Thin films for tissue engineering applications

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8.1 Introduction

Modern surface science has been under development since the late 1960s. Surfaces, interfaces and thin films are planar structures that occur on the boundary of materials or at the junction or interface between two different media. In the late 20th century, the design of thin solid films at the molecular level attracted much attention because of its potential applications in the biological and medical fields. Many studies showed that the modification of surfaces could have an important role in cell and tissue response. In addition, applying thin films has been widely recognized as a promising approach in modifying the surface properties of a biomaterial. Studies have attracted focus on hard tissues and stiff biomaterials (high Young's modulus); however, less attention has been directed toward the field of soft tissues and viscoelastic biomaterials. Furthermore, the significance of thin films in biology and biomaterials research has rapidly increased in recent decades.

Thin films are at the core of many important technologies that have essential roles. Thin films can be applied onto different surfaces with respect to their physicochemical characteristics such as wettability, reactivity, conductivity and corrosion properties. Thin films can be deposited onto different surfaces using a variety of mechanisms including solvent casting, spraying, coating, covalent conjugation, surface-initiated polymerization and self-assembly (ie, electrostatic interactions, hydrogen bonding). These thin films have been produced using a wide variety of biomaterials, including both natural and synthetic materials with diverse architectures and properties. The unique ability of thin films to control various physicochemical characteristics supports the application of this strategy for tissue engineering applications. Thin film components, methods and conditions must be nontoxic and should not affect cellular functions in the body. Thin films are now being applied for osseointegration in terms

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of dental and orthopaedic implants, biodegradable scaffolds and biomimetic materials in the field of tissue engineering and biomedical industries. This chapter describes the fundamentals of thin film process for tissue engineering, including thin film manufacturing, surface modification and molecular engineering. It summarizes current research results in thin film techniques for both soft and hard tissues. This chapter also highlights many pitfalls that researchers may want to consider in future studies. In addition, the use of surface modification methods for study of skeletal and dental tissues is highlighted. Difficulties in using different methods are also discussed in detail.

8.2 Thin film coating technologies for tissue engineering applications

8.2.1 Spin-coating

The spin-coating process has been widely used to deposit uniform thin films on flat substrates. Spin-coating includes four basic steps: deposition, spin-up, spin-off and evaporation.[1,2] The steps involve in this method are summarized in Fig. 8.1. In this technique, an excess of liquid is initially deposited onto the surface, followed by slow rotation. In the spin-up stage, centrifugal force is generated by rotating the substrate, resulting in the liquid flowing radially outward. The centrifugal force can be manipulated to achieve different film thickness on the substrates. In the spin-off stage, the excess liquid flows to the perimeter of the substrate and departs as droplets. The rate of excess liquid removal by spin-off process becomes slower as the film gets thinner. In the fourth stage, evaporation becomes the primary mechanism of thinning.[3] This spin-coating method is capable of coating intraluminal stents, synthetic grafts and stent coverings. In this technique, different therapeutic agents can be mixed with the polymeric matrix to form different therapeutic compositions. There are many studies describing the use of thin film biodegradable coatings for tissue engineering using a variety of biomaterials, methods and techniques.

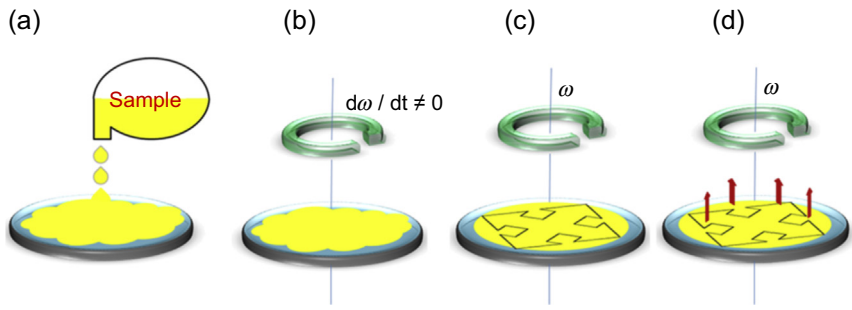

Figure 8.1 Stages of the spin-coating process. (a) Dispensation, (b) Acceleration, (c) Flow dominated, (d) Evaporation dominated.

In the past few years, bioactive thin film coatings have been of great interest owing to the improvement in bone-bonding performance on metallic implants. Mozafari et al.[4] reported the synthesis of bioactive glass−zirconium titanate composite thin films by a solgel spin-coating method. They showed that uniform, multilayer thin films could be successfully obtained through optimization of the process variables and the application of carboxymethyl cellulose as a dispersing agent. They stated that the thickness and roughness of the surface coatings could increase nonlinearly with an increase in the number of the layers, as shown in Fig. 8.2.

The most essential factor in determining the biocompatibility of metallic implants is their corrosion resistance. The same group reported that bioactive glass−zirconium titanate composite thin films could significantly improve the corrosion resistance of metallic implants, as realized by an increase in the corrosion potential and a decrease in the corrosion current density.[5] Fig. 8.3 indicates the electrochemical potentiodynamic polarization curves of the uncoated and coated samples. Also, Table 8.1 summarizes the related electrochemical parameters. As can be seen, the corrosion behaviour of the coated samples signifies notable differences compared with that of the uncoated substrate, whereas the number of thin film layers has no marked influence on the corrosion behaviour of the coated samples. It is an important achievement that the substrate exhibits no passivity, although 316L stainless steel is essentially an active−passive alloy. This is attributed to the considerable concentration of chloride in simulated body fluid (SBF) under the naturally aerated condition, which encourages localised corrosion such as pitting and avoids passivation. It is known that the in vivo corrosion resistance of metallic implants is frequently higher than that under in vitro conditions, owing to the inhibiting effect of organic species such as proteins. However, thin film deposition encourages the stainless-steel substrate polarized in the SBF to be passive. In addition, the coatings advantageously increase the corrosion potential and decrease the corrosion current density of the substrate, which indicates an improvement in corrosion resistance. Thus, it can be concluded that the thin films can act as a physical protective barrier to retard electrolyte access to the metal surface and thereby electrochemical processes. It is expected that this new class of nanocomposite thin films, comprising the bioactive and inert components, will not only enhance bioactivity and biocompatibility but also protect the surface of biomedical implants against wear and corrosion in the body.

8.2.2 Layer-by-layer assembly

In the second half of the 20th century, scientists have been attracted to the design of thin solid films at the molecular level because of their potential for application in the fields of biology and medicine. Two techniques dominated research in this area: Langmuir−Blodgett deposition[6,7] and self-assembled monolayers (SAMs). However, several intrinsic disadvantages of both methods limit their applications in the field of biology. For Langmuir−Blodgett deposition, the problems are the expensive instrumentation and time-consuming process for preparing the films. In addition, limited types of biomolecules can be embedded in the film (Langmuir−Blodgett deposition requires the assembly components to be amphiphilic). For SAMs, because of their

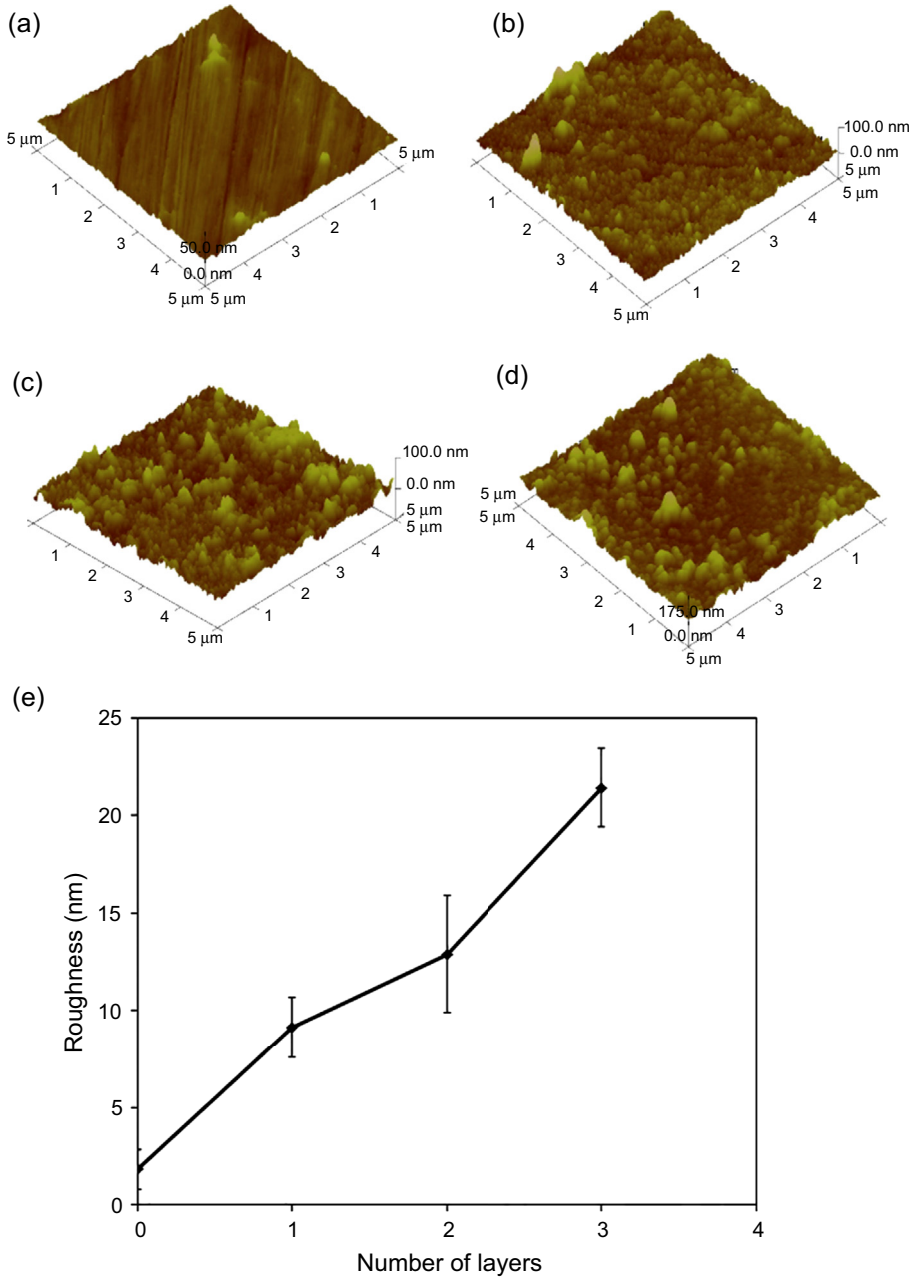

Figure 8.2 Three-dimensional atomic force microscopy images of the surface of (a) uncoated, (b) single-layered, (c) double-layered, and (d) triple-layered coated samples, (e) roughness values of the deposited films.[4]

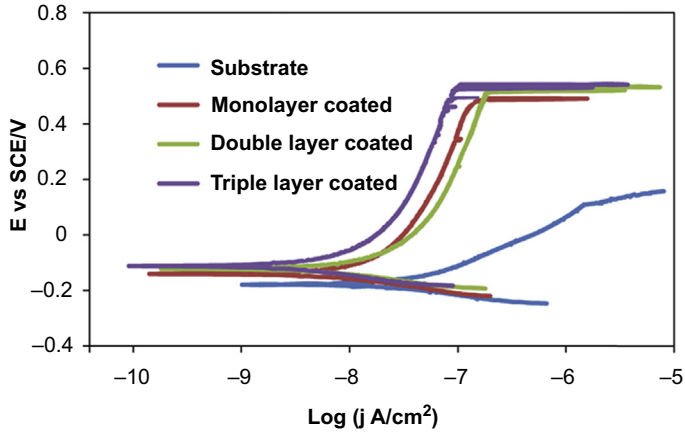

Figure 8.3 Anodic potentiodynamic polarization curves of the samples.[5]

Table 8.1 Corrosion potential (E_{corr}), corrosion current density (j_{corr}), passive current density (j_p), and breakdown potential (E_b). Note that the uncoated sample shows no typical passivity[5]

Sample	E_{corr} (mV(SCE))	j_{corr} (A/cm^2)	j_p (A/cm^2)	E_b (mV(SCE))
Uncoated	−192	4.8×10^{-8}	–	–
Monolayer	−130	1.1×10^{-8}	1.3×10^{-7}	485
Double layer	−114	9.4×10^{-9}	7.9×10^{-8}	518
Triple layer	−105	8.8×10^{-9}	5.1×10^{-8}	531

monolayer nature, a small amount of biological components can be loaded into the thin films. In addition, because SAMs are fabricated only by the adsorption of thiols onto noble metal surfaces or by silanes onto silica surfaces, a limited number of substrate types are applicable with this technique. This strategy is also limited because of the low stability of films under ambient and physiological conditions. An alternative to Langmuir–Blodgett deposition and SAMs is layer-by-layer (LbL) assembly.[8]

LbL deposition is a thin film fabrication technique in which films are formed by depositing alternating layers of oppositely charged materials. Wash steps are added between the different depositing layers. The first implementation of this LbL deposition technique was mentioned in a research by Kirkl and Iler.[9,12] They carried it out in 1966 using microparticles.[10] The method was later revitalized by Decher by the discovery of its applicability to a wide range of polyelectrolytes.[11]

It is known that LbL assembly is a prevalent method for coating substrates with functional thin films. After early studies that reported multilayer assembly,[9,12] it has only been in the past two decades that the field has grown significantly.[13] Generally, LbL assembly is a cyclical process in which a charged material is adsorbed onto a

substrate, and after washing, an oppositely charged material is adsorbed on top of the first layer. This constitutes a single bilayer with a thickness generally on the order of nanometres, and the deposition process can then be repeated until a multilayer film of desired thickness or function has been assembled.[13] For certain applications, the substrate can then be removed, forming macroscopic films such as membranes,[14] or forming microscopic or nanoscopic films such as hollow capsules.[15,16]

In addition to widely used electrostatic interactions for the formation of thin films, other molecular interactions such as covalent, hydrogen bonding and host−guest interactions are currently well established for LbL formation. Furthermore, to achieve different applications, diverse materials such as polymers, nanoparticles, suprastructures, proteins, lipids and nucleic acids can be incorporated into the film.[17] The simplicity, versatility and nanoscale control of the LbL assembly makes this technology to be one of the most widely used technologies for coating both planar and particulate substrates in a diverse range of fields including optics, energy, catalysis, separations and biomedicine (Fig. 8.4(a)).[10] The widespread use of LbL assembly in fields with different standard tools and procedures and the processing requirement of using different corresponded substrates has led to the development of a number of LbL assembly technologies that can be widely applied to porous membranes, particles and biological matters. Examples include dipping,[9] dewetting,[18] roll-to-roll,[19] centrifugation,[20] creaming,[21] calculated saturation,[22] immobilization,[23] spinning,[24] high gravity,[25] spraying,[26] atomization,[27] electrodeposition,[28] magnetic assembly,[29] electrocoupling,[30] filtration,[31] fluidics[32] and fluidized beds.[33] These different methods have often been treated as black boxes, in which the main focus has been on what materials are used (the input) to assemble the thin films (the output), with little focus on the actual assembly method. However, there is now growing realization that the assembly method not only determines the process properties (such as the time, scalability and manual intervention) but also directly affects the physicochemical properties of the films (such as the thickness, homogeneity and interlayer and intralayer film organization), with both sets of properties linked to application-specific performance (Fig. 8.4(b)).[10] The basis of LbL assembly is the sequential exposure of a substrate to the materials that will compose the multilayer films. Assembly technologies used to assemble such films form five distinct categories: (1) immersive, (2) spin, (3) spray, (4) electromagnetic and (5) fluidic assembly (Fig. 8.5).[10] These assembly technologies affect both the process properties and the resultant material properties; therefore, careful choice of the assembly method can be crucial for successful application of the assembled thin films. Furthermore, two main themes can be identified for current developments in assembly technologies: The first is moving away from random diffusion-driven kinetics for layer deposition, and the second is the advancement from manual assembly toward automated systems.

In addition to conventional ways of making LbL assembly, a novel technique to introduce free amino groups onto polyester scaffolds via aminolyzing the ester groups with diamine has been developed.[34] Positively charged chitosan was then deposited onto the aminolyzed poly(L-lactic acid) (PLLA) membrane surface using poly(styrene sulfonate) sodium salt (PSS) as a negatively charged polyelectrolyte through LbL assembly. The LbL deposition process of PSS and chitosan was investigated by

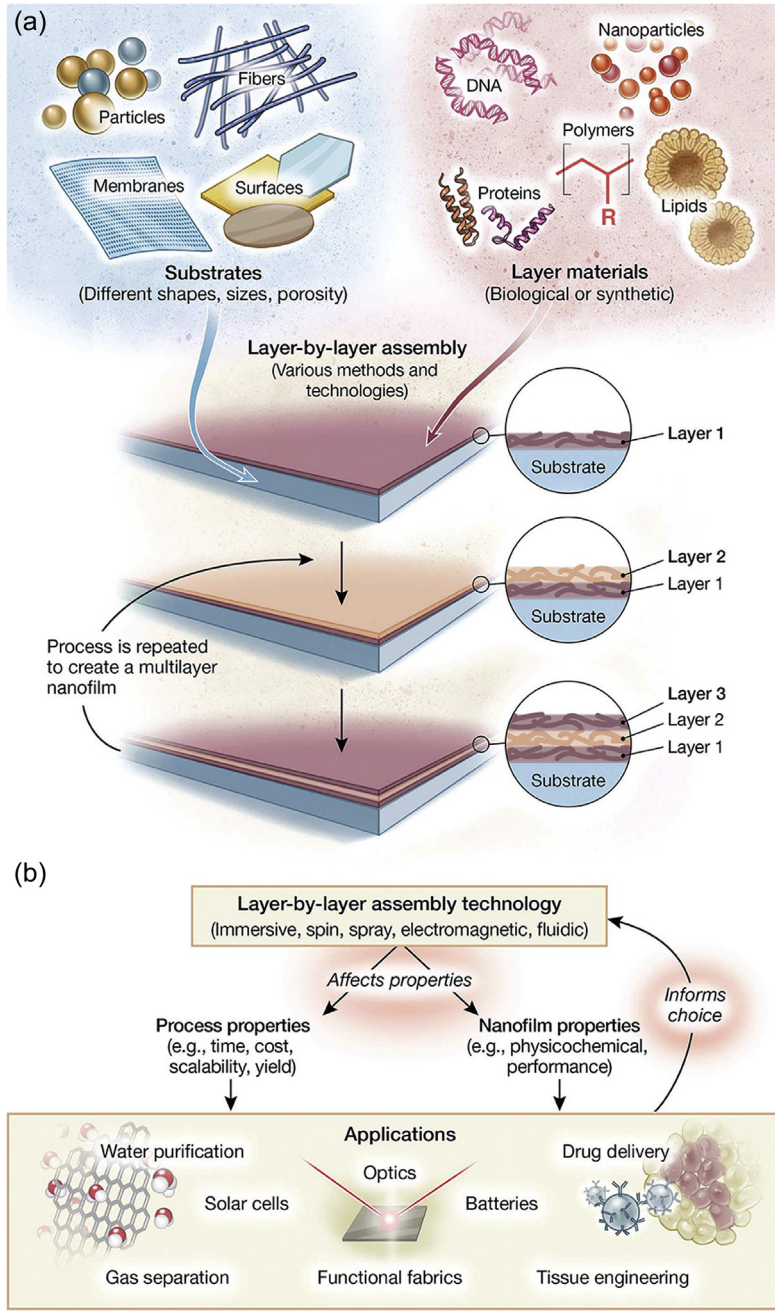

Figure 8.4 Versatility of LbL assembly. (a) Schematic overview of LbL assembly; (b) an overview showing that assembly technology influences film and process properties as well as application areas.[10]

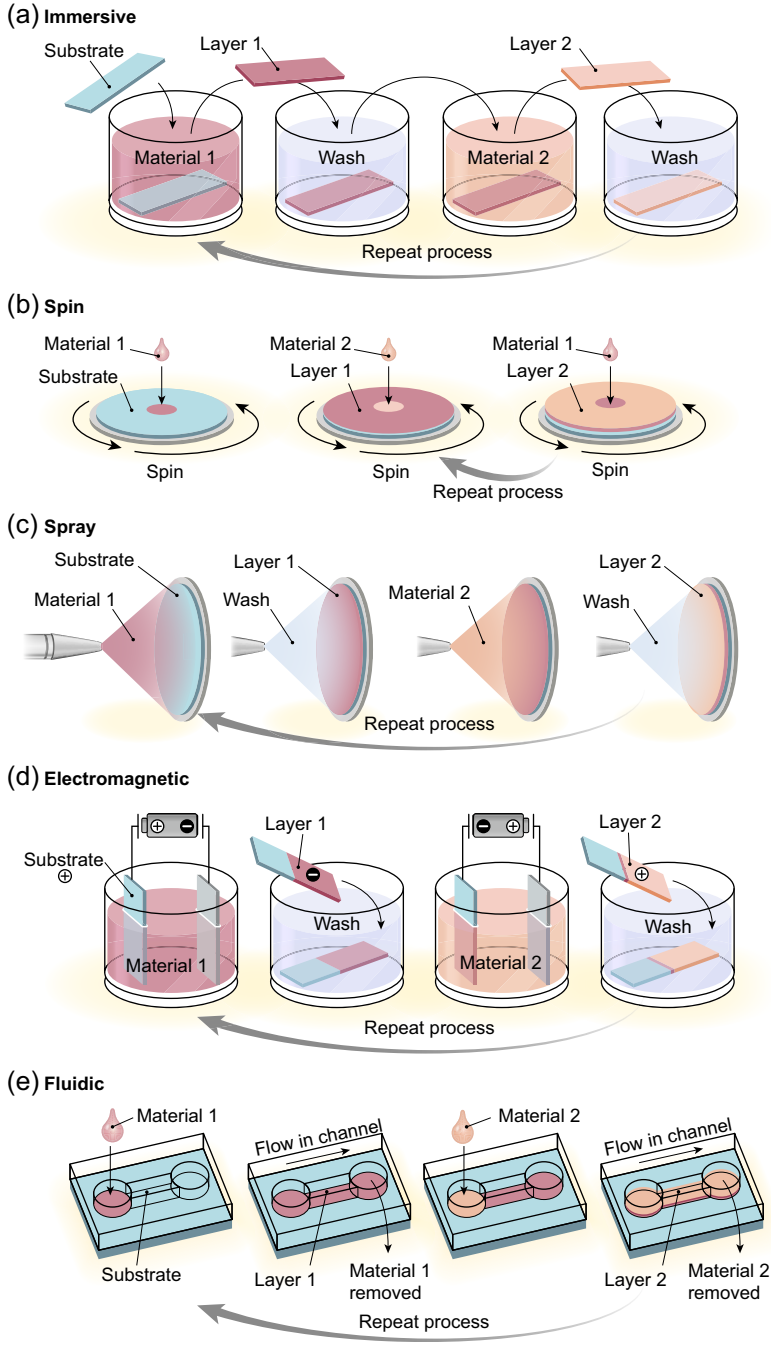

Figure 8.5 Layer-by-layer assembly technologies. (a–e) Schematics of the five major technology categories for LbL assembly.[10]

ultraviolet−visible absorbance spectroscopy, energy transfer by fluorescence spectroscopy and advancing contact angle measurements. The existing coated chitosan on PLLA obviously improved the cytocompatibility of PLLA to human endothelial cells. In addition, different outermost layer structures were studied. The PLLA membranes assembled with three or five bilayers of PSS/chitosan with chitosan as the outermost layer had better cell attachment, activity, spreading and proliferation than those with one bilayer of PSS/chitosan or the control PLLA. The endothelial cells also showed elongated morphology with abundant cytoplasm, which indicated that good spreading on the materials occurred. A confluent cell layer was reached after culturing 4 days. Endothelial cells secreting von Willebrand factor were measured and the endothelial function on the PLLA was confirmed, indicating that good biocompatibility of the PLLA was achieved after LbL assembly.[34]

8.2.3 Dip-coating technology

The physics of dip-coating has been extensively reviewed by Scriven.[35] The researcher divided the batch dip-coating process into five stages: (1) immersion, (2) startup, (3) deposition, (4) drainage and (5) evaporation (Fig. 8.6). With volatile solvents such as alcohol, evaporation normally accompanies startup, deposition and drainage.

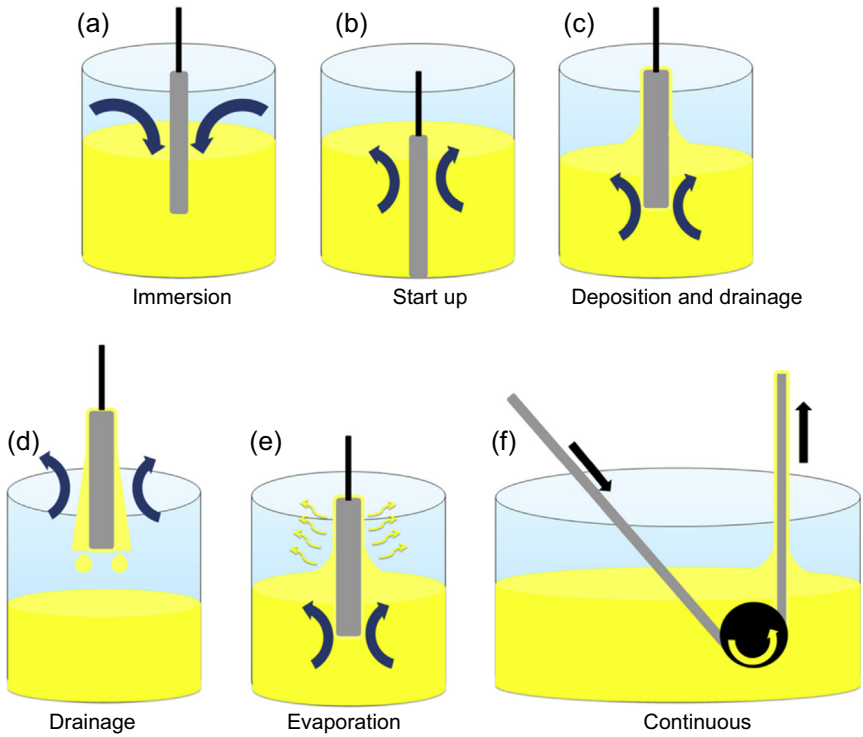

Figure 8.6 Stages of dip-coating process: (a−e) batch; (f) continuous.

The continuous dip-coating process is simpler because it separates immersion from the other stages, essentially eliminates startup, hides drainage in the deposited film and restricts evaporation to the deposition stage. As in dip-coating, evaporation may occur throughout the process. The thickness of the film in the deposition region (Fig. 8.6) is controlled by competition among as many as six forces.[3] The first is the viscous drag upward on the liquid by the moving substrate, force of gravity, resultant force of surface tension in the concave curved meniscus, the inertial force of the boundary layer liquid arriving at the deposition region, the surface tension gradient and disjoining or conjoining pressure (important for films with less than 1 μm thickness).

8.2.3.1 Hydroxyapatite dip-coating

Titanium (Ti) and Ti-based alloys are widely used in dental and orthopaedic implants because of their excellent biocompatibility.[36] However, bone ingrowth and implant fixation and tissue–material integration properties need to be improved to shorten the implant–tissue osseointegration time.[37] Therefore, significant effort in research has been carried out to improve the physical and chemical properties of the surface structure of Ti and its alloys.[38–40] Among the different proposed solutions, hydroxyapatite (HA) (Ca_{10} $[PO_4]_6[OH]_2$) coatings on Ti substrates have attracted much attention over the past few years.[41–43] The excellent biocompatibility of HA is the result of its chemical and biological similarities to human hard tissues.[44] Studies of HA coatings on Ti implants have revealed good fixation to the host bones and an increased amount of bone ingrowth into implants in vivo.[45] In addition to HA, fluorapatite (FA) ($Ca_{10}[PO_4]_6F_2$) coatings have also attracted considerable attention in areas that require long-term chemical and mechanical stability for the thin film layer.[46] Compared with HA, pure FA has a lower biodegradation rate and a similar level of biocompatibility. These important properties demonstrate behaviour for FA as a promising candidate for the fixation of bone and bone ingrowth.[47,48] Moreover, a fluor-hydroxyapatite (FHA) ($Ca_{10}[PO_4]_6[OH,F]_2$) solid solution with HA can be formed on FA to replace the F^- with OH.[49] In reality, F^- itself can prevent dental caries in bacterial and acidic environments, which enhances its application in the dental restoration field.[49,50] In addition, F^- can further promote the mineralization and crystallization of calcium phosphate in the process of bone formation.[51]

To date, most HA and FHA coatings are obtained using plasma-spraying techniques.[41,42] However, poor adherence to the substrate, chemical inhomogeneity and high porosity are the major problems of using the plasma-spraying process. Most of these problems result from the excessively high fabrication temperature.[41,42] In comparison, dip-coating technology offers many advantages including high chemical homogeneity, a fine grain structure, and the low crystallization temperature of the resultant coating. This technology is economically feasible and technically simple to perform.[52,53] Because of these advantages, dip-coating methods have been reported to be widely used for fabricating HA and FHA thin films for tissue engineering applications. In addition, combined with the dip-coating technique, the solgel approach was used to deposit HA and FHA films on Ti substrates. The biological performance of these thin films indicated excellent dissolution behaviour and in vitro cell response.[54]

8.2.3.2 Magnetic scaffolds

In past years, bioengineered scaffolds have been used in bone graft replacement combined with a variety of bioagents. Nevertheless, a problem with using these conventional scaffolds is reloading the scaffold with bioagents after implantation. A conceptually novel solution is magnetic scaffolds. Magnetic scaffolds can attract and take up growth factors and stem cells, or other bioagents can bind to the magnetic particles using a magnetic drive. In a research study, Bock et al.[55] reported on their success in developing a simple and inexpensive technique to transform standard commercial scaffolds made of HA and collagen into magnetic scaffolds. They prepared the scaffolds by dip-coating them in aqueous ferrofluids containing iron oxide nanoparticles coated with various biopolymers. After dip-coating, the nanoparticles were integrated into the structure of the scaffolds. The scaffolds' magnetization values were 15 emu/g at 10 kOe. These values are suitable for generating magnetic gradients, enabling magnetic guiding in the vicinity and inside the scaffold. The magnetic scaffolds maintain their specific porosity and shape and do not experience structural damage during the dip-coating process. According to this study, the magnetic scaffolds did not release magnetic particles under a constant flow of SBF over 8 days. Furthermore, the biocompatibility of the magnetic scaffolds was supported by in vitro adhesion and proliferation of human bone marrow stem cells on the scaffolds. Therefore, this new type of magnetic scaffold is a promising candidate for tissue engineering applications.[55]

In bone tissue engineering, the complete histomorphological and biological maturation of tissues is only achieved if angiogenesis is permanently stimulated by various angiogenic proteins such as growth factors, leading to vascular ingrowth from surrounding tissues in the vicinity of the scaffold.[56,57] During the bone healing process, reestablishing the complete functionality of damaged tissues usually takes longer regeneration times. The temporal control of the tissue regeneration process is important to allow optimal clinical outcomes in the tissue−biomaterial system, and it involves different agents at different times.[58,59] However, in bone graft substitution, such temporal control cannot be achieved with traditional scaffold approaches, in which growth factors are usually seeded in the scaffold before implantation.[60,61]

In some cases, preloading techniques reduce the delivery of localized, controllable, and long-term biochemical stimuli, thus impairing the tissue regeneration potential in the scaffold.[60,62] A controlled delivery that mimics endogenous growth factor production therefore remains a serious issue in the use of conventional scaffolds in tissue engineering.[63−65] This problem has been addressed by fabricating these conceptually new types of bone graft substitutes, which are able to attract and take up the growth factors or other bioagents via a driving magnetic force.[66−69]

In addition, studies have shown that these nanoparticles with biocompatible coatings do not have cytotoxic effects on cell development either in vitro or in vivo,[70] and some magnetic nanoparticles coated with arginylglycylaspartic acid peptides showed excellent biocompatibility in contact with osteoblasts.[71] It was also shown that the change in the magnetic properties of magnetic nanoparticles in the presence of a magnetic field had no influence on cellular toxicity.[72] This suggests that the

magnetization of standard HA−collagen composite scaffolds with magnetic nanoparticles has no adverse effects on cell viability and development. Therefore, magnetic nanoparticles with biocompatible coatings were chosen to magnetize conventional scaffolds and maintain the initial biocompatibility properties of both materials. To reach this objective, an innovative magnetization technique was developed consisting of infilling the nanoparticles in the scaffolds by a simple process of dip-coating in ferrofluid, which is not damaging to biological matter.[55]

8.2.3.3 Bioactive glass−ceramics and biodegradable materials

Biodegradable metals have attracted much attention in the field of biomedical implants owing to their advantages over nonbiodegradable metals such as stainless steel and titanium-based alloys.[73] In particular, magnesium alloys have shown great potential for applications in bone tissue repairing[74] because of their remarkable physical and mechanical properties, such as an elastic modulus similar to human bone,[75] high specific strength and low density. However, magnesium alloys are highly susceptible to corrosion in the biological environment, which could lead to sudden failure of the implants in long-term service.[76] Therefore, an effective approach of surface modification with inorganic coating materials is applied to retard the biodegradation of magnesium alloys.[77] The solgel technique, which offers controlled composition and morphology, high adhesion with metallic substrate, low processing temperature and enhanced bioactivity and so forth, is adoptive to produce ecofriendly anticorrosion coatings.[78] Several investigations have been reported into protecting magnesium alloys with inorganic coating by a combination of solgel and dip-coating techniques.

Bioglass 45S5, a commercially available inorganic material, possesses excellent bioactivity, favourable biocompatibility and controllable biodegradability.[79] The material also exhibits strong interfacial bonding with bone in living organisms and facilitates integration of osseous tissue with the implant, which could promote bone regeneration and has been used clinically as a hard tissue-regenerative biomaterial in orthopaedic surgery. In a research study, Huang et al.[80] reported an improvement in corrosion resistance and bioactivity using mesoporous 45S5 bioactive glass−ceramic (45S5 MBGC) thin films on AZ31 magnesium alloy by dip-coating and the evaporation-induced self-assembly process. Nonmesoporous 45S5 bioactive glass−ceramic (45S5 BGC) thin films were also prepared for comparative investigation. The results of that investigation showed that 45S5 MBGC thin films were crack-free and uniform with a larger special surface area and pore volume and better surface wettability in contrast to 45S5 BGC coatings. In addition, 45S5 MBGC coatings showed good adhesion strength to the AZ31 substrate owing to the chemical bonding interface. Investigation tests in SBF revealed that the pitting corrosion potential and polarization resistance of AZ31 substrate were improved by the 45S5 MBGC coatings. Furthermore, the anticorrosion property decreased the corrosion current density. Consequently, the 45S5 MBGC-coated magnesium alloys had potential for use as biodegradable biomedical implant material.[80] The 45S5 MBGC coatings were more hydrophilic than 45S5 BGC coatings and had more desirable adhesion strength to the AZ31 substrate for chemical bonding in the interface. The electrochemical impedance spectroscopy (EIS) and

potentiodynamic polarization tests verified that 45S5 MBGC coatings can greatly improve the pitting corrosion potential and corrosion resistance of magnesium alloys. Therefore, 45S5 MBGC coatings are a potential material for the development of anticorrosion and bioactivity in magnesium alloys.[80]

To date, research efforts regarding protective thin films have primarily focused on compact textures.[81,82] In fact, studies have confirmed that mesoporous thin films could confer corrosion protection for magnesium alloys. Furthermore, a mesoporous structure is beneficial to the integrity of the outer surface. This mesoporous structure could effectively release stress in the thin film and ameliorate the mismatch between the substrate and coating; thus, crack-free thin films can be successfully prepared. In addition, studies have reported that mesoporous thin films can easily induce apatite formation and have high bone-forming ability owing to the enhancement of cell activity and protein adsorption.[83]

8.2.4 Biomimetic approach

In most cases, bone defects filled with biocompatible materials are encapsulated by fibrous tissue separated from the surrounding bones. It is known that various types of ceramics bond to living bone without forming fibrous tissues around them. Hydroxyapatite, bioactive glass, and glass–ceramic A-W including apatite and wollastonite ($CaO \cdot SiO_2$) are frequently used as bone-restorative materials for clinical applications.[84] Although these biomaterials have excellent mechanical strength, they cannot be used confidently under high load-bearing conditions such as femoral and tibia bones. This phenomenon occurs for two main reasons: their low fracture toughness and their high elastic modulus with respect to human cortical bone.

It is known for various kinds of glasses and glass-ceramics that the essential requirement for them to bond to living bone is the formation of a biologically active bonelike apatite layer on their surfaces in the body.[85] This bonelike apatite layer can be reproduced on their surfaces as a thin film.[86] The mechanism of bonelike apatite formation on their surfaces is associated with their surface chemistry. These findings enable us to form apatite thin films even on surfaces of metals and organic polymers through the biomimetic approach.

All known kinds of bioactive materials bond to living bone through an apatite layer that is formed on their surfaces in the living body. The apatite layer can be reproduced on their surfaces in acellular SBF with ion concentrations nearly equal to those of human blood plasma[87] and are identified as a thin film of carbonate-containing HA with small crystallites and defective structures similar to apatite in natural bone.[88] The mechanism of biomimetic apatite formation on the surfaces of bioactive glass and glass–ceramics in the living body is explained as follows.[89] Calcium release from them increases ionic activity in the surrounding fluid, and hydrated silica on their surfaces provides apatite nucleation sites. After the formation of apatite nuclei, they grow by consuming calcium and phosphate ions, because the body fluid is supersaturated with these ions.[90] These findings provide a biomimetic method for forming a bonelike apatite thin film on different substrates.[91] Simple oxide gels with compositions such as TiO_2, ZrO_2, Nb_2O_5 and Ta_2O_5 also form an apatite thin film coating

on their surfaces in SBF.[91] This shows that even metallic materials based on Ti, Zr, Nb and/or Ta can form apatite layers on their surfaces in the living body and bond to living bone through apatite layers when their surfaces are slightly modified (Fig. 8.7).

In the past few decades, many methods such as physical machining and controlled oxidation have been used to improve the in vivo osseointegration of titanium-based implants. Calcium phosphate—based thin films such as HA have been used frequently on orthopaedic implants. As a new concept in tissue engineering, it has been suggested that HA has distinct luminescence properties allowing rapid identification of phase distribution of biomimetic apatite thin films. In a research study, Sepahvandi et al.[93] reported that the photoluminescence property can be used in the characterization and early detection of biomimetic bonelike apatite formation on the surface of alkaline-treated titanium implants (in SBF solution).

According to their state-of-the-art research, the researchers concluded that the photoluminescence emission peak did not have a significant shift to shorter or higher wavelengths, and the photoluminescence intensity increased as the exposure time increased. This research proved that the observed inherent photoluminescence of biomimetic apatite thin films can be of specific interest for histological probing and bone remodelling monitoring. In that study, the formation of apatite thin film on the surface of implants was confirmed by energy-dispersive X-ray spectroscopy (EDX) analysis, so the appearance of apatite formation after immersion in SBF solution was established by EDX, as shown in Fig. 8.8(a). As can be seen, the EDX spectrum shows the peaks of Ti, Al and V elements related to the titanium implant, and the peaks of P, Ca and C correspond to the newly formed biomimetic apatite thin film. Also, according to the Ca and P peaks of the EDX graph, the Ca—P molar ratio was calculated to be in the range of 2.6, which could be related to nonstoichiometric hydroxy-carbonate apatite.[94,95] In addition, Fig. 8.8(b) shows a scanning electron micrograph of the cross section of the implant after immersion in SBF solution, indicating that the newly formed biomimetic apatite thickness of the specimens is less than 5 μm and also homogeneous and uniform.

8.2.5 Electrophoretic deposition

Electrophoretic deposition (EPD) was discovered in 1808 by the Russian scientist Ruess; it was first used in a practical application in 1933 to deposit thoria particles on a platinum cathode.[96] EPD is a low-cost, flexible and non—line-of-sight coating process which can be used to deposit uniform thin films on substrates of complex shape or surface morphology. Furthermore, EPD can produce thin films of a wide range of thicknesses, from nano to micron, with a high degree of control over thickness and morphology.[97] As for many other ceramic coating techniques, EPD-coated implants need a subsequent densification stage to sinter the thin film. Wei et al.[98] reported a nano particulate dual-coating approach to deposit HA nanoparticles on the surface of metallic substrates through EPD. They also studied the interfacial bond strength of the prepared thin films. Hamagami et al.[99] fabricated highly ordered macroporous apatite thin films onto titanium by EPD. Unfortunately, such coatings deposited by electrophoretic technique have the major drawback of poor adhesion and the need for posttreatment

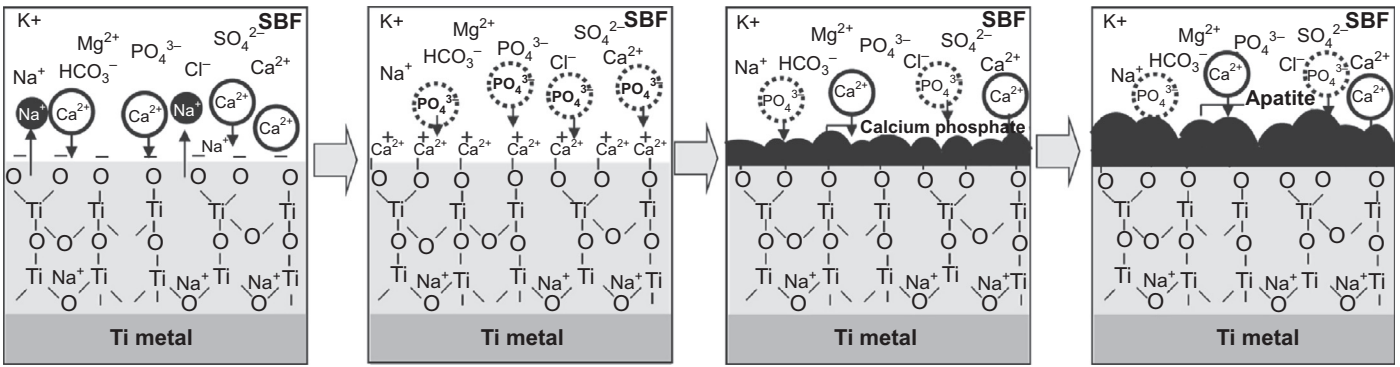

Figure 8.7 Process of apatite formation in simulated body fluid (SBF) on Ti metal subjected to acid NaOH and heat treatment.[92]

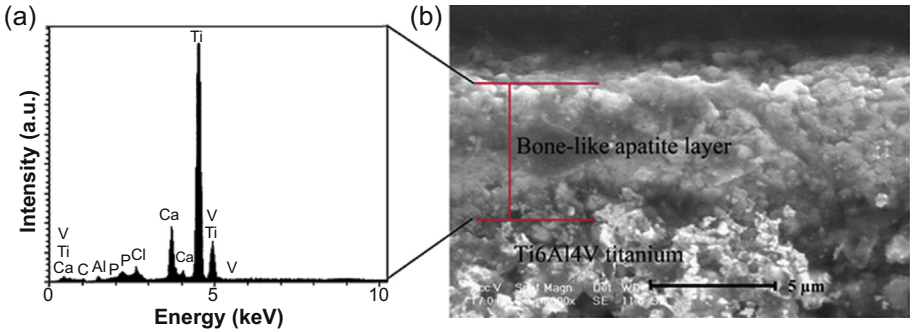

Figure 8.8 (a) Energy-dispersive X-ray pattern and (b) scanning electron micrograph of the cross-section of newly formed biomimetic apatite thin film after 7 days immersion in SBF.[93]

to improve adhesion. The difference in thermal expansion can also cause cracks to generate on the surface of coated layers or at the interface between the thin films and substrates during cooling after sintering.[98] Therefore, relatively low sintering temperatures are desirable for HA-coated systems. Studies have also demonstrated that well-dispersed particles are necessary to produce densely packed thin films because aggregates form loosely bound structures with low green densities and poor sintering behaviour.[100,101] Therefore, it is highly desirable to produce stable suspensions containing fine HA particles. As previously pointed out, the choice of a suitable medium is important for EPD. In fact, EPD is a material processing technique based on the movement of charged particles in liquid suspension and their deposition on a substrate acting as an electrode in the EPD cell, showed in Fig. 8.9. This processing technique is increasingly being considered for the production of nanostructured coatings and layers on a variety of substrates for numerous applications, including wear and oxidation resistance, bioactive coatings for biomedical implants and devices as well as functional coatings for photocatalytic, electronic, magnetic and related applications.

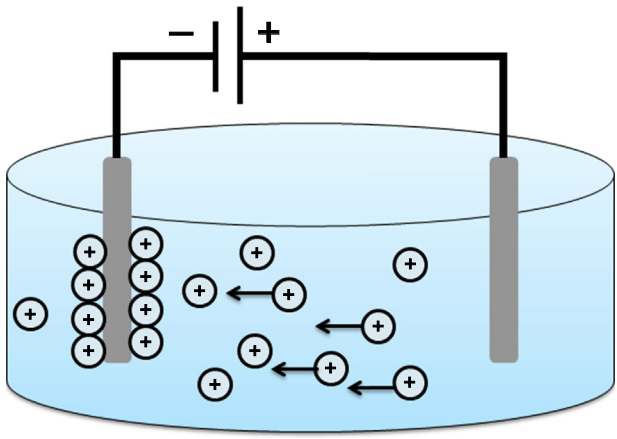

Figure 8.9 Two electrodes for electrodepositing showing positively charged particles in suspension migrating toward the negative electrode.

Carbon nanotubes (CNTs) have been the subject of extensive research over the past 10 years.[102] Because of their impressive structural, electrical and mechanical properties as well as their small size and mass, they have become one of the most promising materials for future development and have opened up a new era in the field of biomaterials science.[102,103] In recent years, considerably effort has been devoted to applying CNTs in the biological and medical fields.[104] Available data suggest that CNT-containing materials may be optimal for tissue engineering applications. This is not only because of their ability to simulate dimensions of proteins that comprise native tissue but also because of their higher reactivity for interactions involved in the cell attachment mechanism.

In a research study, Boccaccini et al.[105] investigated the thin films of highly porous Bioglass-based scaffolds with multiwalled CNT (MCNTs). They fabricated foamlike Bioglass scaffolds by EPD and deposited homogeneous layers of CNT throughout the scaffolds' porous structure. Optimal experimental conditions were determined to be an applied voltage of 15 V and deposition time of 20 min, using a concentrated aqueous suspension of CNT with the addition of a surfactant and iodine. The scaffolds' porous structure remained invariant after the CNT coating. The incorporation of CNTs induced a nanostructured internal surface of the pores that was thought to be beneficial for osteoblast cell attachment and proliferation. Bioactivity of the scaffolds was assessed by immersion studies in SBF for up to 2 weeks and the subsequent determination of HA formation. The presence of CNTs can enhance the bioactive behaviour of the scaffolds because CNTs can serve as a template for the ordered formation of nanostructured HA layers, which does not occur on uncoated Bioglass surfaces.

Several studies have been carried out on the interaction between CNTs and a variety of cells including osteoblasts and have focused on the biocompatibility of CNTs.[106,107] These studies suggest the possibility of using CNTs as an alternative material to treat bone pathologies in combination with bone cells, with the potential for enhanced osteoblast proliferation and bone formation. In addition, next-generation scaffolds could incorporate further functionalities to enhance neotissue formation. For instance, an electric field is known to stimulate the healing of various tissues. In the case of bone regeneration and fracture healing, the use of an electric field is based on the observation that when a bone is subjected to mechanical stresses, deformation of bone is normally accompanied by an electrical signal bearing the strain characteristics. Therefore, a conductive scaffold, eg, incorporating CNT, can potentially be used to stimulate cell growth and tissue regeneration by facilitating physioelectrical signal transfer.

A review of previous work on the EPD of CNT was published.[108] That article showed that EPD is a convenient method to manipulate CNTs and produce reliable CNTs assemblies and layers on planar substrates. For example, Du et al.[109] deposited CNT on metallic substrates by EPD using ethanol−acetone mixed suspensions. Further studies were carried out by Thomas et al.[110] in which homogeneous deposition of CNT assemblies using aqueous suspensions was accomplished on stainless-steel substrates. Incorporation of CNTs into the scaffolds also has a number of special advantages, such as that it encourages cell adhesion and proliferation by inducing nanotopography, it provides a crack-inhibiting mechanism on the scaffold surfaces and it

confers biosensing (electrical conduction) properties while maintaining bioactivity and the interconnected porous network of the scaffold. Moreover, the addition of CNTs to a biocompatible matrix has further advantages for multifunctional (smart) scaffolds because CNT can be used for targeted delivery of growth factors or drugs. In brief, EPD is a technique for the development of complex CNT−ceramic nanocomposite layers and coatings of high structural homogeneity and reproducible properties for tissue engineering.[108,111]

8.2.6 Chemical vapour deposition

In the past few decades, it has been demonstrated that many types of materials can be deposited as films at pressures below 1 atm using chemical vapour deposition (CVD) processes.[112] This is a promising technology for a wide range of biomaterials in the field of tissue engineering because of the great combination of superior properties including hardness, fracture toughness, the low friction coefficient, high chemical resistance and a variety of possible coating substrates. It has been frequently reported that the biocompatibility of CVD surfaces is similar to that of bare titanium implant surfaces. A study[113] demonstrated that thin films of diamond-like carbon containing up to 22 atm% silicon (DLC-Si) deposited onto silicon substrates by low-frequency pulsed direct current plasma-activated CVD (PACVD) showed exceptional characteristics compared with untreated samples. That study showed that biocompatibility tests using MG-63 osteoblast-like cell cultures indicated homogeneous and optimal tissue integration for both the DLC and the DLC-Si surfaces. The pulsed PACVD technique has been shown to produce biocompatible DLC and DLC-Si coatings with potential for tissue engineering applications. Fig. 8.10 represents a schematic of basic process steps during CVD.

In other research activities, CVD polymerization offers several advantages compared with other solvent-based coating processes.[114] First, CVD coatings are conformal, which allows for the easy and uniform modification of nonplanar substrate geometries. Second, although the activation step (which takes place away from the substrate) requires high temperatures, the substrates can be maintained at a controlled temperature (typically room temperature) or below. Third, impurities associated with the use of solvents, initiators or plasticizers can be precluded. Several examples of CVD-based polymer coatings have been reported. Frank and coworkers[115] used CVD to prepare polypeptide chains that are grafted onto a surface. Gleason and coworkers have shown through various examples[116] that even if the monomer of interest does not contain an initiator, polymerization initiators can be introduced together with the monomer through basic process modification. In cases in which hot filaments within the deposition chamber were used for initiation, radical polymerizations often yielded conformal thin films.

8.2.7 Pulsed laser deposition

The pulsed laser deposition (PLD) technique has been used to deposit crystalline HA thin films on titanium substrates.[117,118] The structure and properties of HA thin films

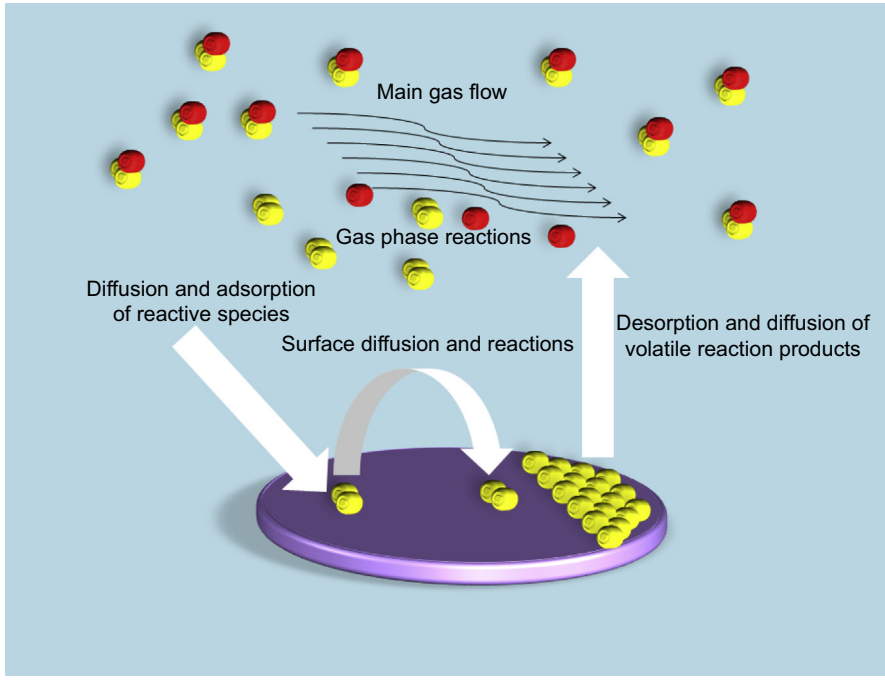

Figure 8.10 Schematic representation of basic process steps during chemical vapour deposition (CVD).

using PLD technique can be tuned with different laser deposition parameters. As an excellent technique for tissue engineering applications, Wang et al.[118] showed that PLD process did not induce a large amount of calcium phosphate phases other than apatite. The process did not also change the behaviour of hydroxyl or phosphate functional groups. In addition, microscopic observations revealed that HA thin films consisted of numerous essentially spheroidal particles of different sizes during solidification, whereas the lateral morphology indicated that columnar and dome-shaped structures both existed in the film. The adhesion strength of the thin film, mostly in the range 30–40 MPa, was closely related to the fractography of the tested sample. The fractured surfaces with higher bond strengths were usually accompanied by a higher degree of deformation and coating–substrate debonding, whereas the fracture of samples with lower bond strengths occurred more frequently within the HA thin films in a more brittle manner.

In addition, two newly developed techniques, matrix-assisted pulsed laser evaporation (MAPLE) and MAPLE direct write (MDW), were suggested to deposit biomaterials thin films. MAPLE involves dissolving the biomaterial in a volatile solvent, freezing for the creation of a solid target and evaporating the target for deposition of the solute inside a vacuum system (using a low-fluorescence pulsed laser). Using shadow masks such as dots or arrays, pattern features with small length scales can be deposited using compound materials on numerous types of substrates.

In comparison, MDW uses a pulsed laser to transfer the target material directly from a ribbon to a substrate, in which the patterns with a spatial resolution can be written directly. Furthermore, pulsed laser ablation has been used to develop improved surface conformation on tissue engineering scaffolds. The in vitro and in vivo biocompatibility of an HA thin film deposited on titanium web (TW) scaffolds was studied.[119] In that study, HA thin films were deposited onto the surface using a pulsed laser operating at a rate of 10 Hz followed by annealing at 380 °C for 1 h. The presence of HA not only along the surface but also in the TW inner region was confirmed by the elementary mapping of calcium, phosphorous and titanium. Finally, the X-ray diffraction (XRD) patterns showed that crystalline HA coated the entire surface of TW scaffolds, indicating the high potential of that technique for tissue engineering applications.

8.2.8 Solgel technique

The use of thin film coatings is one of the most efficient surface modification approaches to improve the performance of metallic implants. Among the various methods used to prepare thin films, solgel deposition has many advantages, such as high homogeneity and simplicity for full coverage of complex structures.[120,121] Generally, solgel-derived thin films are classified into two categories: inorganic oxide and organic−inorganic hybrid thin films. The latter has been developed to overcome the drawbacks of the former, especially brittleness and the need for relatively high-temperature treatment (sintering) after deposition, by introducing an organic component into the inorganic network.[122] According to the literature, organic−inorganic hybrid thin films can better improve the corrosion resistance of metallic implants compared with inorganic thin films[122]; however, the adhesion of inorganic thin films to metallic substrates is better than that of organic−inorganic thin films.[123] In a similar situation, Fedrizzi et al.[124] found that the anticorrosion performance of polyester organic thin films on low-carbon steel improved owing to pretreatment of the substrate by applying an inorganic zirconia coating, which enhanced the film's adhesion to the substrate.

In a research study, Salahinejad et al.[125] investigated a new double-layer solgel thin film coating to improve the corrosion resistance of medical-grade stainless steel in an SBF solution. One effective way to overcome some of the drawbacks of oxide coatings for the corrosion protection of metal surfaces is to incorporate an organic component into the inorganic network, although commonly, film adhesion is disadvantageously affected. In that work, for the first time, by exploiting both inorganic and organic−inorganic coatings, a new double-layer thin film composed of $ZrTiO_4$ as the bottom layer and $ZrTiO_4$−PMMA as the top layer was deposited on a medical-grade stainless-steel substrate via solgel spin-coating. According to potentiodynamic polarization experiments in SBF, the substrate coated with this hybrid thin film exhibited superior corrosion resistance compared with the same substrate coated with purely inorganic $ZrTiO_4$ films.

In a comparable study, Naghib et al.[126] investigated the bioactivation of a 304 stainless-steel surface through 45S5 Bioglass coatings for biomedical applications.

The ability of 45S5 Bioglass to form a bond to living bone tissue and stimulate bone–cell proliferation may be different for melt- and solgel-derived samples. In that research, the differences in corrosion resistance, bioactivity and physical properties between the melt- and solgel-derived 45S5 Bioglass coated on the surface of austenitic 304 stainless steel as a dental and orthopaedic metallic implant were studied. The morphologies of different coated samples were investigated by scanning electron microscopy. Then, electrochemical measurements were performed and compared with uncoated samples. To investigate the bioactivity and surface reactivity of the coated samples, the researchers monitored the samples in SBF solution in vitro and examined their microstructures and electrochemical properties in detail. Immediately after immersion in SBF, reactions occurred on the surface of the coated samples, and the obtained results from XRD and Fourier transform infrared spectroscopy analyses showed typical characteristic peaks of HA crystals. In addition, the coated samples showed enhanced corrosion resistance and bioactivity compared with the uncoated ones. The solgel-derived coated sample had higher corrosion resistance and formed the HA layer more quickly, qualities that are useful for dental and orthopaedic metallic implants.

8.3 Novel innovative strategies for tissue engineering purposes

Studies have reported a novel coating approach to deposit crack-free and nanostructured HA thin films on Ti6Al4V alloys with an Al_2O_3 buffer layer for tissue engineering purposes.[127] In this novel method, the Al_2O_3 buffer layer was deposited by plasma-spraying whereas the HA top layer was applied by dip-coating. The XRD and Raman reflections of alumina buffer layer showed α- to γ-Al_2O_3 phase transformation and the fractographic analysis of the sample revealed the formation of columnar grains in well-melted splats. Surprisingly, the bonding strength between Al_2O_3 coating and Ti6Al4V substrate was estimated to be about 40 MPa. In addition, the microscopic images showed that the HA top layer homogeneously enveloped the troughs and crests of the underneath rough ($R_a = 2.91$-μm) Al_2O_3 surface. It is believed that this novel coating approach to preparing thin films might be useful for rapid cement-less fixations in critical situations for longer periods of time.

In another innovative approach, Kim et al.[128] suggested that nanocrystalline HA thin films can be formed at the surface of Ti by single-step microarc oxidation method using Ca^{2+} and P^{5+} ion-containing electrolytes. They reported that the HA films were 10–25 μm thick and showed strong crystallinity dependence on the $CaCl_2$ concentration in the electrolytes. Also, the formation of an amorphous $CaTiO_3$ interlayer was identified as existing between the HA film and Ti substrates. In contrast to previous research, using K_2HPO_4 for the electrolytes could allow formation of a crystalline HA layer. It is suggested as the most probable mechanism for the HA formation that the high-density hydroxyl groups of $TiO(OH)_2$, formed by the reactions between the amorphous $CaTiO_3$ interlayer and the H^+ ions from the dissolution of the KH_2PO_4,

can have a key role in the nucleation and crystal growth of HA films by attracting Ca^{2+} and P^{5+} ions in the electrolytes. Undoubtedly, this basic research shed light on the creation of the most effective coating thin films by controlling the nucleation and crystal growth of HA on the surface of tissue engineering implants.

Plasma-enhanced CVD (PECVD) for the deposition of nanostructures composed of diphenylalanine has shown promising advantages over conventional methods. This is a solvent-free approach that allows sublimation of the peptide to form dense, uniform arrays of peptide nanostructures on a variety of substrates. The PECVD-deposited D-diphenylalanine nanostructures have a range of chemical and physical properties depending on the specific discharge parameters used during the deposition process.

This technology has been used to deposit two different fluorocarbon precursors (octofluoropropane and hexafluoropropylene oxide) on polycaprolactone scaffolds. These two coating systems were chosen with intention of modifying the scaffold surfaces to be bio-nonreactive while maintaining the desirable bulk properties of the scaffold. Microscopic observations confirmed that fluorocarbon film deposition yielded conformal rather than blanket coatings as the porous scaffold structure was maintained after plasma treatment. The treated scaffolds seeded with human dermal fibroblasts demonstrated that the cells do not attach after 72 h and that the scaffolds are not cytotoxic to the cells. This work demonstrates that conformal fluorocarbon coatings can be deposited on three-dimensional (3D) polymeric scaffolds using PECVD to fabricate 3D bio-nonreactive materials for advanced applications in tissue engineering.

8.4 Conclusion

It is known that biomaterials interact with biological systems through their surfaces. Biocompatibility is a crucial property to minimize unwanted and undesirable responses and permit the surface of the biomaterial to integrate with the host tissues. Therefore, organic and inorganic thin films have attracted much attention because of the following properties: versatility, biocompatibility, biodegradability and integration with tissues. These coated materials can have properties similar to hard and soft tissues, including Young's modulus, fracture stress, elasticity and hardness. To achieve thin film formation for biomedical applications, spin-coating, LbL assembly, dip-coating, EPD, CVD, PLD and the solgel process can be used, as summarized in this chapter. Chemical grafting of molecules onto the surface of biomaterials has also been achieved with such coating technologies as one of the most important advances in the field of tissue engineering. Among different techniques, multilayer thin film coatings based on LbL assembly provides precise control over the physicochemical characteristics of biomaterials on different surfaces. This new type of thin films has the potential to be considered as grafts with new properties to attract drug carriers as the next generation of functional thin films. Furthermore, in vitro and in vivo investigations should be performed to study the biocompatibility and osteoinductivity of these biomaterials. In addition, a suitable biodegradability rate is a

necessary property for the development of tissue engineering implants for enhanced regeneration and must be matched with the rate of neotissue formation. Furthermore, solgel process has been widely used to prepare thin film oxide layers with improved surface characteristics. These thin biocompatible and biodegradable films with an optimized composition and structure on the surface of metallic implants can significantly improve implant integration. It is believed that a combination of these novel coating technologies can effectively solve problems associated with the rapid fixation of implants into natural tissues as well as biocompatibility for longer periods of time in the human body.

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