ethylene glycol) as linker	Antitrombogenic activity tests/ implantation in rats	79
Titanium/PEG and Heparin	carbodiimide covalent coupling method	APTT assay/ HUVECs adhesion and proliferation studies	80

APTT: activated partial thromboplastin time, HUVECs: Human Umbilical Vein Endothelial Cells, HASCs: Human Artery smooth muscle cells, HMA: hexamethylenediamine.

influence inflammatory cell attachment. In a recent study, recombinant CD47 was modified with a poly-lysine tag to the terminus and covalently linked to polyvinyl chloride (PVC) surfaces. PVC modified with CD47 was able to inhibit both platelets and neutrophils adhesion⁸¹. Other interesting examples report instead the immobilization of the human complement receptor 1 (sCR1), that is another important inhibitor of the classical and alternative complement activation pathways^{82, 83}.

2.4 Bio-mimetic surfaces for cardiovascular implants

The polymeric coatings discussed so far presents well-grounded solutions to design advanced blood-compatible material surfaces. However, concerns arise when these coatings are checked *in vivo* as they can be susceptible of cracking and wear leading to undesired host responses such as thrombosis, microembolism and late inflammation reactions^{84, 85}.

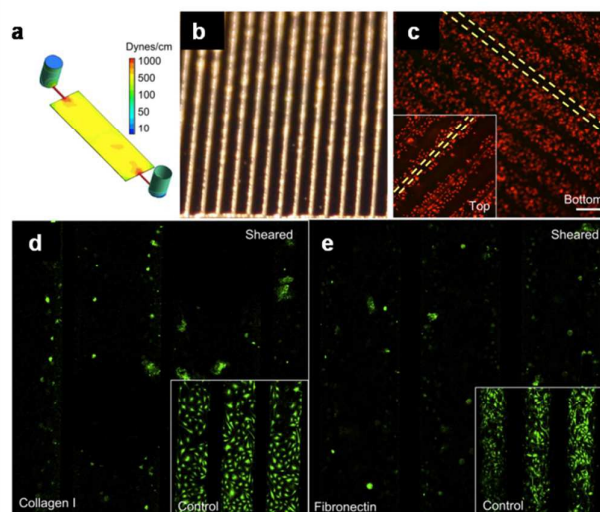


Figure 2. Endothelialization; Micro-topographical manipulation for endothelial cell retention at high shear stress: a) Computational fluid dynamic simulation of carbonized surface channel based bioreactor under chronic ultra-high shear stress. Etched silicon channels (b) were used as a substrate for carbon deposition. c) Fluorescent image showing the retention of endothelial cells at 600 dynes/cm² after 48 h in culture, d & e) Fluorescent images highlighting the absence of plasminogen activator inhibitor (PAI-1), an activator of fibrinolysis in collagen I and fibronectin coated channels respectively under sheared and static conditions. Reprinted with permission from ref 95. Copyright 2014 Elsevier.

For this reason, researchers have started to explore other avenues including the possibility to create bio-mimetic surfaces using endothelial cells (EC). It is well known that EC can play an important role to regulate coagulation, and prevent platelet adhesion and activation⁸⁶. In fact, they can secrete NO that promotes vasodilatation and inhibits clot formation⁸⁷. In addition, ECs can induce fibrinolysis by secreting tissue plasminogen activator to dissolve fibrin clots and thrombomodulin to inhibit the coagulation cascade. For these reasons, strategies are aimed to include a layer of EC on the outer surface of cardiovascular implants. These may be achieved by two different approaches; one can be to implant EC coated medical devices and other one can be to promote in situ endothelialization by exploiting the innate angiogenesis system. In the case of engineered vascular grafts it is important to consider that EC should form a confluent and continuous surface with tight adherent junctions. The integrity of these junctions is important as a disruption of this layer can cause exposure of the sub-endothelial tissue matrix inducing platelet activation and aggregation⁸⁸. Complete endothelial coverage of the surface of cardiovascular devices is fundamental to avoid thrombogenesis post implantation. In order to achieve this goal, it is necessary to modulate the surface properties of cardiovascular implants to increase EC attachment. This can be made possible by working on physical cues such as microtopography⁸⁹ and nanotopography⁹⁰ of the medical device, particularly in the case of metal stents. In fact, topographical cues can modulate not only cell attachment but also morphology and cellular behaviour. Particularly, it exerts a profound effect on the process of endothelialization. Inclusion of cell adhesive peptides derived from the extracellular matrix demonstrates another possible solution to enhance cell adhesion and migration on implant surface^{91, 92}. However, an important thing to consider while biofunctionalizing the outer surface of the implant is that the

affinity of these adhesive peptides can not only promote EC proliferation, but also can induce platelet adhesion, thus eventually making it thrombogenic⁹³. It is thus critical to confirm the stability and nature of the adhering cells once the medical device is implanted *in vivo*. In particular, the endothelial monolayer should be able to sustain the surface shear stress caused by blood flow. For example, in prosthetic heart valves the surface shear stress can exceed 500 dyn/cm² which is much higher than what EC can withstand⁹⁴. In a recent study, Frenzl *et al.* emphasized that endothelial adhesion is not alone sufficient to withstand ultra-high shear stress environment regardless of the adhesive nature, type and concentration of the ligand used on pyrolytic carbon surfaces. This problem was overcome by producing microfabricated trenches on the pyrolytic carbon surfaces that allowed a sensible reduction on the value of shear stress detected by cells, that retained their confluent monolayer when exposed to 600 dyn/cm²⁹⁵. This approach represents an important advance in the design of bio-mimetic surfaces that can retain the monolayer of endothelial cells intact when exposed to higher shear stress (Fig 2).

As an alternative to EC, endothelial progenitor cells (EPCs) that are able to differentiate into functional EC, represent another interesting cell source to promote medical device-based endothelialization⁹⁶. Interestingly, synthetic materials can be coated with specific molecules such as antibodies^{97, 98}, growth factors⁹⁹⁻¹⁰¹, genes^{102, 103} and aptamers¹⁰⁴ to recruit circulating EPCs and generate an autologous endothelium surface.

3. Designing biointeractive coatings to support implant integration in bone

In previous sections, we have discussed the different strategies to suppress adverse host immune responses and increase blood biocompatibility of medical implants. However, these strategies hold limitations when considering prosthesis that have to undergo a process of remodelling to restore functionality of damaged tissues. For example, bone grafts made of polymeric materials should be able to integrate with the host and guide formation of new bone¹⁰⁵. For such applications, several physical cues such as porosity, roughness, topography and chemical conjugation of growth factors needs to be considered to promote bone healing. A lot of effort has been made to design new surfaces and interactive coatings for orthopaedic implants to promote osseointegration. Moreover, surfaces of orthopaedic implants can also be tailored to modulate the immune responses rather than to simply suppress it. In fact, the healing process as well as the integration of the medical device within the bone tissue can be actively controlled by the immune system. This new area of research interest is defined as osteo-immune modulation. Although still in its early stage of development this area of research is progressively gaining importance due to the increased understanding of the complex cellular and molecular interactions between the immune and skeletal systems¹⁰⁶. The following section explores in details the new types of interactive coatings developed for metal and polymer implant-based bone regeneration. In addition, the design of recent immune-modulatory strategies for bone osseointegration will be articulated in an attempt to provide new insights on advanced materials for bone therapy.

3.1 Surface modification with bioactive materials to promote osseointegration

Orthopedic implants such as joint prostheses, plates and screws serve the purpose of providing mechanical and structural support to the damaged area. In addition, they should enhance healing and easily integrate with the surrounding host tissue. Among the materials used metal represent the first choice when mechanical and structural support is essential and some examples of recent strategies to modify the surface of metal orthopaedic implants are reported in Table 2.

One of the widely used approaches is to change the surface properties working on the topographical characteristics of the implants or by introducing specific functional groups. Regarding for example metal implants, an increase in surface roughness can increase the surface area leading to osteoblast differentiation with higher expression of alkaline phosphatase (ALP) and osteocalcin expression respect to smooth surfaces¹⁰⁷. Moreover, the presence of NH₂ or OH groups can increase the wettability improving cell attachment *in vitro* on titanium implants¹⁰⁸. It has also been investigated how the type of functional group can actually influence the conformation of the adsorbed proteins such as fibronectin. The different active conformation of fibronectin can influence the type of integrin used for osteoblast attachment and indirectly influence the relative differentiation¹⁰⁹.

Another possible way to enhance osseointegration can be the deposition on the surface of biomaterials that can mimic the chemical composition of natural bone. In this category, it is possible to include calcium phosphate (CaP) like coatings such as hydroxyapatite (HA). HA coatings can be introduced following several techniques including pulse layer deposition and electrostatic multilayer assemblies using the layer-by-layer technique. These coatings have been widely investigated not only for their osteoinductive properties but also for their potential use as carriers of growth factors, bioactive molecules, and DNA¹¹⁰. In a study by He *et al.*, hydroxyapatite (HA)/collagen coatings were used as carrier of recombinant human bone morphogenetic protein-2 (rhBMP-2) and RGD peptide (RGD). The results showed significant improvement in mesenchymal stem cell (MSC) adhesion, proliferation, and differentiation on collagen-modified HA coatings that can be potentially used for early fixation of bone implants¹⁵. Similarly, in a recent study hydroxyapatite nanoparticles were complexed with chitosan to design nanoscale non-degradable electrostatic multilayers. These were then linked with a degradable poly (β -amino ester) based film having physiological amounts of rhBMP-2 to form an osteoinductive coating for MSC¹¹¹. Still concerns related to the efficacy¹¹² and safety of HA coatings are yet to be overcome mainly due to their possible fragmentation *in vivo*¹¹³ that is responsible for inflammation, delay in osseointegration and osteolysis. This problem can be addressed by reducing thickness of the coating as well as by increasing their homogeneity¹¹⁴.

Other possible coatings to promote osseointegration are the ones made of silicate-based bioceramic that are generally applied using plasma spray methods, showing higher bonding strength to

Table 2. Recent coating strategies to promote osseointegration

Substrate/ Coating	Coating strategy	<i>In vitro/in vivo</i> studies	Ref
Titanium/ Sericin-RGD	Sericin adsorption followed by EDAC/NHS conjugation of RGD	Osteoblast differentiation, Alamar blue assay, RT-PCR	115
Titanium/ Nano CaP crystals	Dual Acid etching	White rabbits tibial and femoral metaphysis implantation	116
Titanium/ Ca-P-Si-Na	Microarc oxidation, Steam hydro-thermal treatment, heat treatment	Rabbits implantation in tibia, Histological analysis, implant push out force tests	117
Stainless Steel (316)/ FN7-10	Passive adsorption	hMSCs differentiation studies, screw implantation in rats	118

FN7-10: recombinant fragment of human fibronectin, hMSCs Human mesenchymal stem cells, EDAC: ethyl (dimethylaminopropyl) carbodiimide and NHS: N-hydroxysulfosuccinimide, CaP: calcium phosphate

titanium implants respect to HA coatings¹¹⁹. This aspect is important as poor binding could lead to detachment of the coating from the titanium implants limiting their long-term stability once implanted *in vivo*. However, silicate based materials such as CaSiO₃ present high dissolution rate in biological fluids and attempt has been made to increase their chemical stability by incorporating Zn ions in their structure¹²⁰.

A possible alternative to CaP and silicate coatings is the fixation of bioadhesive coatings on the implants surfaces by entrapping or adsorbing ECM proteins, peptides¹²¹, growth factor¹²² or DNA¹²³. To achieve these objectives several available techniques can be considered including immobilization, hydrogels and layer-by-layer coatings. Immobilizations of peptides that contain cell binding domains represent one of the most investigated strategies to improve osseointegration in orthopaedic implants. Such peptides are more stable than large proteins and are able to withstand harsh treatments without losing their biological activity¹²⁴. Among them, the ones containing RGD sequences have been largely investigated, however their lack of selectivity in integrins binding reduce their potentiality as enhancer of osseointegration¹²⁵. To achieve better results different peptides displaying a more selective integrin binding have been proposed as alternative to the RGD sequence¹²⁶. For instance, titanium implants have been coated with a triple helical peptide, GFOGER derived from α_1 (I) chain of type I collagen that has specific affinity for integrin $\alpha_2\beta_1$. The Ti surfaces treated with the peptide showed higher ALP activity and calcium content compared to the ones uncoated¹²⁷.

Immobilization can be extended not only to peptides but also to cytokines and growth factors although their biological activity can be reduced after this process. For this reason in general it is necessary to introduce a layer on the implant surface that can be used to link the growth factor. Kashiwagi *et al.* reported the formation of a bioactive layer of artificial protein on titanium surface with the help of titanium binding motifs fused to the N-terminal of BMP-2¹²⁸. In a similar study Naskar *et al.* immobilized silk fibroins on titanium surface to facilitate the initial cell adhesion followed by improved cell spreading and better mineralization in order to achieve enhanced osseointegration. The immunological responses along with the effect of cytokines on osteoblast adhesion and function were investigated showing that the silk fibroin from non-mulberry source could be potentially used for better osteogenesis on orthopaedic implants¹²⁹. Apart from immobilization of adhesive proteins or peptides, polymeric hydrogels can be an alternative method to coat orthopaedic implants with bioactive molecules. A gel layer can be simply obtained by soaking the orthopaedic device into polymeric solutions that contain the biomolecule of interest prior hydrogel formation. This simple technique can be applied to coat implant with complex geometry although this method cannot be considered reproducible as variability can occur from batch to batch. In a study by Stadlinger *et al.*, various hydrogels were tested as coating of titanium implants loaded with TGF- β 1/BMP-4 growth factors using collagen, decorin and chondroitin sulphate. The results indicate that a collagen/chondroitin sulphate coating was the most effective among the tested hydrogels in promoting osseointegration¹³⁰.

Layer-by-layer coating is another widely used strategy. It consists of alternate repeated soaking of implants into polyelectrolyte solutions with opposite charges. This technique allows a better control over the loading efficiency and the release kinetics of the entrapped biomolecule respect to hydrogel coatings. In fact, several important parameters can be chosen such as the number of layers, type of polyelectrolyte and the concentration of the biomolecule. The layer-by-layer approach have been studied Macdonald *et al.* to promote delivery of different growth factors such as VEGF and BMP-2^{122, 131} (Fig 3). Here, a layer by layer coating was used as carrier for the dual release of rhBMP-2 and rhVEGF₁₆₅. The first one was released over a period of 2 weeks, while rhVEGF₁₆₅ eluted from the film over the first 8 days. The sequential delivery of growth factors has been used to target both blood vessel formation and bone formation. Most importantly, both growth factors retained their efficacy and an improvement in bone formation was observed compared to the system with the delivery of only one growth factor¹³².

3.2 Surface modification with osteoimmunomodulating factors to promote osseointegration

The immune and the skeletal system share several regulatory pathways. Extensive research over the last two decades has demonstrated the influence of the immune system on the regulation over bone healing^{106, 133}. This important cross-talk among

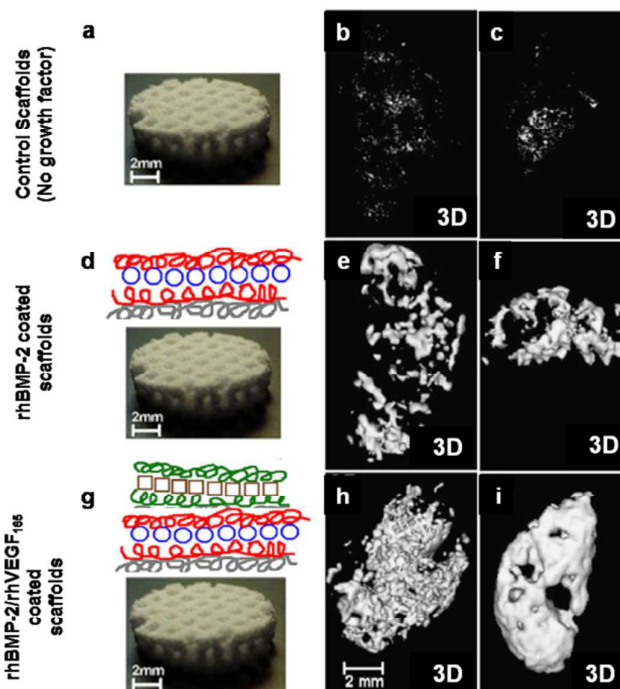


Figure 3: Osseointegration using growth factor. a) Bare macroporous polycaprolactone/b-tricalcium phosphate (50wt% PCL/50wt% bTCP) waffle cylinder scaffold, with diameter of 10 mm and height of 2.5 mm. b & c) Three dimensional (3D) μ CT reconstructions of intramuscular implanted scaffolds in rats. No detectable bone formation was found after 4 and 9 weeks in bare scaffolds. d) Schematic of polyelectrolyte multilayer films (PMF) using the layer-by-layer method entrapping rhBMP-2 on the surface of the scaffold. e & f) 3D μ CT scans of scaffold with PEM releasing rhBMP-2 was able to promote bone formation only to the periphery of the scaffold. g) PEM coating containing both rhBMP-2 and rhVEGF₁₆₅. h & i) 3D μ CT scans of scaffold releasing both growth factor showed ectopically bone formation at 4 weeks with a significant increase after 9 weeks. Reprinted with permission from ref. 131 Copyright 2011 Elsevier.

the two systems could be a useful tool for the design of a new generation of “immuno-informed” biomaterials capable of positively interact with the immune system rather than trying to dampen its effect. In fact, the innate immune system can play an important role in bone repair and maintaining its homeostasis. Among the cells of the innate immunity, macrophages have been identified as key elements for bone repair¹³⁴. According to the micro-environment, macrophages can display different polarization states into the pro-inflammatory M1 type and the anti-inflammatory M2 type. M2 population can be further subdivided into M2a (anti-inflammatory), M2b (immune-regulatory) and M2c (remodelling) mainly due to their surface receptor expression and cytokine secretory profile⁴. However, there is plasticity among macrophage phenotypes, meaning that they possess a continuum of phenotypes for distinct biological functions.

Macrophage polarization can be influenced by biochemical and biophysical cues that are more inherently to the material properties of the surface of the implants. In fact, macrophages rely for their migratory, phagocytic and mechano-sensing activities mainly on short lived focal complexes, point contacts and podosomes¹³⁵. In this regard, topographical cues including surface roughness, specific surface area, scale and shape of the feature³⁵ represent example of biophysical parameters that can be finely tuned to control macrophage cytoskeleton mediated mechanisms and influencing their response in bone healing. A lot of studies have been oriented in understanding the relation between macrophage phenotype and topographical cues of biomaterial surfaces^{136,137}. For example, Chen *et al.* found out that surface with 1 μm wide gratings showed the lowest inflammatory response compared to nanogratings and planar controls¹³⁷ (Fig 4). Same results were confirmed in other studies where topographical features of different polymeric microfibers in the range of 1 to 5 μm showed well acceptance after implantation *in vivo* respect to other with higher gratings¹³⁸. Apart from topographical characteristics, also mechanical properties can profoundly influence macrophage phenotype¹³⁹. In fact, macrophage elasticity can be modified by the substrate stiffness. For example Blakney *et al.* showed that hydrogel stiffness did not impact macrophage attachment, but was able to influence their morphology and corresponding phenotype. In fact, hydrogels with lower stiffness led to reduced macrophage activation and a less expression of TNF- α , IL-1 β , and IL-6¹⁴⁰. Considering all these factors to design better orthopaedic implants, scientists are now trying to decipher all the unique surface properties that control macrophage polarization in an attempt to enhance the process of osseointegration. For instance, titanium topography of orthopaedic implants is one of the factors that has been an object of intense research over the years. In a recent study by Calvo-Guirado *et al.*, biomechanical comparison of bone response to commercially pure titanium screws with four different types of surface topographies was investigated. In specific, the surface of the titanium implants were sandblasted, acid-etched or treated with a combination of the two techniques along with a four group in which also a discrete crystal deposition (DCD) of HA was applied. Implants were placed in the tibial metaphysis of 30 rabbits and this study indicated that only titanium with increased surface roughness showed statistically significant increase in osseointegration, 56 days after implantation¹⁴¹. Apart from conventional HA coatings used in the previous study, ceramic containing bioactive elements such as Mg, Si and Sr have also been proved to have immunomodulatory effects that can enhance *in vivo* osteogenesis. For example clinostatite coatings (MgSiO₃) or bioactive ceramic having Mg, Si and Sr (Sr₂MgSi₂O₇) are capable of controlling macrophage differentiation into the M2 lineage while inhibiting inflammation and osteoclastic activity^{142,143}. Similarly, titanium surface hydrophilicity has been proven to accelerate implant osseointegration in humans¹⁴⁴. In a recent study, the mechanism behind this modulatory effect has been further investigated by evaluating the inflammatory cytokine expression profile in a murine macrophage cell line. Results illustrated how the increased hydrophilicity can decline the pro-inflammatory response by activating NF- κB signalling¹⁴⁵. Importantly, MSCs co-cultured with macrophages in 3D scaffold led to a significant decrease in the secretion of soluble factors related with inflammation and chemotaxis including IL-6 and MCP-1 compared to 2D settings¹⁴⁶. To further investigate the influence of macrophage over tissue remodelling, post implantation, other studies have been carried out. Brown *et al.* explored whether the presence of a cellular component in ECM scaffolds influence macrophage phenotype.

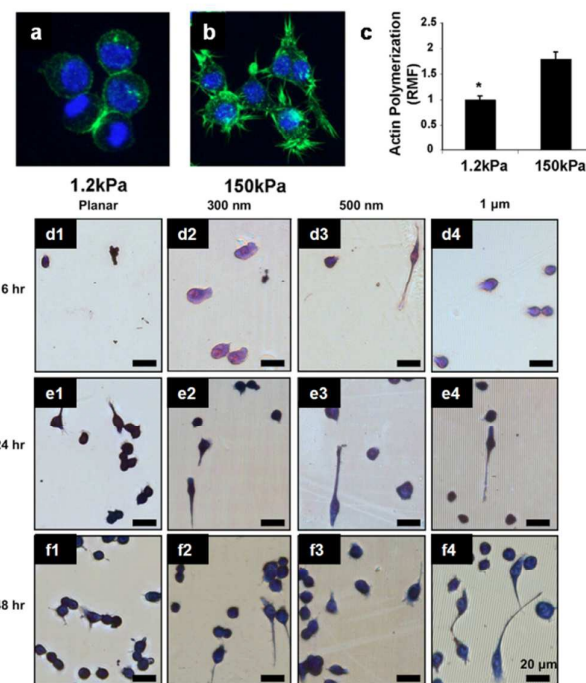


Figure 4: Influence of elasticity and topography on macrophage polarization. a & b) confocal images of macrophages cultured on substrate with different elasticity showing a decreased presence of filopodia in less rigid surface (1.2 kPa). b) Quantification of actin polymerization in macrophages cultured in different elastic surfaces. d) Photomicrographs of adherent macrophage cells on PDMS gratings and controls of constant height (at 350 nm): planar control (Column 1), 300 nm gratings (Column 2), 500 nm gratings (Column 3) and 1 μm gratings (column 4). Figure a-c reprinted with the permission from ref 139 and figure d-f from ref 137. Copyright 2010 Elsevier.

Acellular matrices were shown to elicit a predominantly M2 type response and resulted in constructive remodelling, while those containing a cellular component presented M1 polarization response leading to deposition of dense connective tissue and scarring¹⁴⁷. Very recently, a novel approach to assess osteogenesis *in vitro* has been proposed by Chen *et al.*¹⁴⁸. In their study the osteogenic capacity of cobalt incorporated with β -tricalcium phosphate (TCP) was assessed using a coculture of MSCs and macrophages and compared with the traditional “one cell type” method. Macrophage phenotype switched to M1 in response to TCP. The group also performed *in vivo* studies to support their *in vitro* findings. This study represents an important example that combines both immune and skeletal system together and represents a smart approach for *in vitro* testing of orthopedic implants and predicts their behaviour once implanted *in vivo*.

4. Conclusion and Future Directions

To conclude, the review highlights the currently available strategies to control the surface properties of biomedical implants for cardiovascular and bone therapy applications and possible solutions to control the adverse host responses. Emerging approaches to control host immune responses against the foreign medical implants using bioactive molecules and polymers are also underscored. It can be stated without any reservation that significant advancements have been made in developing different

REVIEW

types of bioinert surfaces to prevent non specific protein adsorption. With respect areas where we still need to improve is to close the gap between *in vitro* and *in vivo* study outcomes. The main cause for this mismatch with respect to cardiovascular implants is probably due to the incomplete understanding of the specific mechanisms involved in the cross-talk between the immune system and the coagulation mechanism. A better understanding of the underlying pathways, which bridge the gap between the two systems, will significantly contribute to improve the biomaterial coating strategies, suppress thrombosis and host complement activation systems. Since thrombus formation involves several complex mechanisms, surface modifications that address only one possible way to block its formation does not present a holistic strategy to manufacture successful cardiovascular implants. It is imperative to integrate several coating strategies with complementary strengths to build smarter next-generation biomaterial-based medical implants.

In near future, strategies to engineer the ideal bioinert coatings can be harnessed from the nature considering the vast number of non-biofouling surfaces present. In a recent study by Leslie *et al.*, such a concept has been put forward and demonstrated by fabricating a bioinspired, omniphobic coating surface capable of repelling blood and suppressing biofilm formation¹⁴⁹. To do this, a slippery liquid-infused porous surface (SLIPS) was synthesized following the *Nepenthes* pitcher plant's example, that uses a layer of liquid water to create a low friction surface that prevents attachment of insects. Such bioinspired materials thus signify an innovative approach that may lead to the discovery of successful coatings in the field of biomedical devices.

Our second objective was to shed lights on the current research works in the field of surface chemistry and material interfaces to control host biological responses and tissue integration. This is extremely important in case of orthopaedic implants, in which osseointegration is a necessary step to accelerate bone healing. We believe the innovative coatings illustrated in this review, particularly the ones which consider immune responses as a useful resource to promote bone healing, has immense potential for future clinical applications.

Ideally, the next generation orthopaedic implants should be able to deliver the optimal amounts of growth factors within the bone healing area while exerting a possible control over the immune responses. One possible way could be the entrapment on the surface of growth factors recruiting M1 macrophages over an initial period of time to initiate vascularization followed by the sustained release of other cytokines to recruit M2 macrophages, that will help the formation of a mature and stable vasculature. However, very little is known about the specific roles of the two macrophage phenotypes M1 and M2 in the different musculoskeletal diseases and healing phases. Growing understanding of these mechanisms to regulate macrophage polarization will help pave the way in developing more advanced orthopaedic implants with emerging surface bioengineering techniques. Microfabricating *organ-on-a-chip in vitro* models capable of mimicking the cross-talk between osteoblast and macrophage could play a highly beneficial role in the advancement of these technologies.

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