Surface engineering of biomaterials: optimizing interactions between biomaterials and host tissues and organs

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Abstract

Interfaces between biomaterials and living system are critical in regulating their interactions. Poor biocontact properties always limited the performance of biomaterials in biological environment. Surface engineering aims to control the interface interaction to further enhance the desired behavior of biomaterials. Upon implantation of biomaterials into the biological environment, a series of host responses are initiated. Non-specific protein adsorption on biomaterials is the essential stage of all biological reactions that associated with implants failure, device-related infections and blood-coagulation. In this review, we first focused on surface modification techniques to eliminate protein adsorption by emphasizing PEGylation of both macroscopic surface and nanoparticle system. Next, recent developments in surface engineering of biomaterials to optimize interactions between biomaterials and specific host tissue and organs are discussed. Optimizing the biocontact property of blood-contact devices can improve their hemocompatibility and maintain vascular homeostasis. Surface modifications of orthopedic and dental implants confer improved osteointegration and tribology performance. Controlling the surface chemistry and topography, and immobilizing biomolecules can aid the expansion and direct the differentiation of stem cells.

Keywords

Anti-biofouling, Blood-coagulation, Biomaterials, Regulation of stem cells, Surface engineering

Abbreviations

PEG, polyethylene glycol; **PHMB,** poly (hexamethylene biguanide) hydrochloride; **PVDF,** polyvinylidene difluoride;**PLA,** polylactide; **PLGA,** poly(lactide-co-glycolide);**PDA,** polydopamine; **PEI,** polyethyleneimine;**PET,** polyethylene terephthalate; **PDMS,**polydimethylsiloxane; **HA,** hydroxyapatite; **UHMWPE,**ultra-high molecular weight polyethylene; **PVPA,** poly (vinylphosphonic acid); **PTFE,** polytetrafluoroethylene;**CoCrMo alloys,** Cobalt-Chrome-Molybdenum alloys; **EC,**endothelial cell; **SMC,** smooth muscle cell; **hMSCs,**human mesenchymal stem cells; **ECM,** extracellular matrix;**BSA,** bovine serum albumin; **RGD,** Arg-Gly-Asp;**REDV,** Arg-Glu-Asp-Val; **YIRSR,** Tyr–Ile–Gly–Ser–Arg;**NPs,** nanoparticles

Introduction

Biomaterials have been widely used in various healthcare applications such as implants, blood-contacting devices, tissue engineering and regenerative medicine, drug delivery and biosensors. However, their performance can be suboptimal in some cases due to the unsatisfactory interactions between the biomaterials and living matters such as cells, blood flow and host tissue. Surface engineering of biomaterials aims to enhance their performance in contact with biological environment by combining the benefits of modified surface and retained bulk properties of the substrate. The engineered surface will construct a new interface to contact with biological substances that forms the biointerface (Figure 1A). Surface engineering in terms of surface treatments on original surface and surface coating of additional layer can achieve alteration on surface composition, topography and chemistry (Figure 1B). By deliberate selection and employment of surface engineering techniques, specific objectives can be achieved as required.

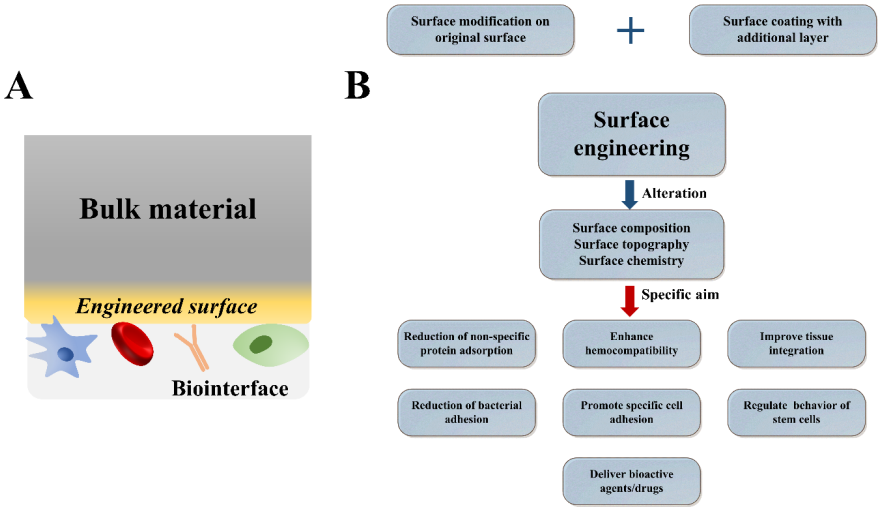

Figure 1. (A) Basic concept of surface engineering of biomaterials to control the interaction between living matter and biomaterials. (B) Surface engineering includes modification on original surface and additional layer coating to control over surface properties. By altering surface characteristics, various purposes can be fulfilled including enhanced biocompatibility, antibacterial ability, cells regulation and delivery of bioactive agents for specific applications.

When extracorporeal devices such as orthopedic implants, drug-eluting stents, tissue engineering scaffolds and microfluidics first contact with the living matter of human body, the body would elicit a foreign body response involving inflammation, blood coagulation, fibrous encapsulation, and rejection in extreme cases[1].

Protein adsorption is the first major event in the interaction between the living matter and implanted devices. Subsequent events such as cellular activities and signaling pathways initiation are largely dependent on their interactions with the deposited protein layer. For example, the complement system can be activated by protein adsorption. Blood-coagulation process will be initiated for wound healing. Neutrophils are responsible for the acute inflammatory response; they will migrate to the interface and degrade the foreign objective. Monocytes will be recruited to the biomaterials-tissue interface and differentiated into macrophages attempting to eliminate foreign objects, which marks the chronic inflammatory response. Macrophages uptake the debris as well as injured tissue and clear them through phagocytosis. However, with a large mass of foreign objective, a “frustrated phagocytosis” occurs resulting in aggregation of macrophages to form multinucleated foreign body giant cells. Fibroblasts will be activated and secrets collagen fibers aligned parallel to the surface of biomaterials that forms a fibrous capsule. Fibrous encapsulation is always formed around the implant to screen it from the body. Those undesirable reactions result in destruction of local tissue as well

as implants failure. Such non-specific protein adsorption is governed by protein properties such as protein structure, polarity and charge distribution, and features of biomaterial surface including chemistry and topography as well as environmental conditions including pH and temperature[2]. Engineering biomaterials with an anti-fouling surface will create a protein-resistance layer to improve their performance. Herein, we first described surface engineering methods to construct anti-fouling surface by underscoring the use of PEG in both macroscopic surface and nanoparticle system.

The adsorption of plasma protein will benefit the bacterial adhesion as well. Upon binding to the surface, bacteria will proliferate rapidly and secrete extracellular matrix leading to biofilm formation (Figure 2). The biofilm is a colony of immobilized bacteria on the surface of biomaterial that exhibits a robust structure. Bacterial biofilms are much harder to eradicate by antibiotics than circulating bacteria[3]. The biofilm formation results in device-related infections limiting the success of implant and medical interventions. Anti-biofouling surface formation is known as the "passive" strategy to address the problems of bacterial adhesion. "Active" strategy by construction of anti-bacterial surface is discussed as well. Various bactericidal substances are incorporated in surface modifications including silver ions, antimicrobial peptides, antibiotics and antibacterial polymers.

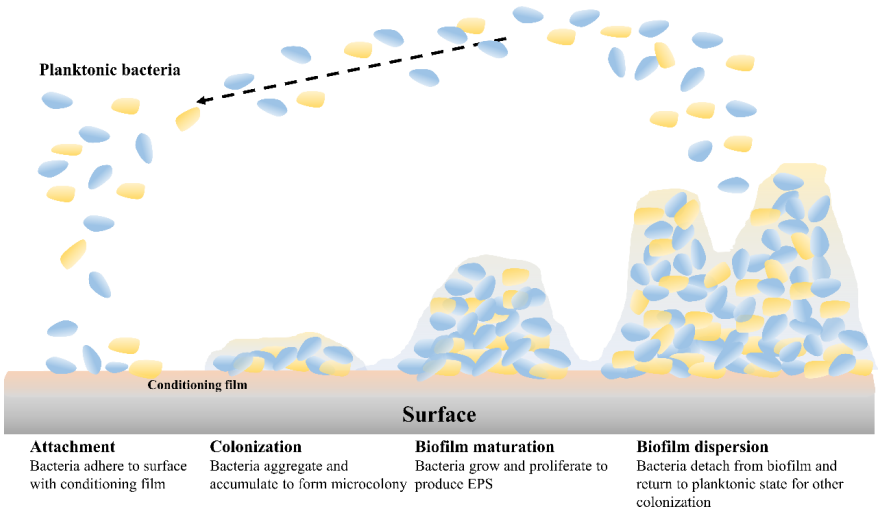

Figure 2. Biofilm formation process. Conditioning film is formed upon protein adsorption. Bacteria adhere and proliferate on the surface to produce extracellular polymeric substances (EPS).

There is a high demand of medical device and implants especially for blood contacting devices as well as orthopedic and dental implants. Here, we focused on how surface engineering techniques on blood-contacting devices and hard tissue implants improve their biocontact performance. Current surface modifications to optimize the antithrombogenicity of biomaterials mainly include physiochemical treatments such as surface patterning, plasma treatment and surface coating especially with heparin, and biofunctionalization that relies on incorporating bioactive agents. Surface engineering of biomaterials for hard tissue applications typically focusing on promoting implant-tissue integration and enhancing the corrosion and wear resistance.

Stem cell-based strategies offers a great potential to tissue engineering and regenerative medicine owing to their self-renewal ability and multipotency to differentiate into multiple linages. Normally, stem cells are isolated from their original microenvironment and processed through *in vitro* expansion prior to seeding on scaffolds for engineered tissue production. Notably, the substrate in which the stem cells are cultured is required to encourage their proliferation and expansion while maintain their multipotency. Subsequently, the large population of stem cells is favored for producing engineered tissue through desired differentiation. Therefore, deliberate selection of the biomaterials and proper surface modifications are critical to stem cells regulation.

Commonly used surface engineering methods are summarized in Table 1. Physicochemical methods alters surface characteristics by physical texturing and/or chemical reactions including acid etching/oxidation, grafting of functional groups, surface coating by deposition and ionizing irradiation treatments and surface patterning by lithography[1]. Biological methods are mainly based on biomolecules immobilization either by physical adsorption or covalent bonding. Not all surface engineering techniques are applicable and favorable to all biocontact scenarios. Therefore, this review will be application targeted that recent advances in surface modifications to address associated problems in optimizing different interactions between biomaterial and living matter are focused.

Table 1. Summary of common surface engineering techniques used for biomaterials.

	Techniques	Characteristics
Physiochemical methods	**Blending**	Simple adsorption of functionalized additives to surface
	Acid etching	Surface roughening Surface oxidation
	Plasma treatments	"Dry" surface engineering technique Effective and universal method for all types of organic surfaces Introduction of reactive functional groups on the surface
	Plasma sputtering & etching	Materials/impurities removal Surface roughening
	Plasma polymerization	Thin polymer films deposition Good adhesion between the substrate and deposited layer
	Photon irradiation	Feasible to small and localized area Highly accurate surface topography altering Polar surface functional groups generation by controlled surface photo-oxidation
	Ion-beam deposition	Surface patterning Effective in controlling hydrophilic/hydrophobic balance Optimal durability of the modified surface
	Lithography Photolithography Ion lithography Electron lithography	Surface micro- & nano-structuring
	Thin film coating	Physical adsorption through weak forces (hydrogen bonding, van der Waals forces & electrostatic interaction)
	Dip coating	Simple and effective Homogeneous & smooth layer coating Controllable film thickness
	Spin coating	Controllable film thickness
	Langmuir-Blodgett Films	Possible multi-layer deposition with controlled internal structure

	Techniques	Characteristics
	Layer-by-layer assembly	Multi-layer deposition based on electrostatic interactions Suitable for various topography and structure
	Covalent immobilization	Strong adhesion to the surface Reactive functional groups on surface required Surface pre-activation of chemically inert surface required
Biological methods (Biomolecules (BMs) immobilization)	**Physical adsorption**	No chemical modification included Unstable & reversable interactions between BMs and surface Potential steric hindrance to proteins & peptides with long sequence
	Covalent immobilization	Strong attachment of BMs to surface Surface functional groups required

Optimizing protein adsorption and antimicrobial properties

The biological response varies considerably across the *in vivo*applications resulting in different requirements of biomaterials design. However, all undesirable biological reactions are associated with a series events know an "biofouling" that started with protein adsorption following by other biomolecules and cell adhesion. Therefore, creating a non-biofouling surface with minimal non-specific protein adsorption is of great importance to avoid undesirable biological response. There are two main conditions in which form non-adhesive surfaces as suggested: strongly hydrophilic or strongly hydrophobic[4]. Construction of hydrophilic surfaces of biomaterials is more favored in biomedical applications. Various polymers have been studied to generate a hydrophilic and protein-resistance surfaces. Among them, PEG has been the most widely used polymers for antifouling applications. PEG is a water-soluble amphiphilic polyether and termed as the "gold standard" of antifouling polymers[5].

PEG was coupled to a titanium oxide (TiO_2) surface by a 3,4-dihydroxyphenylalanine (DOPA) derivative as cross-linker[6]. The DOPA was used to construct a hydroxylated surface to graft PEG via an amination reaction. The antiadhesive property of PEG functionalized TiO_2 surface was assessed through the protein adsorption of BSA. Results revealed a reduction of BSA adsorption by a factor of 4 on PEG-surface compared to bare surface. Similarly, PEG was grafted to PVDF porous membranes via an amination reaction with reactive graphene oxide (GO) additives[7]. The antifouling ability and hydrophilicity of PEGylated PVDF/GO surface were significantly enhanced. The flux recovery rate (FRR) of PEGylated surface was 90.2% with a total fouling rate (R_t) of 20.7%, whereas the FRR of original PVDF/GO surface was 86% with a R_t of 26.7%. In one demonstration, a click reaction was conducted on silicon surface to create amine terminated layer for coating PEG with improved grafting density and uniformity[8]. The PEGylated silicon surface showed no fouling of human serum albumin and relatively lower adsorption of lysozyme. Silicon based biomaterials have been widely used for the development of ophthalmic devices such as contact lenses and intraocular lenses[9]. However, silicon-based contact lenses are always associated with limited wettability and excessive protein adsorption leading to ocular discomfort[10]. PEG coating was applied to intraocular lenses for improving hydrophilicity and antifouling property[11]. There is a commercial PEG based contact lens coating technology, Tangible Hydra-PEG, to improve the lubricity and antifouling ability of contact lens.

In addition to macroscopic surfaces, PEG can be incorporated to nanoparticle system to confer protein-repellent properties. NPs are widely used in nanomedicine and drug delivery applications. Whereas the protein corona formed on the surface of nanoparticle can induce fast uptake by macrophages, the reduced targeting efficiency and lower cancer cell uptake specifically for anticancer drug delivery[12]. PEG can be grafted on a wide range of NPs such as inorganic NPs, magnetic metallic NPs, polymeric NPs and nanoscale metal-organic frameworks. Mesoporous silica NPs was first surface modified with PEI-coated carbon dots for effective transepithelial transport and then coated with PEG for better mucus permeability and oral bioavailability[13]. PEG was coated on biodegradable PLGA NPs to improve the mucus permeability and retention of NPs as well[14]. The *in vivo*animal studies indicated a considerably improved colorectal retention of PEG-modified PLGA NPs compared to pristine PLGA NPs. The retention of PEG-modified PLGA NPs reached 2-hours post-administration in contrast to 15 min of bare PLGA NPs. A sequential antifouling surface can be constructed on porous silica NPs by grafting PEG via a photo-triggered system. PEG was conjugated to PEI surface with biotin conjugates as targeting molecules via a photo-cleavable ortho-nitrobenzyl linker; and PEI was conjugated to the surface of silica NPs. PEGylation afforded the antifouling property of NPs and avoided the clearance by macrophages. Upon light irradiation, the outer PEG layer would be detached from PEI surface leaving the negatively charged carboxylic acids. Together with positive charged amine groups on the surface, a zwitterionic surface was generated that preserves targeting efficiency of biotin and offers further antifouling property.

Zeolitic imidazolate framework (ZIF-8) NPs with encapsulated doxorubincin (DOX) was modified by PEG in one-pot[15]. PEGylation endowed improved colloidal stability of ZIF-8 NPs in both water and cell culture medium. There was a pH-sensitive drug releasing behavior of DOX@ZIF-8/PEG NPs and higher cytotoxicity to hepatocellular carcinoma cells than free DOX suggesting an enhanced cancer cell targeting ability. Drugs can be conjugated to PEG directly prior to decorate the surface of drug carrier. Curcumin was coupled to the hydroxyl groups of PEG to form drug conjugates; then the PEGylated curcumin was physically attached to magnetic Fe_3O_4 NPs. Such PEG modified drug delivery system possessed higher drug loading efficiency and a pH-sensitive drug releasing profile.

Construction of an anti-fouling surface using aforementioned techniques can benefit the reduction in bacterial adhesion. However, unlike the "passive" strategy relying on the production of low adhesive surface, an "active" approach involves an antibacterial surface based on the incorporation of antibacterial agents[1].

Metal NPs or ions can be incorporated in antibacterial surface. Silver related antibacterial activity has been widely studied. There are numbers of silver incorporated commercial products in healthcare such as Silverlon® surgical dressing and Palindrome Precision SI-silver ion antimicrobial dialysis catheter. Silver has been coated on single-walled carbon nanotubes (SWCNT) to achieve antibacterial activities[16]. Results showed a stronger bactericidal activity against foodborne pathogens of PEGylated silver coating than non-PEGylated silver coating. The *in ovo*administration of PEG/Ag-SWCNT indicated undetectable toxic effects on development of chicken embryo. Silver doped HA coating was deposited on NiTi alloys through electrodeposition to obtain an antibacterial and bioactive surface for orthopedic applications[17]. A composite coating composed of nano-HA and silver NPs on Ti6Al4V dental implants was developed for enhanced biocompatibility and additional antimicrobial property[18]. Since the HA coating was a porous layer, the antibacterial ability of silver layer would not be masked. There was some initial release of silver ions in the first 24 h immersion in cell culture media followed by a slow release. The initial release could be clinically beneficial for an early infection control.

Quaternary ammonium compounds (QACs) are antimicrobial materials and effectively against various bacteria. The most accepted mechanism of the antibacterial ability of QACs is the disruption of cell membrane due to the sufficiently long cationic polymer chains[20]. Another explanation is the disruption of divalent cations on cell membranes by the highly charged surface[21]. However, both explanations lead to concern about the potential cytotoxicity to human cells by QACs, which quite limited their progress in clinic[3,22].

Antimicrobial peptides (AMPs) are widely used in antibacterial coatings due to their broad-spectrum antimicrobial activity. Magainin I (Mag) has been bonded to TiO_2 surfaces via PEG crosslinker[6]. There

was a significant reduction in *Listeria ivanovii* adhesion to PEG-Mag modified surface compared to bare surface by a factor close to 2. Even though some bacteria were adhered to modified surface, such bacteria exhibited abnormal morphology revealing a detrimental effect from the antimicrobial surface. Another AMP, ε-poly-L-lysine (EPL), was dip-coated to 3D PCL/HA scaffolds to confer antibacterial property[23]. The EPL modification endowed a notable improvement in hydrophilicity and broad-spectrum antibacterial activities of PCL/HA scaffolds. Such antimicrobial activities against*S. aureus* , *E. coli* and *S. mutans* . can retain for 3 days.

Various antibiotics can be immobilized on the surface of biomaterials to directly achieve bactericidal effects. Triclosan has been encapsulated into multilayer films composed of PEG, PCL, chitosan and PAA[24]. Such films were deposited on PDMS substrates by layer-by-layer self-assembly. Zone of inhibition (ZOI) and bacterial LIVE/DEAD staining assays verified the high efficiency of this antibiotics delivery system. The ZOI against *E. coli* was 15 mm while ZOI against *S. aureus* was 3 mm. The multilayer film allowed a sustained release of triclosan up to 7 days enabling long-term antibacterial function. Importantly, the sustained antibiotics release was stimuli-responsive that can be triggered by pH and bacteria stimuli, which may address the problems of resistant bacteria. As implanted the triclosan loaded multilayer coated substrates in rabbit models, the implants related infection was considerably eliminated with an infection rate of 16.7% in comparison with infection rate of 83.3% in multilayer alone modified group.

Polymers with bactericide effects can also be employed as antibacterial coating such as chitosan and PHMB. Acid treated carbon nanotubes were incorporated in PCL fibers to enhance mechanical strength as well as to create negatively charged surface[25]. Chitosan as a positively charged polysaccharide can be strongly immobilized to the surface of PCL fibers through electrostatic attraction. ZOI assay confirmed the acquired antibacterial function of PCL fibrous mats attributed to chitosan with ZOI against *E. coli* of 11.15 ± 0.21 mm and ZOI against *S. aureus* of 8.38 ± 0.19 mm. A hemostatic and antibacterial sodium alginate/gelatin sponge was fabricated by surface engineering with PHMB and hyaluronic acid by alternately spraying them on the sponge layer-by-layer. Hyaluronic acid was deposited on top of PHMB to endow the surface with better biocompatibility. When encountered with Gram-positive bacteria such as *S. aureus* , the bacteria secreted hyaluronidase would degrade the hyaluronic acid layer leading to subsequent exposure of PHMB to perform the bactericide function. This bacteria-stimulated antibacterial sponge showed an on-demand strategy and exhibited excellent *in vivo* anti-infection performance. However, its antibacterial property against Gram-negative bacteria such as *E. coli* was quite limited due to the masking effect of hyaluronic acid layer on PHMB.

Optimizing interactions between biomaterials and blood

A cascade of biological events can be initiated at the blood-material interface leading to thrombosis and intimal hyperplasia. Thrombus formation can be initiated intrinsically by surface interactions with adsorbed proteins or extrinsically by clotting factors derived from damaged tissue. The interaction between clotting factors and platelet surface receptors leads to platelet activation. The cleavage of prothrombin via prothrombinase formation in which those two pathways converged into one common pathway generates thrombin. The common pathway converts fibrinogen to fibrin that forms a hemostatic clot. The intrinsic pathway also known as contact-clotting pathway, is considered as a more critical pathway in biomaterial-associated blood coagulation (Figure 3).

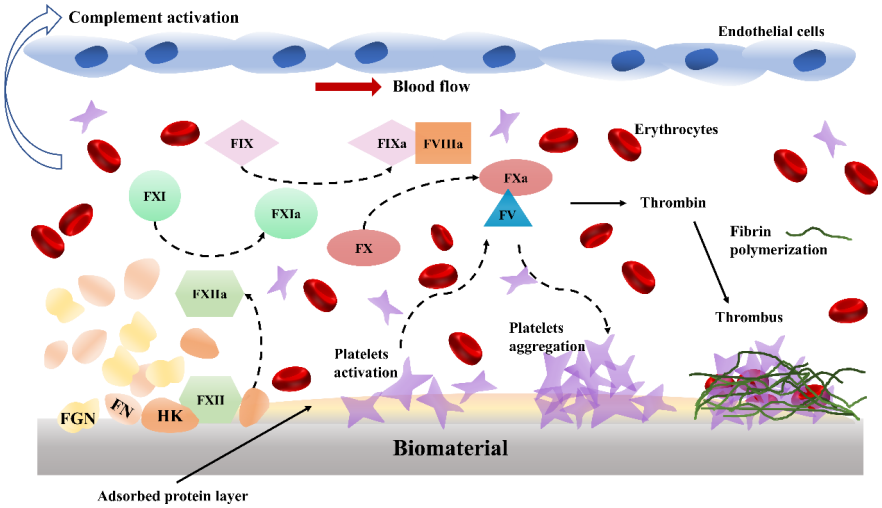

Figure 3. Schematic representation of intrinsic blood-coagulation mechanism. Upon the implantation of biomaterials, proteins from plasma and ECM will be adsorbed on the surface rapidly resulting in a thin protein film deposition and subsequent restructure of the blood-material interface[26]. Plasma protein in high concentration will first be adsorbed onto the surface such as fibrinogen (FGN) and fibronectin (FN). Those proteins will eventually be replaced by trace proteins with high affinity such as high molecular weight kininogen (HK) and clotting Factor XII (FXII), known as "Vroman effect"[27]. The complement system is also triggered resulting in immune response to the biomaterial. Once adsorbed on the surface, FXII undergoes conformational change and is activated to FXIIa. Activated FXIIa will sequentially activate other clotting factors: FXI and FIX. FIXa complexed with cofactor FVIIIa will further activate FX to FXa leading to thrombin generation and conversion of fibrinogen to insoluble fibrin. Platelets will be activated and adhere to the surface through protein adsorption. Activated platelets release other clotting factors to facilitate the thrombin formation and further platelets activation and aggregation. Thrombin further promotes the polymerization of fibrin. Together with platelets aggregation, an insoluble thrombus is formed.

Designing surfaces of blood-contacting biomaterials should consider the protein adsorption, thrombin generation, platelet adhesion and cellular behavior at the interface especially ECs and SMCs to improve grafts patency and thrombogenicity reduction. The intact vascular endothelium is responsible for anticoagulant properties and vascular protective functions. Endothelium contains prostacyclin and nitric oxide (NO) that exhibit signal-inhibit effects to inhibit platelet aggregation and activation as well as the proliferation SMCs[27]. Overgrowth of SMCs is an early stage of intimal hyperplasia formation. Inspired by the thromboresistive nature of the vascular endothelium, achieving fully endothelialization on the luminal surface either through*in vitro* or *in situ* approaches has been highlighted as the ultimate solution[26].

Topography on the micron- and nanometer scale of the surface plays a crucial role in antithrombogenicity. For example, picosecond laser ablation technology was adopted to micropattern PEG-functionalized PLA vascular grafts with parallel microgrooves with varying geometries[28]. It was found that all microstructured surfaces were non-toxic and non-hemolytic. A specific feature with 20 to 25 μm wide and 6 to 7 μm deep favored the adhesion of EC. The hydrophobicity of patterned surface was significantly increased with the water contact angel changed from 71.1 ± 0.2° to 112 ± 1° after laser ablation. Since PEG element was homogenously incorporated in the substrate, the topographic change would contribute to the increased hydrophobicity instead of the removal of PEG from the top layer. However, higher platelet adhesion on patterned surface may be attributed to the increased surface roughness due to the presence of nanopores after micropatterning. It was believed by the authors that under*in vivo* conditions, the platelet adhesion on microstructured surface would be mitigated due to the micro shear gradient produced by hemodynamics around the patterns.

It was suggested that the heparin-like molecule (heparan sulfate) residing on vascular endothelium plays a key role in thromboresistance[29]. Heparinization of biomaterials has been widely used in clinical practice to improve hemocompatibility. Various heparinized blood-contacting devices are currently in market[30] such as Palindrome Precision H-heparin coated dialysis catheter (Medtronic) and Affinity Pixie Arterial Filter (Medtronic).

A biodegradable PLA vascular stent was fabricated by 3D printing and heparinized through PDA/PEI intermediates to improve hemocompatibility and anticoagulation property[31]. The surface of PLA is lack of functional groups that limits its heparinization potential. Mussel-inspired natural PDA can bind to substrates under mild aqueous conditions instead of organic solvents. Since the amine groups provided by PDA are insufficient, amine-rich PEI was introduced onto the surface to effectively conjugate with heparin. Heparinization resulted in significant increase in stent flexibility as evaluated by a three-point bending test (1.00 +- 0.11 N of heparin-coated stents v.s. 1.39 +- 0.24 N of bare PLA stents). It was confirmed that those heparinized stents suppressed SMCs proliferation while promoted ECs proliferation. The *in vitro* adhesion tests showed that fewer fibrinogen and platelets attached to heparinized stents compared to PDA/PEI coated ones, which reveals their anti-thrombogenic properties. When implanted those stents in porcine models, the heparinized stents showed the most promising lumen patency with inhibited neointima hyperplasia and lowest area restenosis.

Traditional drug-eluting stents rely on the incorporation of cytotoxic or cytostatic drugs such as paclitaxel for inhibiting the migration and proliferation of SMCs[32]. However, those drug-eluting stents are always associated with delayed re-endothelialization due to the suppressed growth of ECs. Co-immobilization of two or more biomolecules into the vascular grafts is developed to obtain complementary or synergistic functions in SMCs suppression while ECs promotion. However, bioactive molecules with relatively distinct therapeutic effects will impair the combined efficacy due to the absent interactions between those molecules, which further hinders their practical use[33].

An endothelium mimicking coating was developed through the sequential conjugation of heparin and nitride oxide (NO)-releasing substance on 316L stainless steel stents[34]. There are other studies that investigated the combined effects of NO and heparin on healing outcomes of vascular grafts based on employment of NO donors[35]. Whereas the safe therapeutic dose of NO remains uncertain and the half-lives of those NO donors are unsatisfactory, limiting their applications in long-term devices[34]. The NO-releasing compound used in this study was selenocystamine (SeCA) to realize *in situ*catalytic generation of NO. The bioactivity of both biomolecules was retained and not affected by each other. The heparin/SeCA treated stents combined the anticoagulant function by heparin and anti-platelet adhesion by NO-releasing. The migration and growth of SMCs were effectively suppressed, whereas the growth of ECs was promoted. When implanted the heparin/SeCA coated stents in iliac arteries of rabbits, the enhanced re-endothelilization and suppressed restenosis were achieved.

Nitinol (NiTi), known as shape memory alloys, is widely designed for self-expanding vascular stents to prevent the possible plastic deformation in vessels due to the balloon expandable stents. However, excessive nickel ion releasing from the nitinol can lead to cellular inflammation[36]. A nanocomposite coating composed of TiO_2 nanotubes and chitosan-heparin particles, was developed to obtain improved hemocompatibility as well as enhanced corrosion resistance. The TiO_2 nanotubes were deposited on NiTi alloy by electrochemical anodization followed by chitosan-heparin NPs coating via an intermediate dip-coated PEI layer. Those nanoparticles can act as drug carriers for sustained release of heparin. There was a continuous release of heparin for 2 weeks after the initial release. It was reported that the anodization of highly ordered nanotubular structure to nitinol surface would improve its corrosion resistance and reduce nickel ions releasing[37]. The TiO_2 nanotubes layer effectively reduced the release of nickel ions, while the nanoparticles coating also inhibited those ions releasing. Compared to bare metallic and anodized stents, the chitosan-heparin incorporation resulted in significant reduced hemolysis ratio and platelet adhesion as well as enhanced the attachment, spreading and proliferation of ECs. Whereas the effects of this nanocomposite coated nitinol stents on SMCs were not determined.

Many researchers aimed to promote the adhesion and growth of ECs, yet obtained limited re-endothelialization. Possible reason can be the ignorance of the competitive growth between ECs and SMCs[34,38]. Therefore, the efficient surface engineering techniques on ECs proliferation are suggested to perform a co-culture assay of ECs and SMCs. For the aforementioned heparin/SeCA treated stents, they exhibited a synergistic effect on ECs over SMCs. ECM peptides can be incorporated to vascular grafts to influence cellular behavior and output specific interactions to surrounding. Several biomolecules incorporated in vascular grafts such as RGD peptides are not cell-specific, that raises the concern about competition between ECs and SMCs. The REDV polypeptide is specifically recognized by ECs making it an ECs-specific biomolecule. Xue *et al.* covalently immobilized REDV peptides on nitinol reinforced PET microfibrous grafts through PDA NPs[38]. Such surface modification on microfilaments produced hierarchical micro/nanostructures that benefit cell attachment and proliferation. REDV immobilization grafts improved the hemocompatibility with untraceable hemolysis rate as well as ECs proliferation and increased release of NO. Besides single peptide, Peng *et al.* studied the effects of multiple-peptides (YIGSR, RGD, and REDV) immobilization of silk fibroin scaffolds on ECs[39]. YIGSR-modified scaffolds showed the highest cell migration rate compared to RGD- and REDV-modified scaffolds. Whereas dual-peptides (YIGSR+RGD) significantly enhanced the proliferation of ECs compared to other dual-peptides combination.

Optimizing interactions between biomaterials and hard tissue (orthopedic & dental)

Metals and metallic alloys are widely used in biomedical applications especially for load bearing and hard tissue prosthesis. Titanium and its alloys are well-established biomaterials for dental and orthopedic implants due to their excellent mechanical strength, light-weight, biocompatibility and corrosion resistance. However, the surface of titanium alloys is bioinert, which limits their potential in promising osteogenesis and osseointegration[40]. Recent advances in surface engineering of titanium alloys mainly focus on improving the bioactive interactions between implants and host bones through nanoscale functional coatings such as titanium oxide layer and bioactive calcium phosphate deposition.

Microarc oxidation (MAO) can produce porous titanium oxide coating on metallic implants. A novel hierarchical implant surface with micro/nanomorphology was developed by a duplex coating process. A titanium oxide layer was first generated by MAO, and then the coating was electrochemically reduced in alkaline solution (MAO-AK)[41]. Such modified titanium promoted adhesion and proliferation of seeded canine bone marrow stem cells. Besides, those stem cells were guided towards osteogenic differentiation by MAO-AK modified titanium. As implanted into canine femurs for 10 weeks, accelerated bone formation and higher bone-implant contact ratio were noticed in MAO-AK treated titanium compared to MAO only treated implants. Yang and Huang developed multiform TiO_2nano-network coated titanium implants through a simple electrochemical anodization process[42]. The pore size in this TiO_2 coating ranged from a few nanometers to a few hundreds of nanometers, which provided a large number of cell adhesion sites for the formation of focal adhesion complex. Such surface modified titanium implants promoted the osteogenic differentiation of human bone marrow hMSCs.

Different oxidizing atmosphere of titanium implants can result in surface deposition composed of various phases. It was investigated that surface oxidization of titanium in air leading to the rutile bioactive phase (TiO_2) deposition. In contrast, under pure oxygen atmosphere, titanium monoxide (TiO) also formed on the surface besides TiO_2[43]. High concentration of oxygen in pure oxygen atmosphere may induce a rapid oxidation process, thus forming an oxide layer on the surface which inhibits further oxidation. On the contrary, less oxygen in air allows more diffusion of oxygen across the titanium surface which leading to a gradual and sufficient titanium oxidation process. Different atmosphere treatments showed no significant effects on surface topography. Whereas the hydrophilicity of air-treated surface was significantly higher than that treated by pure oxygen. Similarly, air-treated implants were more efficient in apatite forming, cell attachment and proliferation, which suggests that air is more promising for the titanium implants oxidation

compared with pure oxygen for better biofunctionalization outcomes.

Jeong *et al.* studied the effects of nonthermal atmospheric pressure plasma treatment (NTAPP)-treated titanium dental implant surface on oral soft tissue integration and control of cytokine release[44]. The inflammatory cytokine release is essential to physiological functions; however, overproduction may cause the destruction of surrounding soft tissue. The topographic features of titanium surface were not altered due to NTAPP treatment, whereas higher hydrophilicity and surface energy were detected. Inflamed cells on NTAPP-treated samples exhibited lower cytokine release compared with those seeded on untreated implants. However, higher cytokine level of inflamed cells was observed when compared with normal cells on NTAPP-treated implants. Which suggests that such surface engineered titanium implants may control the cytokine release necessary for proper inflammation response instead of a complete reduction in cytokine release.

Hydroxyapatite (HA) as an example of calcium phosphate, is an osteoconductive biomaterial that closely resembles the mineral phase in native bones[45]. HA coatings have been used for fixation of titanium hip replacements for over 20 years. Recent research focuses on adopting HA as a base layer and incorporating other functional molecules for diverse functions such as healing acceleration and infection reduction. Sarkar and Bose coated titanium implants (Ti6Al4V) with HA via plasma spraying to achieve better osseointegration for load-bearing bone-defect repair after osteosarcoma resection[46]. Plasma spraying is the most common method to apply HA coating that creates a rough and porous microstructure benefiting bone fixation. Besides, a localized dual-drug delivery system was constructed by applying curcumin and vitamin K2 on the surface of coated implants through simple physical adsorption for postoperative chemoprevention. The surface roughness was significantly increased upon HA deposition. The drug included HA-coated implants showed excellent performance in inhibiting *in vitro* osteosarcoma cell proliferation, which indicates their chemopreventive effect. That could address the difficulty in bone regeneration in tumor environment and prevent tumor recurrence. To assess the *in vivo*osseointegration ability, drug releasing HA-coated titanium implants were inserted in distal femur of rats. Dual-drug incorporated implants showed prominently improved bone-implant integration compared to HA only coated implants. Combining localized drug delivery with enhanced biocompatible titanium implants is effective for repairing tumor-associated bone defects.

Engineering of titanium implants with TiO_2 nanotubes can improve surface chemistry and hydrophilicity, hence better cell attachment. However, the bioactivity brought by such nanoscale surface modification is reported to be insufficient compared to calcium phosphate (CaP) coating[47]. And in some cases, CaP coating encounters low adhesion strength to substrates and occasional *in vivo* delamination problems. Bose *et al.*applied strontium ions and silicon ions doped calcium phosphate coating on TiO_2 nanotubes modified porous titanium implants by biomimetic coating[48]. The TiO_2 nanotubes were first fabricated onto the titanium surface via electrochemical anodization. The surface modified metallic implants were immersed in SBF solutions at physiologic temperature and pH to grow homogenous CaP apatite layer on the surface. Histological evaluation showed evident and more osteoid formation and tissue ingrowth at the interface of CaP/ TiO_2 coated Ti implants than Ti implants with nanotubes alone. Such effects were more pronounced in early healing stage (4 weeks). Push out tests after 4-weeks implantation showed a higher shear modulus of CaP/ TiO_2 coated implants than TiO_2 alone coated ones (80 MPa v.s 26 MPa), which reveals a better tissue adherence and mechanical interlocking.

Besides the dual-coating of TiO_2 nanotubes and CaP onto titanium implants, there was a nanocomposite coating developed and applied to Ti6Al4V aiming for better corrosion resistance and osseointegration[49]. A PMMA-silica hybrid coating was synthesized by radical polymerization and deposited on Ti6Al4V by dip-coating. The PMMA-silica coated titanium implant presented a homogenous, relatively smooth and crack free surface with a roughness value of 1.3 +- 0.1 nm. The silica addition not only significantly increased the coating adhesion to the substrate, but contributed to notable improvement in coating durability ($>$ 100 days). As stated by authors, the PMMA-silica treated titanium implants exhibited an anticorrosive performance that are superior to other reported anticorrosion coating on Ti6Al4V implants, for instances, SiO_2-HA coating and PCL-HA coating.

The main cause of failure in joint replacements is implant loosening due to the inflammation response induced

by wear debris (Figure 4). A thin layer of polyamide was coated on UHMWPE to strength the surface for reduction in wear debris[50]. The polyamide coated UHMWPE showed significantly higher antibacterial property than uncoated implants as well as enhanced wound healing effect. CoCrMo alloys are mostly used in join replacement due to their relatively high corrosion resistance and optimal mechanical properties. To improve their tribology performance, more wear-resistant materials can be coated on the bearing surface. Lohberger et al. studied the biological effects of ceramic surface coating on CoCrMo alloys[51]. A 5.5 +- 1.5 μm thick TiN layer was deposited on CoCrMo alloys using physical vapor deposition. The TiN coating was considered to be anti-allergic, wear-reducing and biocompatible coating. Releasing of particles and metal ions due to corrosion and abrasion was reduced through the TiN coating. Human osteoblasts seeded on TiN coated alloys exhibited improved cell viability and adhesion properties.

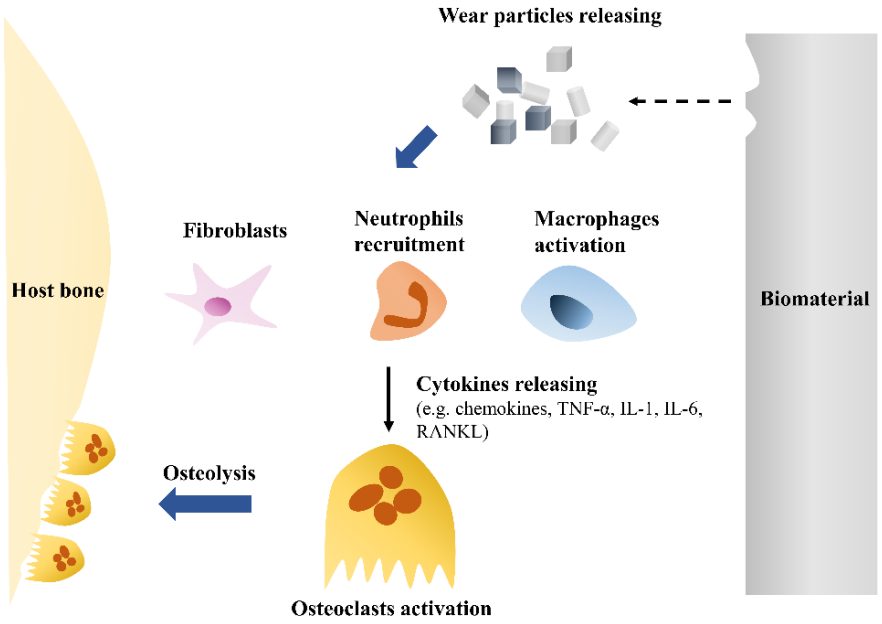

Figure . Wear-debris induced osteolysis. Wear debris releasing from implants initiates inflammatory response. Various cells such as neutrophils, macrophages and fibroblasts will be activated and recruited and release inflammatory cytokines. Osteoclast progenitor cells will be initiated to differentiate into osteoclasts. Osteoclasts are activated and responsible for osteocytic osteolysis.

Current research on improving wear resistance of metallic alloys also focuses on super-lubricous coating that mimics natural cartilage function. PVPA is a hydrophilic polymer with a high density of phosphate groups on the polymer backbone. Phosphate groups have a strong affinity to metallic surfaces such as aluminum and titanium[52]. PVPA was once deposited on Ti6Al4V surface by the evaporation-induced self-assembly method to construct a cartilage-like super-lubricous surface[53]. The friction coefficient in the interface between PVPA-modified Ti6Al4V and PTFE ball in the ball-on-disc machine showed a significant reduction in friction coefficient (˜70%) than unmodified implants. The coefficient was approximately 0.006 under a contact pressure of 44.2 MPa (initial pressure), which suggests its superlubricity. Such low friction coefficient can even maintain over a long period (over 8 h). The wear particles in the interface were superlow owing to the coating stability and most importantly, the fluid-like manner of the PVPA coating that allows fast exchange of the water molecules.

Optimizing interactions between biomaterials and stem cells

An ideal surface would promote the interactions between biomaterials and stem cells to achieve the expansion of stem cells without compromised potency, and differentiation of stem cells with maintained differentiated phenotypes. Niche is the native microenvironment where stem cells residing in that regulates the behavior of stem cells including adhesion, proliferation and differentiation through various intrinsic signaling pathways. Recent studies also focused on biomimicking such environment in terms of comparable mechanical and biochemical properties via biofunctionalization of various proteins, peptides and growth factors.

Embryonic stem cells (ESCs) are considered as pluripotent that can be differentiated into almost all different cell lineages. Human induced pluripotent stem cells (iPSCs) are derived from somatic cells through reprogramming. Unlike ESCs, iPSCs originated from human autologous cells can bypass certain ethical issues and exhibit lower immune response[54]. However, a feeder layer is frequently required to culture pluripotent stem cells and support their pluripotency. Mouse embryonic fibroblasts (MEF) and Matrigel are typically used as feeder layers; yet use of xenogeneic cell source and mouse sarcomas derived products brings about the risk of potential disease and pathogen transmission.

UV/ozone surface treatment has been applied to polystyrene substrates to construct feeder layer-free system for iPSCs[55]. The polymer chains of polystyrene were decomposed into shorter fragments through UV treatment, and formed functional carboxylic acid groups on surface. Results showed that a more hydrophilic and cell-adhesive surface was generated. Such changes in surface chemistry resulted in promoted attachment and proliferation of iPSCs. The pluripotency of iPSCs was well maintained as indicated by the comparable Nanog expression of iPSCs cultured on UV-treated PS to those on MEF feeder layer.

A vitronectin peptide (VN)-decorated nanofibrous niche was developed to promote *in vitro* culture and osteogenic differentiation of human iPSCs[56]. VN was immobilized to the PCL scaffolds through an intermediate carboxymethyl chitosan (CMC) layer. Grafting of CMC and VN tuned the initial super hydrophobic PCL surface to hydrophilic with water contact angle changed from $122.3 \pm 3.91^{\circ}$ to $23.8 \pm 1.0^{\circ}$. The peptide-decorated nanofibrous scaffolds well supported the proliferation of iPSCs with maintained pluripotency. Upon osteogenic induction by adding osteoinductive medium, iPSCs showed enhanced osteogenic differentiation in the feeder layer-free culture system. Decoration of VN to PDA-coated tissue culture plates via CMC conjugation not only stabilized long-term pluripotency of hESCs and hiPSCS, but supported reprogramming of human somatic cells (human urine derived cells and human umbilical cord blood cells) into hiPSCs under defined conditions[57].

An iron-containing porphyrin, hemin, was dip-coated on serum albumin (SA) electrospun scaffolds to confer conductivity resembling the electroresponsive nature of neurons[58]. Human iPSCs derived neural stem cells (NSCs) were cultured on surface treated scaffolds. Hemin doped SA scaffolds exhibited higher cell attachment and viability than non-doped scaffolds. Whereas no significant difference in NSCs differentiation was found. The electrical stimulation of hemin-doped scaffolds resulted in enhanced neuronal differentiation and maturation. Fibroblast growth factors-2 (FGF-2) was non-covalently bind to hemin-doped scaffolds. Although through a non-covalent binding, there was a strong binding of FGF-2 to SA scaffolds with a slow release profile. The FGF-2 incorporation led to higher cell proliferation yet lower neuronal differentiation than other respective groups without FGF-2. That is quite consistent with the prediction that FGF-2 mainly functions in the proliferation of NSCs.

Adult stem cells can be harvested from various sites such as bone marrow and adipose tissue that constitute an alternative stem cell source. Those stem cells possess multipotency that can be differentiated to various cell lineages unlike the pluripotency of embryonic stem cells. Various bioactive molecules including fibronectin, collagen, RGD peptides and designed peptide (R-peptide) were coated on glass substrates to study their effects on cellular behavior of bone marrow derived-MSCs[59]. R-peptide exhibits a sequence of GRKKRRQRRRGGGRGD by linking RGD peptide with basic domain of Tat protein (recognized as heparin binding domain). Well-established filopodia and focal adhesions of hMSCs were found on fibronectin and R-peptide coated substrates indicating enhanced cell attachment. There was appreciable difference in the proliferation

rate of hMSCs between R-peptide coated substrate and other coated substrates, which suggests R-peptide as a promising sequence for controlling proliferation and attachment of hMSCs.

FGF-2 and chitosan were conjugated to tissue culture polystyrene after the chemical vapor deposition (CVD) of parylene onto the surface for ADSCs culturing[60]. The CVD copolymerization process led to improved coating durability in terms of adhesive strength and thermal stability; and offered functional groups including amine and thiol groups to bind chitosan and FGF-2. Chitosan promoted the self-assembled cellular spheroids formation; FGF-2 enhanced the proliferation of ADSCs. Through a layer-by-layer assembly technique that based on alternating exposure of precharged PLGA/nanoHA membrane to polyelectrolytes, 14 layers of multipeptides can be grafted on surface based on a 3D peptide gradient[61]. Peptides functionalized PCL/nanoHA enhanced proliferation and osteogenic differentiation of bone marrow derived-hMSCs; upon *in vivo*implantation, the scaffolds showed enhanced osteoconductivity and improved bone healing.

Nanopatterning of platinum bulk metallic glass (Pt-BMG) was achieved by thermoplastic forming to study the effect of nano-topography on differentiation of adipose derived-hMSCs[62]. Nanorods of a nominal diameter of 200 nm were patterned on the surface by thermoplastic nanomolding. The surface roughness was significantly increased from 14.1 $\pm$ 2.8 nm to 231.7 $\pm$ 47 nm. The elemental surface composition and modulus remained unchanged. Results showed that nanopatterned Pt-BMG directed adipogenic differentiation of hMSCs, whereas flat Pt-BMG induced osteogenic differentiation. Many studies suggested that stiffer substrate guides preferential osteogenic differentiation. However, when increasing the stiffness of nanopatterned Pt-BMG, no difference in osteogenic differentiation was observed suggesting that the osteogenic differentiation of Pt-BMG was dominated by topography. Nanotopography can influence cellular behavior by interacting with integrin-receptors and the formation of focal adhesion. Focal adhesions are essential in sensing the stiffness of substrates and regulating intracellular signaling transductions[63]. Previous studies suggested that higher number of focal adhesions can lead to improved osteogenic differentiation[64]. While more focal adhesions were formed on flat Pt-BMG than nanopatterned one.

A nano-roughened PDMS surface was developed by chemical etching of a polystyrene mold using acetone and rapid prototyping of PDMS[65]. The surface roughness increases as raising the acetone concentration and etching time. Whereas no defined correlation was found between surface roughness and surface wettability. Protein adsorption was favored on more roughened surface as indicated by a significant increase in fibronectin adsorption on nano-roughened PDMS than native PDMS. The surface wettability was also increased due to fibronectin coating. The nano-roughened and protein coated PDMS enabled adhesion and proliferation of bone marrow-derived MSCs, which makes it potential for PDMS-based lab-on-a-chip devices.

Conclusion and future perspectives

The primary aim of surface engineering of biomaterials is improving their biological performance by controlling over the interaction between the surface and living system. It is suggested that physicochemical cues of the surface are intrinsically linked revealing that the alteration of the surface topography will lead to localized changes in surface chemistry. Surface engineering methods usually are combined to obtain optimal results. For example, chemically non-reactive surface requires pre-activation via surface oxidation, functional groups introduction or ionizing irradiation for further surface grafting or biomolecules immobilization.

Non-specific protein deposition underlies all undesirable biological reactions and triggers other biomolecules and cell adhesion accounting for "biofouling". PEG is considered as the "gold standard" in reducing bioadhesion and widely applied in anti-biofouling applications not only in biomedical applications but marine applications. However, PEG can suffer oxidative damage that limits its non-fouling feature for long-term applications[66]. Development of non-PEGylated hydrophilic surface with comparable protein-resistant property to PEGylated surface but better thermal and oxidative stability is of great interest. For example, dextran as a natural phosphorylcholines is studied as a PEG alternative for antifouling surface coating of biomaterials in long-term applications[1]. Zwitterionic polymers are another alternatives for antifouling sur-

face modifications that exhibit even stronger hydration effect than PEG[66]. A curcumin loaded zwitterionic polymersome was incorporated in PDMS contact lenses to improve the antibiofouling and antimicrobial properties[9]. Bacteria acidify the local environment like tumor cells. Creation of stimuli-responsive antibacterial surface such as pH-sensitive can offer an on-demand strategy to address the resistant bacteria. Silver-releasing coatings are widely adopted due to their bactericidal ability. However, there are concerns arisen from their potential side effects to proteins. Such effects seem to be limited in applications with easy excretion of silver such as urinary catheters, or where the benefits outweigh the risk, such as skin wound dressings[3].

Thrombosis and intimal hyperplasia account to the major causes of blood-contacting device failure due to the unsatisfactory hemocompatibility. Achieving fully endothelialization on the luminal surface is termed as the ultimate solution for anti-coagulation. When designing proper surface of blood-contacting devices, the competitive growth of ECs and SMCs should be considered. Heparinization is a common accepted technique to enhance the antithrombogenicity of biomaterial. The mechanisms by which heparinized devices modulated those cellular behavior remains unclear. Possible reasons could be the interchanges between heparin and thrombospondin that impairs migration and proliferation of SMCs; and binding between heparin and angiogenesis growth factors that accelerates endothelialization[67]. Metallic implants are currently used in many hard tissue applications especially in load bearing conditions. Improving the tribology performance of biomaterials through surface coating can mitigate the abrasive debris and enhance the corrosion resistance. Super-lubricous coating offers a new perspective in surface engineered implants for articulating joint with relatively low wear generation.

Retaining the pluripotency in cell culture stage and maintaining the differentiated phenotype of stem cells are both critical. Stem cells can respond to the mechanical cues generated by the surface engineered substrate and convert them into biochemical cues. Biomolecules immobilization such as growth factors and peptides can provide direct biological cues to stem cells. Full-length proteins are prone to undergo conformational change and proteolytic degradation induced by surface properties. On the contrary, peptides are preferred owing to higher stability and easier control of surface density[68]. Intermediate crosslinker is favored to conjugate biomolecules to the surface due to the avoided direct contact between biomolecules and biomaterials. However, the mechanisms of interactions between cell and biomaterials surface are not fully defined yet since there are a few cell-ligand interactions identified as present. Moreover, the behavior of engineered surface can vary across*in vitro* and *in vivo* studies since living body presents a dynamic and more complex environment. For example, platelet adhesion to micropatterned surface can be mitigated in *in vivo* due to the hemodynamics. Which suggests that long-term *in vivo* effects of surface engineering are necessary to fully understand the performance of biomaterials.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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