Bio-inspired nanofunctionalisation of biomaterial surfaces: a review

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Abstract: The surfaces of biomaterials determine their efficacy and hence, have an important role in clinical applications. Through bio-inspired surface nanofunctionalisation, the surface properties of biomaterials such as physical morphology and chemical composition can be tailored using biomimicry. It is a powerful tool for improving the interactions between the physiological environment and biomaterial surfaces. Therefore, research on bio-inspired nanofunctionalised surfaces has attracted much attention in recent years. This review focuses on the recent bio-inspired strategies based on the structure of the extracellular matrix (ECM) and composition of mussel-inspired polydopamine (PDA). The design, preparation, and properties of ECM and PDA-inspired nanofunctionalised biomaterial surfaces are reviewed. I have also highlighted the effects of these bio-inspired nanofunctionalised biomaterial surfaces on bone regeneration, cartilage repair, and antibacterial activities.

1 Introduction

The surface functionalisation of biomaterials is of critical importance in tissue engineering. The functions of the surfaces of biomaterials are the key factors in determining its biological performance, such as cell adhesion, cell proliferation, cell differentiation, and bacterial resistance. Generally, the specific biological interactions between biomaterials and surrounding tissues are determined by their physical topography and chemical composition [1–3]. Firstly, biomaterials with specific physical topographies at the micro or nano-levels participate in the biochemical reactions occurring in the physiological environment to form specific surfaces/interfaces after being implanted into the body [4]. Finally, the specific surfaces/interfaces form a mimic the extracellular matrix (ECM) 3D microenvironment, which accommodates cell growth. These specific surfaces/interfaces exhibit unique physical, chemical and biological properties, affecting the cellular processes on the surfaces, such as cell adhesion, proliferation, migration and differentiation.

In addition, most of the biological tissues react with the chemical composition on the biomaterial surfaces inside the body. Hence, the chemical composition of the biomaterials can also determine the surface properties, such as surface wettability and surface charge, which can be used to manipulate the cellular interactions on the surfaces [5]. Therefore, it is necessary to functionalise biomaterial surfaces for enhancing their biological performance.

The construction of biomaterials is often inspired by nature to enable them to exhibit ideal structures and functions. Bio-inspired nanofunctionalisation, which combines the biomimicry and nanotechnology, is a powerful approach to modify the biomaterial surfaces [6–9]. Over the past decades, various strategies have been developed to functionalise the biomaterial surfaces, such as electrochemical deposition (ED) [10], electropulse treatment [11], plasma spraying [12], layer-by-layer assembly [13], self-assembly monolayers [14], and additive manufacturing [15–17]. However, most of these strategies are utilised to construct a surface with a single function and hence, their applications and biological performance are limited. The environment around the human tissue is complicated and dynamic [18]. Biomaterial surfaces with a single function cannot adapt to this complicated environment, resulting in a chronic repair process. However, biomaterials at a nanoscale designed using biomimetics can mimic the structures and multi-functionality of natural components very well, such as the ECM, bone, DNA, and proteins. Bio-inspired nanofunctionalised biomedical surfaces are thought to integrate with surrounding tissues more effectively than conventional biomaterial surfaces [19–21].

In general, the following principles have been adopted in the design of bio-inspired nanofunctionalised surfaces: (i) biomimetic nanostructures; (ii) multifunctional synergistic system; (iii) smart stimuli-responsive system. In this review, I aim to present an overview of the latest progress in the development of the bio-inspired nanofunctionalised surfaces based on the structure of ECM and composition of mussel-inspired polydopamine (PDA). In the first part of this review, I will summarise the design strategies of the bio-inspired nanofunctionalised surfaces and the following parts will review their biomedical applications of the bio-inspired nanofunctionalised surfaces. The last part will introduce the outlooks on the bio-inspired nanofunctionalised surfaces.

2 Design strategies for creating the bio-inspired nanofunctionalised surfaces

2.1 Bio-inspired structures

In nature, various materials with fascinating properties can be used for the construction of bio-inspired materials. For example, the surface wettability could be inspired from surface structures of biological materials, such as butterfly wings [22], eyes of flies [23], spider silk [24], and lotus leaves [25]. In particular, it is important to mimic the structures found in the living organisms as they directly react with biomaterials. ECM is composed of a well-organised hierarchically nanostructure, adhesion proteins, and polysaccharides (Fig. 1a) [26]. This well-organised hierarchically nanostructure is constructed with protein fibres, which provides support for cell growth. Adhesion proteins, such as fibronectin and laminin, covering on the nanostructure manipulate cell adhesion, migration, proliferation and differentiation. Polysaccharides present in the nanostructure provide a pliable substrate for cell growth and delivery of growth factors. Dvir et al. illustrated the ECMs of different tissues such as heart, liver, and bone (Figs. 1b–d) [26].
They demonstrated that the morphologies of different tissues are controlled by the different composition and nanostructures of the ECMs. Therefore, the design of artificial ECMs on the biomaterial surfaces is important in tissue engineering. Bio-inspired biomaterial surfaces based on artificial ECMs can be produced by surface nanofunctionalisation techniques, such as electrospinning [27], layer-by-layer assembly [28], ED [29], and alkali treatment [30]. The details of these techniques have been widely reviewed.

A class of bio-inspired structure is nanofibrous structure, which mimics the nanofibrillar structure of ECM. Bio-inspired nanofibrous structures are mostly composed of biodegradable polymers, such as collagen [31, 32], chitosan (CS) [33], and poly(lactic-co-glycolic acid) [34], which serve as an artificial ECM for cell adhesion, proliferation and differentiation. Moreover, the diameter of nanofibrous structures between 50 and 500 nm can significantly reinforce the interactions of cell and the nanofibrous surface [35]. The general techniques to fabricate bio-inspired nanofibrous structure include electrospinning, phase separation, and self-assemble.

The bio-inspired porous structure has recently received increasing attention, which plays an important role in tissue regeneration [36]. Especially, bone-mimicking porous structures are widely designed for enhancing the efficiency of tissue regeneration of the biomaterials [37]. It is because porous structures can allow the tissue and blood vessels to grow in. Thus, the porous structure can also promote the integration degree of tissue and biomaterial surface [38].

### 2.2 Bio-inspired composite

In addition to the bio-inspired structure design, the composition on the biomaterial surface can also be designed to mimic from nature. The bio-inspired composition should be biocompatible and non-toxic, and should have desired biological functions. Marine mussels have attracted considerable attention due to their specific adhesive proteins, which allow them to adhere to both wet/dry surfaces [39]. The specific adhesive proteins, such as *Mytilus edulis* foot protein 5 (Mefp-5), that is secreted by mussels contains 3,4-dihydroxy-L-phenylalanine (DOPA) and lysine amino acids (Fig. 2). Inspired by mussels, various materials containing DOPA and its derivatives have been designed to obtain strong adhesive properties. PDA, with a structure similar to the specific adhesive proteins, has been used to modify a number of biomaterial surfaces [40–42]. In particular, PDA has good cell affinity and tissue adhesive capability [43–46]. Therefore, PDA is a promising bio-inspired composite for biomaterial surface modification.

Typically, mussel-inspired biomaterial surface functionalisation can be performed by immersing the biomaterials into a dopamine solution.
solution at basic pH. During this process, dopamine is polymerised oxidatively and spontaneously forms a thin PDA film on the biomaterial surface [47]. Recently, Du et al. reported that UV can also trigger dopamine polymerisation without any oxidising agents even in neutral and acidic conditions [48]. UV irradiation can induce the generation of reactive oxygen species, such as singlet oxygen, superoxide radicals, and hydroxyl radicals, which can trigger the dopamine polymerisation. Thus, PDA coating is an effective and simple strategy for biomaterial surface functionalisation.

Gelatine is a biopolymer with outstanding biocompatibility and biodegradability derived from partial hydrolysis of collagen while remaining partial amino acid sequences of collagen. Thus, gelatine can promote the cell adhesion, proliferation and differentiation, which have been widely used to construct bioactive surfaces as the cell matrix [49–52]. Particularly, gelatine is the major component of bone. Biomaterial surfaces are functionalised by gelatine can effectively improve the bio-mineralisation and osteoinductivity [53–55].

Hydroxyapatite (HA) is the main mineral component with nano/microstructure of natural bone. It has been well demonstrated that HA can significantly promote the biocompatibility and osteoconductivity of biomaterials [56, 57]. Thus, HA has been widely used to functionalise biomaterial surfaces [58, 59]. Moreover, HA also exhibits outstanding biocompatibility with other tissues [60, 61], which make it an ideal bio-inspired composite for the surface functionalisation of biomaterials.

Silk fibroin is a natural protein-polymer derived from domestic silkworm with good biocompatibility, oxygen and water vapour permeability, mechanical strength and biodegradability [62]. With a long history, silk fibroin has been used as sutures. Recently, various properties of silk fibroin are exploited in biomedical areas, such as the regeneration of bone, vascular, and cartilage [63, 64]. Thus, silk fibroin is also a common bio-inspired composite for surface functionalisation of biomaterials.

### 3 Bio-inspired nanofunctionalised surfaces for bone regeneration

Over the few decades, biomedical implants have been used in bone tissue engineering, such as in the preparation of metallic scaffolds, and calcium phosphate (CaP) scaffolds. Although these scaffolds have excellent biocompatibility and suitable mechanical strength, their osteoinductivity is not good enough. Bio-inspired surface nanofunctionalisation is an efficient way for enhancement of their osteoinductivity.

Titanium (Ti) and Ti alloys scaffolds are widely used in bone tissue engineering. Although they are biologically inert, their surfaces can be activated easily through chemical treatments. A direct and simple approach to achieving this is alkali treatment. During the alkali treatment, an alkali titanate layer is formed on the Ti surface. After the activation, a bone-like apatite coating was formed on the Ti surface. The uniform and nano/microstructure apatite coating can mimic the ECM structure in bone tissue, which provides a microenvironment for growth of bone-related cell growth.

The typical methods for fabricating a uniform apatite coating on Ti surface include biomimetic mineralisation and ED. Biomimetic mineralisation is a powerful approach for enhancing the osteoinductivity of metallic scaffolds. Simulated body fluid (SBF), a solution with similar ion concentrations and pH value as that of the human body, is usually used for biomimetic mineralisation. During the biomimetic mineralisation process, a bone-like apatite is formed on the surfaces of metallic scaffolds. Bae et al. soaked chemically treated Ti in SBF which led to the formation of an HA coating on its surface [65]. Meanwhile, the bone morphogenetic protein-2 (BMP-2) nanocomplexes were deposited along with HA. Thus, they created an environment on the Ti surface, which has not only the nanostructure of bones but also the growth factor delivery capability. Their results demonstrated that the osteoblast cells on the functionalised Ti surface have high osteogenic activity. Cai et al. used PDA to modify the surface of Ti6Al-4V and then biomimetically induced the growth of nanostructured HA coatings on the surface in SBF [66]. After the formation of nanostructured HA coatings, BMP-2 peptide was adsorbed onto the HA coatings. They also constructed a nanostructured surface with growth delivery system. Human osteosarcoma cells exhibited high alkaline phosphatase (ALP) activity, and expression of osteogenic genes in vitro. Except for HA, other CaP coatings, such as octacalcium phosphate and dicalcium phosphate, could also be formed in different kind of SBFs [67].

Biomimetic mineralisation can also be implemented on the inorganic/organic scaffolds. Xie and co-workers prepared CS/graphene oxide (GO) hybrid scaffold [68]. GO has a high surface area with wrinkled structure and oxygen-containing functional groups. Thus, the octacalcium phosphate (OCP) was biomimetically mineralised on the surface of CS/GO hybrid scaffolds by immersing the scaffold into a supersaturated calcium and phosphate solution. The nanostructured OCP coating and the micro porous scaffold together created a hierarchical ECM structure, which can promote the cell adhesion and growth (Fig. 3). Suarez-González et al. incubated an alginate scaffold in modified SBF [69]. After 4 weeks, the surface of the alginate scaffold achieved a bone-like HA coating. Human mesenchymal stem cells (MSCs) were seeded onto the mineralised surface, and showed high proliferation activity.

ED is another promising way to prepare CaP coatings on Ti surfaces for enhancing the osteoinductivity. ED can not only control the composition and uniformity of the CaP coatings but also be used for the metallic implants with complex geometries. Lu et al. reported that varying atmospheric conditions can control the CaP crystal growth on the surface of metallic implants by ED [70]. Their results showed that HA coatings are formed on the surface in a nitrogen-rich atmosphere, while OCP coatings are formed on the surface in a carbon dioxide-rich atmosphere. They also reported that the morphologies of CaP coatings can be controlled using CS during ED [71]. With the assistance of CS, CaP fibres are formed on the substrate by ED. Their findings provide a foundation for the construction of nanostructured CaPs on biomaterial surfaces. Zhao et al. prepared macroporous titane nanowire on a Ti surface via a hydrothermal method [72]. Afterwards, they coated HA nanoparticles (HA-NPs) onto the surface of the titane nanowire by ED. The macroporous titane nanowire and highly oriented HA-NPs constructed hierarchical scaffolds, which mimics the nature’s ECM for tissue growth. Human osteoblast-like MG63 cells on the hierarchical scaffolds exhibited high osteoinductivity and differentiation (Fig. 4).

Mussel-inspired PDA functionalisation is another effective strategy to promote the osteoinductivity of biomaterials [73]. PDA-functionalised biomaterial surfaces have been demonstrated to enhance the CaP crystallisation and loading of growth factors. Ryu et al. used PDA to assist the HA formation on ceramics, metals, semiconductors, and polymers [40]. Owing to the presence of catechol moieties, PDA films can adhere to these substrates. Meanwhile, the catechol moieties enrich the surface with calcium ions in SBF, which promotes the nucleation of HA crystals. Thus, with the assistance of PDA, a hierarchical structured CaP could be formed on various biomaterials. Their study provided a universal biomimetic mineralisation strategy for bone tissue engineering. Jo et al. also used PDA to modify the surface of poly(ε-caprolactone) (PCL) scaffolds [74]. Afterwards, the PDA-modified PCL scaffolds were immersed in SBF for 14 days. After that, HA was formed on the surfaces of the PCL scaffolds. They found that the HA/PDA layers enhanced the adhesion of preosteoblasts onto the PCL coatings.

Moreover, the PDA films on the biomaterial surfaces can enhance the efficiency of the osteogenic growth factors, such as bone morphogenetic protein-2 (BMP-2). Wang et al. prepared PDA microcapsules on the Ti surface using layer-by-layer assembly with CS (Fig. 5) [75]. The PDA microcapsules can mimic a natural ECM for achieving cell adhesion and BMP-2 immobilisation. The cationic groups on the PDA can react with the amino and thiol groups of BMP-2 via Schiff-base and Michael-type addition reactions. Thus, the nanostructured PDA microcapsules have a high BMP-2 loading capability. On the other hand, it has been
demonstrated that BMP-2 can be released sustainably from the nanostructured PDA microcapsules. Their results revealed that bone marrow stromal cells (BMSCs) on the nanostructured PDA microcapsules coated Ti surfaces display high proliferation and differentiation level. Cho et al. coated PDA films on the surface of poly(L-lactide) (PLLA) nanofibres, and immobilised BMP-2 on the PDA-coated nanofibres for bone regeneration [41]. They found that PDA stably retained 90% of the BMP-2 on the nanofibre surface. Their in vitro study conducted by this group showed that MSCs possess ALP activity and calcium mineralisation capacity on the BMP-2-immobilised-nanofibre surfaces. Moreover, BMP-2 could be sustainably released from the PDA films on the nanofibre surfaces. Thus, the in vivo study demonstrated that almost 77.8% of new bone was formed at the defect area with a small dose of BMP-2, as compared to other groups without BMP-2.

PDA provides a universal platform for surface modification of biomaterials used in bone regeneration due to its adhesiveness and biocompatibility. However, PDA lacks the property of osteoinductivity. Xie et al. developed a pulse electrochemical driven layer-by-layer (PED-LbL) assembly method to induce HA-NPs onto PDA films, in-situ, to enhance the osteoinductivity of PDA films [29]. PED-LbL assembly consists of oxidative and reductive processes. HA-NPs and PDA (HA-PDA) were alternately synthesised in situ to form HA-PDA multilayer nanofilms on the Ti surface via the switch of oxidative and reductive conditions (Fig. 6a). Moreover, the HA-PDA multilayer nanofilms can also act as a reservoir for immobilisation and delivery of BMP-2. In vivo studies have shown that the HA-NPs promote the bone-to-sample contact and bone area ratio of pure PDA films on Ti surface (Figs. 6b–d). Furthermore, the osteoinductivity of the HA-PDA multilayer nanofilms could be effectively improved by the delivery of BMP-2. Jiang et al. coated a PDA film on the surface of HA-NPs (PDA@HA-NPs), and then assembled the PDA@HA-NPs on the surface of the implant [76]. Meanwhile, BMP-2 was loaded on the PDA@HA-NPs. In their study, they observed that PDA not only promoted the strong bonding between HA-NPs and implant surface but also served as a platform for...
Bone area (BA) ratio. S, sample; CB, cortical bone; NB, new bone; OC, osteocytes of implantation PDA microcapsules and CS assembly Biosurf. Biotribol. Histological sections of the implant with different surface modi-

Fig. 5 Schematic illustration of the preparation process of the PDA microcapsules on the Ti surface by layer-by-layer assembly with CS [73].

- a Polystyrene microspheres (PS-MS) templates
- b Sulfonated PS-MS (SPS-MS)
- c PDA coated SPS-MS
- d Layer-by-Layer assembly film of PDA coated SPS-MS and CS
- e PDA microcapsules and CS assembly films
- f BMP-2 was loaded on the films
- h-g Mechanism of the interaction between BMP-2 and PDA

Fig. 6 Preparation process and biocompatibility of BMP-(HA-PDA)10-Ti scaffold

- a Schematic diagram of the PED-LbL assembly process [29]
- b Histological sections of the implant with different surface modifications after 12 weeks of implantation
- c Bone-to-sample contact (BC) ratio
- d Bone area (BA) ratio: S, sample; CB, cortical bone; NB, new bone; OC, osteocytes

growth factors. In vivo studies have revealed that PDA@HA-NPs significantly enhanced the osteoinductivity of the implants. In a similar report, PDA nanoparticles (PDA-NPs) and HA-NPs were prepared on the substrate by LbL assembly [77]. PDA promoted the adhesion of HA-NPs, which were alternately assembled with PDA-NPs on the substrate surface to form a porous and hierarchical structure. Moreover, this porous and hierarchical structure was also used to immobilise BMP-2. The results demonstrated that BMSCs favourably to adhere to the PDA-NPs and HA-NPs assembly surfaces. BMP-2-loaded PDA-NPs and HA-NPs assembly surface exhibited excellent osteoinductivity in vivo.

4 Bio-inspired nanofunctionalised surfaces for cartilage repair

Articular cartilage is a key tissue in the bones as it supports and distributes loads. However, articular cartilage is avascular, which is the reason behind its poor regenerative capability after injury [78]. The conventional cartilage treatments are mostly autogenic and allogenic cartilage transplants. However, setbacks such as limited availability of cartilage in the body for autografts and the body’s immune response to allografts are unavoidable. Generally speaking, cartilage grafts should meet the following requirements [79]: (i) biocompatibility; (ii) presence of interconnected pores for cell ingrowth and nutrients diffusion; (iii) mechanical properties similar to actual cartilage tissues; and (iv) biomimetic surface for integration with surrounding tissues.

In recent years, hydrogels have been considered as promising candidates for cartilage regeneration due to their high water content and structural similarities with natural tissues [80, 81]. Hydrogels are biocompatible, have tunable mechanical properties and porous structures, which provide a biomimetic based ECM for cartilage repair. Moreover, hydrogels can load biomolecules, such as peptides and growth factors, and release them into the defect areas. Most of the hydrogels used for cartilage repair consist of natural or synthetic polymers. Natural polymer-based hydrogels, such as gelatine, CS, chondroitin sulphate (CHS), hyaluronic acid and collagen, are biodegradable, biocompatible, and cheap to manufacture [82–85]. However, the mechanical properties of natural polymer-based hydrogels are insufficient to match the natural cartilage [86]. Synthetic polymer-based hydrogels, such as poly(lactide-co-glycolide) (PLGA), PCL, poly(vinyl alcohol) (PVA), have high mechanical properties and tunable physical and chemical properties [87, 88]. For example, Ng et al. [89] prepared a macroporous PVA hydrogel scaffold. The compressive moduli of the PVA hydrogel scaffold could be controlled in the range of the natural articular cartilage. However, most of them lack bioactivity, and are not able to support cell adhesion, survival, and reorganisation. Although combining the natural and synthetic polymers could obtain hydrogels with bioactivity and sufficient mechanical strength, the surface properties of these hydrogels should also be considered [90–92]. Surface properties can manipulate cell recruitment, adhesion, and migration at the tissue-hydrogel interface [93]. Moreover, cell response to hydrogels occurs on the surfaces resembling ECM found in physiological environment. Thus, designing biomimetic ECM environment on the hydrogel surfaces is important for cartilage repair.

Bio-inspired nanofunctionalisation is a promising biomimetic approach to recreate the ECM environment on the hydrogel surfaces to induce the specific cellular responses and cartilage regeneration. Bio-inspired nanofunctionalisation of hydrogel surfaces is usually based on the surface composition, such as cell-binding peptides. Arginine-glycine-aspartic acid (RGD) is the most frequently used peptide to enhance the biomolecular recognition of hydrogel surfaces by the cells [94, 95]. Kim et al. [96] prepared CHS-based hydrogel by copolymerising the RGD-modified polyethylene glycol (PEG) and methacrylated CHS. The results showed that the expression of the gene, lubricin, found in chondrocytes is increased by the RGD moieties on the CHS-based hydrogels. Park and Lee [97] prepared an RGD-alginate/hyaluronic hydrogel with primary chondrocytes.
by including specific interactions between the RGD and cells. RGD on the hydrogels not only provides cross-linking sites for the polymer network but also provides adhesive sites for the primary chondrocytes. Their results showed that the RGD-alginate/hyaluronate hydrogel with primary chondrocytes improved the secretion of glycosaminoglycans (GAG) secretion and chondrogenic gene expression of the cells in vivo. Park et al. [98] fabricated an RGD-conjugated CS-pluronic (CP) hydrogel by chemical cross-linking RGD onto the surface of the CP hydrogel. The bovine chondrocytes grown on these hydrogels exhibited high levels of viability, proliferation, and GAG secretion. Since GAGs are essential components of the ECM of cartilage tissues, this suggests that the RGD-functionalised hydrogel can promote cartilage regeneration.

In addition, mussel-inspired surface nanofunctionalisation might open avenues for enhancing the cartilage-inducing capability of hydrogels. Recently, PDA-functionalised hydrogels have attracted increasing attention for cartilage regeneration. PDA-functionalised hydrogels exhibit high adhesive ability to various materials, including soft tissues, which could prevent the detachment of hydrogel from the implantation site. Unlike the RGD, PDA could be used as a network of the hydrogel, which endows the entire hydrogel surfaces with catechol groups. Karimi et al. [99] designed a multifunctional hydrogel based on PDA by reduction of graphene oxide (rGO), and polyacrylamide (PAM). First, GO was reduced to rGO with the oxidation of dopamine. Afterwards, the PDA-rGO-PAM hydrogel was prepared with the polymerisation of arylamide in the PDA/rGO solution. The PDA-rGO-PAM hydrogel is stretchable and tough due to the presence of covalent bonds in the PAM network and the non-covalent interactions between PDA, GO and PAM. rGO, which was uniformly dispersed in the hydrogel by PDA, endowed the hydrogel with good conductivity. Moreover, the free catechol groups of PDA imparted the hydrogel with self-healing powers and self-adhesiveness. Therefore, the PDA-rGO-PAM hydrogel could be used for cellular electrical stimulation (ES) and for implanted electrodes (Figs. 7a and b). Especially, in vivo studies have shown that the PDA-rGO-PAM hydrogel could well integrate with surrounding tissues and immobilise the transforming growth factor to improve cartilage regeneration (Fig. 7c). In their other report [100], they designed a PDA-CHS-PAM hydrogel with tissue adhesiveness for cartilage regeneration without growth factors. CHS is the main component of the ECM, which can upregulate the chondrogenic differentiation of cells. PDA provided adhesive catechol groups on the hydrogel which enabled recruitment of cells and enhanced the efficiency of CHS, resulting in the cartilage regeneration without chondrogenic growth factors.

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**Fig. 7 Biomedical applications of the hydrogels**

a Hydrogel for cellular ES
a1 Schematic diagram of high throughput ES device
a2 Proliferation of BMSC under different conditions
a3 SEM image of BMSC morphology on the hydrogel
b Hydrogel as the implanted electrode
b1-2 Schematic illustration of hydrogel electrodes in vivo
b3 EMG signal recorded by the hydrogel electrodes in vivo
c Hydrogel for cartilage regeneration, (c1-2) Hydrogel in the osteochondral defect, (c3-5) Histological images of the osteochondral defect after 6 weeks [99]
5 Bio-inspired nanofunctionalised surfaces for an antibacterial effect

Bacterial adhesion on biomaterial surfaces is a serious problem in clinical studies. In recent decades, various antibacterial biomaterial surfaces have been designed to prevent bacterial attachment and biofilm formation. The major factors influencing the strategies for the design of antibacterial surfaces are antibacterial agent release, contact-active antibacterial surfaces, and bacteria resistance properties.

The general approaches for fabrication of antibacterial surfaces include surface chemical modification and physical modification [101]. Among these approaches, bio-inspired nanofunctionalised antibacterial surfaces have attracted much attention in recent years.

Natural surfaces, such as plant leaves, and insect wings, are super-hydrophobic, and low-adhesive, which have inspired the design of antibacterial surfaces [102]. The general approaches for fabrication of antibacterial surfaces include surface chemical modification and physical modification [101]. Among these approaches, bio-inspired nanofunctionalised antibacterial surfaces have attracted much attention in recent years.

Ma et al. [103] reported that the hierarchical surface nanostructure of the taro leaf is superhydrophobic, which highly repels to bacterial adhesion. Pechook et al. [104] developed a bioinspired super-hydrophobic surface via self-assembly of paraffin or fluorinated wax crystals to prevent bacterial attachment and biofilm formation. They demonstrated that these bioinspired superhydrophobic surfaces passively reduce biofilms formation by 95.6 to 99.9% in 7 days. Bruzaud et al. [105] prepared different group-terminated (fluorocarbon or hydrocarbon) PEDOT superhydrophobic coatings with controllable topographies on stainless-steel surfaces via electrodeposition for reducing bacterial adhesion. They found that the surface chemistry is not the only factor that affects the antibacterial adhesion on the superhydrophobic surfaces. Antibacterial adhesion also requires controllable surface topographies. However, some surfaces with nanostructure cannot efficiently resist bacterial fouling. Ivanova et al. [106] reported that the attachment of Pseudomonas aeruginosa cells onto the cicada wings with nanopillar arrays could not be prevented. Consequently, the attached cells are penetrated by the nanopillars within a short duration of time. They also modified the surface chemistry of the nanopillars by coating them with a gold film, and found that modified surface chemistry did not affect the antibacterial activity of the surface.

Mussel-inspired PDA modification is another approach to fabricate antibacterial surfaces. PDA has been reported to directly act against bacteria in a solution of dopamine solution [107]. There are two hypothetical mechanisms. One is that PDA can polymerise on the surface of the bacteria, which blocked the bacterial proliferation. The other one is that PDA layer acts as a barrier to cut off the nutrient delivery into the bacteria. However, the intrinsic
antibacterial nature of PDA coatings on the biomaterial surface has not been well investigated.

Most of the antibacterial strategies of mussel-inspired PDA-functionalised surface include immobilising antibacterial agents on the surfaces utilising its outstanding adhesive property of PDA. Gan et al. [108] developed a tough, and antibacterial hydrogel, which displayed strong cell affinity, based on mussel-inspired functionalisation. The antibacterial hydrogel was prepared by copolymerisation of mussel-inspired methacrylamide dopamine (MADA), acrylic acid, and 2-(dimethylamino)ethyl methacrylate (DMAEMA) with the interpenetration of quaternised CS (QCS) (Fig. 8). Mussel-inspired MADA has an adhesive property which favours bacteria attachment, and QCS is an antibacterial bio-polymer due to its abundant positively charged groups. Thus, the surface of the hydrogel exhibited contact-active antibacterial activity.

The results demonstrated that the bacterialicidal ratios of the mussel-inspired antibacterial hydrogel against *S. epidermidis* and *E. coli* reached 87%, which is higher than that of antibacterial hydrogel without mussel-inspired MADA. Interestingly, the mussel-inspired antibacterial hydrogel also exhibited high toughness due to the chemical and physical cross-linking network. Han et al. [109] sequentially assembled vancomycin-loaded oxidised sodium alginate (Van-OAlg) and dexamethasone-loaded CS/bovine serum albumin nanoparticles (Dex-CB-NPs) on the mussel-inspired PDA-functionalised Ti surface. The mussel-inspired PDA functionalisation endows the Ti surface with adhesive activity, which allows the successful assembly of Van-OAlg and Dex-CB-NPs. Thus, the vancomycin can be sustainably released for 25 days from the mussel-inspired PDA-functionalised surface for long-term antibacterial effect.

Yeroslavsky et al. [110] developed a tough, and antibacterial hydrogel containing mussel-inspired dopamine-functionalisation. Wang et al. [111] constructed nano- Ag contained alginate/CS ultrathin film on Ti-6Al-4V surfaces via mussel-inspired PDA nanofunctionalisation. PDA was used to functionalise the Ti-6Al-4V surfaces via Shiff-base reactions. Compared to the Lst physically adsorbed on the untreated PDA surfaces, covalently immobilised Lst killed the Staphylococcus aureus within 15 min, which effectively prevented the biofilm formation. Xu et al. [111] prepared PDA-coated polystyrene culture plates. Then, they immobilised dexamethasone and minocycline-encapsulated liposomes on the PDA-coated surfaces. With the adhesive activity of PDA coatings, the sustained release of dexamethasone and minocycline occurred from the surface, which prevented the adhesion and proliferation of *Porphyromonas gingivalis* and *Streptococcus mutans*.

Additionally, nanofunctional surfaces for tissue repair also usually integrate antibacterial functions. The dual-functional surfaces can prevent the infection of biomaterials during the infection during the tissue regeneration process. Forte et al. [112] used PDA to functionalise the CaP surfaces. PDA acts as a reducing agent and can reduce the silver ions to the silver nanoparticles (Ag NPs) on the CaP surfaces in a silver nitrate solution. Their results showed that the Ag NPs deposited on the PDA-functionalised CaP surfaces have high antibacterial activities and osteoblast viability. Owing to the adhesive property of PDA, this method can be applied on various substrates. Xie et al. [113] developed a pulse electrochemical layer-by-layer assembly method to alternately deposit the HA NPs and Ag NPs on the Ti surface with the assistance of polypyrrole (PPy) (Fig. 9). Under electrochemical oxidative conditions, pyrrole is polymerised to PPy NPs, while PO4 doped into the PPy backbone to neutralise its positive charge. PPy NPs further form PPy coatings on Ti surface. Under electrochemical reductive condition, PO4 doped PPy NPs act as a template to attract Ca2+ into the electrolyte. Meanwhile, Ag+ is chelated by PPy. Finally, HA NPs and Ag NPs are simultaneously co-reduced on the Ti surfaces. The HA-Ag-PPy multilayer coatings demonstrated high osteoconductive and antibacterial activities against *E. coli* and *S. epidermidis*. In their other study, BMP/CS/Ag/HA coatings were prepared on Ti surfaces by ED (Fig. 10) [114]. Ag NPs and HA were co-deposited on the Ti surfaces with CS, and then BMP was immobilised on the coatings by electrostatic interaction. The BMP/CS/Ag/HA coatings were capable of sustainably releasing BMP and Ag+ for 25 days. Antibacterial tests showed that the BMP/CS/Ag/HA coatings have high antibacterial activities. Moreover, Ag NPs in the coatings showed no cytotoxicity. In vivo studies indicated that the

Fig. 10 Preparation process, antibacterial activity, and biocompatibility of the BMP/CS/Ag/HA coatings

a Schematic diagram of the preparation process
b *E. coli*

c *S. epidermidis* suspensions after culturing for 24 h

d Histological section of BMP/Ag/HA-Ti after 12-week implantation

e Amplified image of (d). Green stars, new bone; blue arrows, osteocytes

BMP/CS/Ag/HA coatings could improve new bone formation. Wang et al. [115] constructed nano-Ag contained alginate/CS ultrathin film on Ti-6Al-4V surfaces via mussel-inspired PDA nanofunctionalisation. PDA was used to functionalise the Ti-6Al-4V surfaces. The PDA coatings acted as an anchor layer to immobilise the nano-Ag containing alginate/CS ultrathin film. The mussel-inspired PDA nanofunctionalised surfaces successfully inhibited the growth of *E. coli* and *S. aureus*, while promoting the proliferation and viability of BMSC.

6 Conclusion and outlook

Bio-inspired surface nanofunctionalisation is important for biomedical engineering, and it is rapidly developing. In the past decade, various nanofunctionalisation strategies have been designed to fabricate bio-inspired biomaterial surfaces, which provide a potential solution to solve the clinical problems associated with biomaterials. These bio-inspired biomaterial surfaces mimic not only the physical structures, but also the chemical compositions from nature, which can be utilised effectively for applications in the fields of bone/cartilage repair, antibacterial agents, wound regeneration etc. However, there are still some challenges. For example, although artificial ECM structures and functions are widely used for enhancing the biological performance of biomaterial surface, these characteristics need to be optimised for specific tissues. However, the traditional methods for fabricating and optimising different the bio-inspired functional surfaces are time-consuming. Therefore, developing high throughput tools for fabricating different bio-inspired functional surfaces, and screening out the surfaces most favoured by cells will be useful for future biomaterial surface design.
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