Three-Dimensional (3D) and Drug-Eluting Nanofiber Coating for Prosthetic Implants

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Abstract Failure of osseointegration and implant infection are the two main causes of implant failure and loosening. There is an urgent need for orthopedic implants that promote rapid osseointegration and prevent infection, particularly when placed in bone compromised by disease or physiology of the patients. This chapter reviews current and potential future use of biologic and drug-eluting coatings for orthopedic implants to facilitate osseointegration and prevent implant infection. The potential application of porous and drug-eluting coaxial nanofiber as a means of alternative implant surface coating was discussed.

Keywords Osseointegration · Infection · Periprosthetic implant · Implant coating Three-dimensional (3D) nanofibers · Automatic electrospun nanofibers collector Cellular activity · Electrospinning · Corona discharge · Porous structure · Coaxial Drug delivery

Abbreviations

AL	Aseptic loosening
AMP	Antimicrobial peptides
CS	Chondroitin sulfate
DAC	Defensive antibacterial coating
Doxy	Doxycycline
ECM	Extracellular matrices
HA	Hydroxyapatite
LBL	Layer-by-layer
MSCs	Mesenchymal stem cells

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NFs	Nanofibers
PAA	Poly(acrylic acid)
PCL	Polycaprolactone
PEO	Poly(ethylene oxide)
PJI	Prosthetic joint infection
PLGA	Poly(lactic-*co*-glycolic acid)
PVP	Polyvinyl pyrrolidone
rhBMP-2	Recombinant human bone morphogenetic protein-2
rhBMP-4	Recombinant human bone morphogenetic protein-4
THA	Total hip arthroplasties
Ti	Titanium
TiColl	Type-I collagen-coated titanium
TJA	Total joint arthroplasty
TKA	Total knee arthroplasties
$VEGF_{165}$	Recombinant human vascular endothelial growth factor
β-TCP	β-tricalcium

Background

Introduction

Total joint arthroplasty (TJA) is a life-improving intervention for millions of people all over the world. It is for total replacement of hip, knee, ankle, elbow, and shoulder by orthopedic implant/prosthesis. In the USA alone, there were approximately 332,000 total hip arthroplasties (THA), and over 700,000 total knee arthroplasties (TKA) performed in 2010 [1, 2]. The number of TJA continues to increase significantly along with an aging population. It may reach 572,000 and 3,480,000 for hip and knee arthroplasties, respectively, by 2030 [2, 3]. Besides, the procedure for other joints, including ankles, elbow, and shoulders, are also available, and also increasing performed.

TJA is one of the most successful clinical procedures. It helps patients with functional restoration, pain and stiffness relief, thereby improving patients' quality of life. Several surveys reported that the patients who received TKA expressed 90–95% of satisfaction rate [4–9]. The survival rate of the implants within 10–15 years was greater than 90% [4–9], which indicates the reliability and durability of the implants.

A biocompatible and bioactive orthopedic implant is one of the keys that determine the success of the TJA. An implant should be sufficiently inert to avoid triggering systemic immune/inflammatory reactions; and in the meanwhile it should stimulate the integration of the implant to the surrounding tissues. The most widely used implant materials are titanium (Ti) and its alloys. It has notable biocompatibility and lower stress shielding comparing to other metallic materials. Therefore, the Ti

alloy has become the gold standard in cementless implants [10]. Focusing on the orthopedic implants of TJA, this chapter will introduce the potential complications of TJA and current strategies on how to address issues by utilizing implant coatings (design, materials, and drugs).

Complications of Total Joint Arthroplasty: Osseointegration Insufficiency and Infection

The TJA is a well-established orthopedic procedure in both surgical technique and implant design. Nevertheless, a small portion of patients has poor outcomes, like implant loosening or infection. As a result, they may need a revision surgery. The revision rate, within the first 2 years after TKA, is approximately 3% [4, 11]. This percentage, although seems insignificant, would lead to considerable medical costs considering the total number of TKA procedures each year.

A successful TJA requires the orthopedic implant to be appropriately stabilized (primary stability) through mechanical press-fit during the surgery [10]. It is followed by native bone ingrowth to bridge the gaps between the implant surface and periprosthetic tissues. Subsequently, osteoblast-like cells deposit on the interface of the implant and surrounding bone and start active proliferation (first 10–12 days after implantation) [10]. Through a series of spatiotemporal cellular activities, the extracellular matrices (ECM) are finally mineralized to a mature ECM (28 days after implantation), which is composed of ~65% minerals, like hydroxyapatite (HA) and ~35% of organic components, mainly type-I collagen [12]. Thus, the implant could be further stabilized to the host bone, which is called secondary stability [10, 13]. The bone continues remodeling until it completely integrates with the implant to restore the function. It is a dynamic and long-term healing process. If any one of these steps is delayed or disrupted, it may lead to TJA failure. For instance, if the implant was not fixed perfectly at beginning, it would initiate micromotion and enlarge the gap between the implants and bones. Later, more and more wear debris may be accumulated in the gap, leading to macrophage-induced osteolysis [14–16].

The reasons of implant failure include aseptic loosening (AL) and septic loosening, also referred to as periprosthetic joint infection (PJI) [2]. AL is the leading cause of TJA failure and its incidence continues to increase [17]. Rapid and sufficient osseointegration can enhance implant stability and increase the implant life. In 1950s, Dr. Brånemark et al. first presented the concept of "osseointegration." It was based on the observation of the formation of a direct interface between a Ti implant surface and periprosthetic bone [10, 18]. The clinical definition of the osseointegration is that alloplastic materials are rigidly fixed to bone and maintained during load bearing [10]. The lack of sufficient osseointegration causes implants micromotion, instability, osteolysis, and loosening [14, 16]. Ryd et al. reported that early implant loosening in both hips and knees might result in implant failure [19]. Kärrholm et al. also concluded that the subsidence of the implant could increase the risk of AL

[20]. The physiochemical and bioactive characters of the implant surface have a big impact on the consequence of the osseointegration.

PJI is another leading cause of TJA failure. The annual incidence of PJI after TJA is more than 2% among the Medicare population [21]. This is a large number considering ~581,000 total knees and more than 193,000 total hips are performed each year in the USA alone. The direct cost/hospital cost to treat PJI in the American health care system was $566 million in 2009 [2, 22]. In general, the first 2 years has the highest risk of PJI in that roughly 60–70% PJI happens in this period [2, 21, 23, 24]. The incidence of implant infection is even higher after revision surgery than after primary surgery. The average cost of treatment PJI in revision surgery is 3–6 times higher than the primary implantation.

The PJI is mainly initiated through introducing bacteria like *Staphylococcus aureus*, a leading pathogen (60%) of PJI, during the surgery [25, 26]. The bacteria contaminate the orthopedic implant surface or periprosthetic tissue, and quickly colonize on the implant surface. Later, the infection spreads and progresses to adjacent tissues during early onset infections (within first few months after implantation). Hematogenous spreading is another path for PJI. The overall rate of hematogenous spreading is low however, the patient may remain at the risk of hematogenous infection throughout the life of the implant and is one of the causes of late-onset PJI (over 1 year after implantation) [27]. Biofilm is a complex community of one type or multiple types of microorganisms that forms on a surface of the implant. It may start to take shape at any time, including during the late-onset PJIs [23]. The formation of the biofilm makes the treatment of infection more difficult and complicated. The biofilm protects the cells from the treatment of antibiotics and the action of the immune system [23, 28] because of their low growth rate, antibiotic resistance property, and the protective extracellular matrix [29, 30].

The PJI treatment that aims to control the infection and restore the function of joints can be reached by many different medical and surgical strategies. It includes antibacterial treatment without surgery, debridement with implant retention, and resection of the implant without reimplantation or with reimplantation through one-stage arthroplasty or two-stage arthroplasty exchange, and amputation [21]. Prophylactic systematic administration of antibiotics is a routine treatment to prevent infection. However, long-term use of antibiotics may lead to drug resistance. In addition, the systematic antibiotic treatment would be less effective when the biofilm was formed on the implant surface. To prevent implant infection, various strategies have been attempted, either by implant surface fabrication or incorporation of antibiotics into the implant devices [31].

Recent Implant Coating Developments: Advantages and Disadvantages

The aim of the TJA is to restore or improve the pre-morbid function. Over the past 25 years, the orthopedic implant concept or design has progressed from the restoration of the mechanical functions of bone tissue to regenerative medicine. Researchers

have no longer been satisfied with the Ti or its alloy implants for the lack of the bioactive property. An increasing number of scientists have been focusing on adding biological properties of the implants to enhance the bone healing. Implant surface coating is one of the strategies to modify the physiochemical properties of the implant surface, and to reach locally pharmacological treatments [10, 32]. Recent developments in implant surface coating technologies for the osseointegration enhancement and infection inhibition are summarized and discussed below.

Hydroxyapatite (HA) Coating

HA coating fabricated by plasma spray is a common coating in the clinic. It has been used clinically since 1987 [14]. The HA coating has similar component to the bone, which provides calcium and phosphate for new bone formation. Clinical studies indicated that HA coating not only bridges the interface of implant and bone, but also enhances the osseointegration of cementless metallic implants within bone [33, 34]. In a canine study, the formation of new bone was discovered at distance of 400 μm from the HA coated implant, which was inserted in the femoral condyles of mature dogs [35]. This finding proved the osteoconductive capability of HA coating [35]. However, there were also controversial reports stating that no differences were found between HA coated and non-coated implants for the long-term clinical outcomes [36, 37]. The HA coating may impair initial osseointegration because it lacks a physiological surface [38]; It's brittle in nature [39] and the poor adhesion strength [40] additionally effect the clinical outcomes.

Recently, the traditional HA coating has been used as a drug delivery device for the local delivery of growth factors, peptides, antibacterial drugs, and DNA [41–46]. He J. et al. [41] developed a porous HA coating infiltrated with collagen, RGD peptide, and recombinant human bone morphogenetic protein-2 (rhBMP-2) for Ti alloy implant. The collagen/rhBMP-2-modified HA coating increased the attachment, proliferation, and differentiation of mesenchymal stem cells (MSCs) in vitro. It also significantly accelerated bone growth rate after implantation into dog femora. Thus, the modification of the HA coating by embedding osteogenic factors becomes an effective method to enhance osseointegration at bone–implant surface [41].

With the aim to inhibit implant-associated infection, the HA coating was used to load with antibacterial drugs, such as silver [46, 47], antibiotics [48], and antimicrobial peptides (AMP) [49]. Silver has a broad antibacterial spectrum. The bactericidal effect of silver coating is through the interaction of silver with the membranes, proteins, and DNA of bacteria [50]. Moreover, silver can interrupt the formed biofilm [14]. Thus, silver is an effective bactericide, which has been applied to the HA coating. For example, Chen W. et al. used co-sputtering technology to create a silver-HA coating on Ti implant surface [47]. The silver-HA coating significantly reduced the attachment of *S. epidermidis* and *S. aureus* when compared to uncoated surfaces. Moreover, the silver-HA coating did not induce in vitro cytotoxicity. In another study, although the silver-HA coating fabricated by plasma spray inhibited bacterial colonization, it showed cytotoxic effect [46]. The silver-HA coating

reduced the viability and osteogenic differentiation of human fetal osteoblast cells. Fortunately, adding strontium to the silver-HA coating offsets the negative effects, and even improved the performance when compared to pure HA coating [46]. Thus, the silver embedded HA coating is a multifunctional surface, which enhances osseointegration and inhibits infection. Like silver, other alternative inorganic antibacterial elements, including copper, zinc, nitrogen, and gold, can be applied to HA coating for infection inhibition in the future [51, 52].

Systemic administration of antibiotics is common clinical practice to prevent infection for TJA patients. However, the effectiveness may be reduced because of relatively low dose in the implant site, and the risk of antibiotic resistance occurred after long time use. Therefore, local delivery of antibiotics is expected to directly eliminate the bacteria on the implant surface and hence even more effective when combined with the systemic antibiotic treatment. HA coatings have embedded various types of antibiotics, such as gentamicin, tobramycin, and vancomycin [53–55]. The antibiotic-embedded HA coatings have shown effective antibacterial properties [53–55]. A biodegradable, poly (lactic-*co*-glycolic acid) (PLGA), gentamicin-embedded HA coating on cementless hip implant was developed by Neut D. et al. for the prevention of PJI [53]. The PLGA-gentamicin-HA coated pin reduced staphylococcal infection rate in a bacterially contaminated medullary canal of rabbit; and didn't impair the bone ingrowth rate through a condylar defects of Beagle dog model [53].

Although HA coatings embedded with osteogenic or antibacterial agents can enhance osseointegration and prevent infection, there are some unsolved issues regarding the efficacies of local drug delivery. The first issue is the methodology of embedding agents into HA coating. The plasma spray is a high temperature procedure and may lead to the inactivation of embedded drugs, such as growth factors and antibiotics during the procedure. Therefore, the types of drugs that can be incorporated within the HA coating is limited. The second issue is uncontrollability of drug release [56]. In HA coating, physical absorption is the mechanism of agent embedding. The weak bonding force results in a burst release of embedded agents. A study showed that most antibiotics were released from HA coating within 1-h incubation [57]. Besides the HA coating, many coating technologies have been developed to extend and control the release of embedded drugs to enhance osseointegration and inhibit infection. They can be classified into three categories: hydrogel coating, layer-by-layer (LBL) coatings, and immobilization [58], which are introduced in the following sections.

Hydrogel Coating

Hydrogel networks are generally obtained by chemical or physical cross-linking, ultraviolet (UV) irradiation, and electrochemical polymerization. Hydrogel coatings are usually achieved by simply immersing implants into a hydrogel solution and drying out afterward. Hydrogel coatings have been easily applied to many types of implants for stabilizing the implant through bridge of the bone–implant interface

[14]. In addition, a broad range of drugs can be easily added into hydrogel solution before coating. Many studies demonstrated that Ti implants coated with type-I collagen promoted osseointegration [59–62]. Sartori M. et al. developed a type-I collagen-coated titanium (TiColl) screw [60]. The TiColl screws increased bone–implant contact and bone ingrowth in the femoral condyles of healthy and osteopenic rats. The results proved that the TiColl coating enhanced osseointegration even in the physiologically compromised animals. Stadlinger B. et al. combined chondroitin sulfate (CS) and recombinant human bone morphogenetic protein-4 (rhBMP-4) to the type-I collagen coating on Ti implant [61]. The in vivo results showed that the highest bone–implant contact was formed on CS-collagen-coated implant, followed by collagen-coated implant and CS-rhBMP-4-collagen-coated implant [61].

Chitosan is a derivative of chitin, which is a popular polymer material in tissue engineering because of its good biocompatibility and antibacterial property. It has been reported that either chitosan alone [63] or combined with other polymers such as poly(acrylic acid) (PAA) [64] and polyvinyl pyrrolidone (PVP) [65] can form hydrogels to carry antibiotics. For example, a drug-eluting chitosan-vancomycin coating on Ti foil was biocompatible and bactericidal. It reduced the infection risk in antibacterial tests [63]. Recently, an antibiotic-loaded fast-resorbable hydrogel coating (defensive antibacterial coating, DAC) has been applied in an European clinical trial for THA and TKA [66]. The DAC composes of covalently linked hyaluronan and poly-D,L-lactide with antibiotics. In a clinical trial, the DAC was used by simply spreading on the hip/knee prosthesis surface during the surgery. The results showed that the DAC reduced the rate of early surgical site infection. There were no detectable side effects after THA and TKA with a cementless or hybrid implant [66].

The limitation of hydrogel coating as a drug-eluting device is the burst drug release. Like the DAC, it completely degraded within 72 h with 100% antibiotics released [66]. It can be applied as antibacterial coating to prevent early onset infection but is not suitable for the inhibition of the late-onset infection.

Layer-by-Layer (LBL) Coatings

Layer-by-layer (LBL) coating is by depositing layers of polyelectrolyte solutions with opposite charges in an alternating fashion on the implant surface, resulting in a thin film that can be used to load a variety of biomolecules [58]. The number of layers, concentration of molecules in the solution, and chemical properties of the polyelectrolyte solution can be modified to reach an optimal drug loading efficiency and release kinetics. Various growth factors have been deposited on the implant via LBL coating technology [44, 67, 68]. Shah NJ et al. [67] developed [poly (β-amin ester)/polyanion/growth factor/polyanion] LBL tetralayer coating on polycaprolactone/β-tricalcium (PCL/β-TCP) scaffolds. With the aim to mimic the healing process, this coating not only extended the release time of recombinant human vascular endothelial growth factor ($VEGF_{165}$) and BMP-2, but also delivered the two growth factors at different times. As a result, the implant coating facilitated blood vessel ingrowth and bone formation in vitro [67].

Bactericidal LBL coating has been developed by incorporation of polyelectrolyte multilayer films with silver [69], gentamicin [70], and vancomycin [71]. The LBL coating can control drug densities and release profiles. For example, it has been reported that polyelectrolyte multilayers on Ti implants showed a sustained release of bioactive gentamicin over 1 month [70]. The coating has been demonstrated to be bactericidal against *S. aureus* and biocompatible in vitro. An in vivo study has shown that the coated Ti implant successfully decreased the degree of infection in a rabbit *S. aureus* bone infection model [70].

Overall, the LBL coating is a promising technique for massive drug loading and controllable drug release. It can be applied to prevent late-stage infection and inhibit biofilm formation. However, the broad application of LBL techniques has been limited due to several technical challenges. Firstly, the fabrication of LBL coating is labor intensive and expensive. In order to reduce initial drug burst release and to extend releasing duration, usually a few hundreds layers would be required. Secondly, the LBL coating is performed in acidic solution, which may cause toxicity to tissue [58].

Immobilization of Drugs on the Implant Surface

An alternative strategy for long-term drug delivery is to immobilize drugs directly on the implant surface. Osteogenic peptides are the most common used agents that have been used for the improvement of osseointegration [58, 72–74]. Peptide GFOGER, derived from type-I collagen, was proven to promote osteogenic differentiation through binding to the α2β1 integrin receptor on the surface of osteoblast-like cells [72]. Wojtowicz et al. immobilized the GFOFER peptides on the surface of PCL scaffolds via passive absorption [72]. The implantation of GROFER-coated PCL scaffolds was performed in rat femoral defects model. The results showed that the GROFER-coated PCL scaffold effectively promoted bone repair with significant bone volume increase after 12-week implantation [72]. The peptides can be easily absorbed on the polymer surface but is difficult to attach on the metallic surface via physical absorption. Therefore, a strong covalent bonding between drugs and metallic surface needs to be formed to immobilize the drugs. For example, in order to improve the cell adhesion on Ti implant, modified cyclic-RGD peptide with phosphonic acid anchors was developed. The phosphonic acid anchor can bond to titanium oxide and indirectly immobilize the RGD peptides to the metal surface [74].

To inhibit infection, immobilization of vancomycin (Vanc) on the Ti or Ti6Al4V(Vanc-Ti) implant surface through covalent bonding was reported [75–77]. In this way, the Vanc-Ti coating presented the antibiotic for a long period. It inhibited *S. aureus* colonization up to 11 months in vitro [75]; and even inhibited *S. epidermidis* biofilm formation [76]. Although the immobilization technology achieved long-term drug delivery, the immobilized antibiotics or peptides should remain function in their tethered form, which limits the application of many types agents.

Other Coatings

Other coatings focus on modifying the surface structure of the implant. Porous implant surface coatings have been used clinically on ceramic or metallic implants since 1970 to assist osteoconduction [14, 78]. Various types of porous structures have been developed and investigated, such as open pores and highly interconnected porous structure. The most commonly used porous coating is trabecular metal (Ti) [14]. The trabecular metal with high porosity (80%) allows rapid bone ingrowth and implant stabilization as reported in in vivo studies [79]. However, clinical revision rates of patients using these porous implants are not reduced [80]. Thus, more clinical investigations are needed to evaluate the long-term survival rate of the implants with porous coatings. Currently, several engineered implant surfaces with micro- or nanostructure have been developed [81, 82]. This micro- or nanoscale surface with increased porous structure enhances the cell adhesion and bony ingrowth. At the same time the rough surface increases the friction force and enhances the osseointegration. A cell-favored surface may also attract bacterial attachment. It is a challenge to balance promoting host cells growth and inhibiting bacterial growth.

Future Direction

The implant surface coatings for enhancing osteointegration and inhibiting infection have been closely related and stated as a "race for the surface" by Gristina [83]. The host cells and bacteria will be racing for the implant surface right after implantation. The ideal implant surface should promote strong osseointegration by facilitating host cells attachment to the surface and meanwhile inhibit the bacterial colonization. Thus, the strategies for osteointegration and anti-infection coating could be combined together to reach a multifunctional coating. In addition, the applicable coating should be simpler for preparation and economic for fabrication.

Electrospun Nanofibers (NFs) Coating to Enhance Osseointegration

Characters and Current Researches in Electrospinning

The native ECM of bone tissues is a nanofibrous collagen network. The fundamental unit of the bone is mineralized and highly ordered collagen I fibrils, only a few nanometers thick [84] with collagen. The collagen I fibrils are aligned and arranged to form a higher order structure seen in a mature bone matrix [85–87]. One of the promising technologies that can be used to mimic bone nanoscale ECM structure is electrospinning [88, 89].

Electrospinning, developed in the early 1930s, has been applied in various industrial products, such as highly efficient filters, lightweight and protective cloth, and battery cells, as well as tissue engineering and regenerative medicine [90]. It uses an electrical charge to exceed the surface tension of a charged polymer solution, resulting in the formation of micro- or nanoscale fibers [91]. With the expanding availability of nature, synthetic or combined polymer materials, electrospun NFs have been applied to tissue engineering because of its unique characteristics, such as high surface area and porosity [89]. Recent studies indicated that the attachment, proliferation, and differentiation of bone cells can be enhanced by the physiochemical and microstructural properties of electrospun NFs [92, 93]. The potential application of implants with NF coating for the enhancement of osseointegration is promising but often is overlooked [89]. More efforts are obviously required to better understand the dynamic interplay between the physiochemical and microstructural natures of NFs and the fate of bone cells [89].

Limitations (Dense and Compact Structure)

The porous structure of the NF scaffolds is critical for its application in tissue engineering and regenerative medicine. The nutrition and waste of cells should be transported through the pores [94]. Cells growth and differentiation demand a porous structure of the local environment. It is well known that different cells require different pore sizes [94]. For example, vascularization happens at pore sizes over 300 μm in the bone tissue [95]; while fibroblasts prefer a pore size of 6–20 μm [96]. Thus, ideal electrospun NFs scaffold should have three-dimensional shape and macroscale pores, which provide sufficient space for cell infiltration and differentiation [97]. From this aspect, one main limitation of current electrospinning technology is that the electrospun NFs are firmly packed that only provide a superficial porous structure due to the sheet-like assembly process [98]. This inevitable event impedes cell infiltration and growth throughout the NF mats [98]. There are no satisfactory resolutions of this technical barrier. The fabrication of loose, thick, and bulky scaffolds (3D scaffolds) with controllable microstructures remains a technical challenge [99].

Current 3D NFs Fabrication Techniques

Many efforts have been explored in past decades to fabricate 3D porous and looser NF scaffolds. The first strategy to form the 3D NF scaffold is by simply stacking, folding, or rolling multiple thin NF films [100]. For example, by layer-by-layer interval stacking, micro- and nanofiber membrane were formed into a sandwich-like 3D scaffold. The nanofiber layers assisted cell adhesion and proliferation; while the microfiber layers with larger pores helped cell infiltration [100]. Levorson et al.

found that this scaffold has increased the chondrogenic differentiation of MSCs in vitro [101]. However, the microstructure of the layer-by-layer stacking NFs is still dense and compact.

Adding porogens, including salt particles [98, 102], ice crystal, and washable polymers [103], is an alternative strategy to fabricate 3D porous NFs. The embedded porogens can quickly built-up the NFs volume during the electrospinning. The porogens will be then washed away after electrospinning, leaving numerous larger pores in the formed NFs. Salt leaching uses salt particles as the porogens. Nam et al. introduced NaCl crystal (diameter: 90–106 μm) to the Taylor Cone by a sheath surrounding the spinneret [98]. Formed 3D PCL NFs characterized a uniform porous structure with average pore size of 200 μm. The highly porous structure facilitated CFK2 cell infiltration to the depth of 4 mm [98]. The salt leaching method can be used to control the pore size; while the requirement of multiple steps and the modification of electrospun device together make fabrication far more complicated.

Cryogenic electrospinning, by embedding ice crystal as porogens, was first reported by Simonet et al. in 2007, and later was termed as cryogenic electrospinning in 2009 by Leong et al. [104, 105]. From condensing humidity, the ice crystals are formed simultaneously with NFs deposition by a low-temperature fiber collector device; and the crystals will be then removed by freeze-drying procedure [105]. The porosity of formed 3D NFs was four times higher than the traditional NFs [105]. Correctly balancing between the fiber and ice crystal formation is the key to achieve the 3D NFs by cryogenic electrospinning. Unlike the salt leaching, another limitation of this method is relatively smaller and uncontrollable pore size. Another removable polymer porogen is poly (ethylene oxide) (PEO) [106]. PEO is a water-soluble material electrospun with PCL polymer to achieve a combination of PEO NFs and PCL NFs. Later, the PEO NFs were washed away leaving pure and porous PCL NFs. The porosity could be adjusted by the ratio of PCL and PEO NFs. However, it is difficult to increase the pore size and scale up the formed NF volume through this approach.

It has been demonstrated that the fabrication of NF collector surface design is one of the most effective approaches to create 3D fibers with desired fibrous structure and patterns [102]. Some advanced NF collector modification techniques have been reported recently, such as rolling or stacking collectors [100, 107], liquid bath collector [108, 109], and micro-patterned collector. The liquid bath collector design increased the dispersion effect and decreased the fiber bonding through collecting NFs in various liquid solutions, such as water and organic solvents [103, 110]. Yang et al. collected 3D cotton-like poly(lactic-*co*-glycolic acid) (PLGA)/PCL NFs in ethanol bath for bone regeneration in vivo [109]. The super loose and uncompressed NFs scaffolds were found to enhance the chondrogenic differentiation both in vitro and in vivo. Yarn, a bundle of aligned nanofibers, is formed in a liquid bath as well. Usually, the NFs deposit on the surface of the liquid bath, and through water vortex, the NFs are pulled and twisted into a continuous yarn. The yarns could be further collected by a rotating collector to compose a 3D nanoyarn scaffold [111]. The nanoyarn scaffolds have been studied in tendon tissue regeneration [111] and cartilage tissue regeneration [112] for the improvement of cell penetration and

vascularization. Overall, the NFs formed in the liquid bath collector have homogenous structure, but they are difficult to scale up.

Micro-patterned collector is another collection technique allowing the formation of highly porous NFs. Li and Xia arranged conductive and nonconductive void spaces to make a patterned collector [113]. NFs were aligned across a nonconductive void. These methods make it possible to form 3D NFs scaffolds in certain forms. However, the processing is relatively complex, slow, and difficult to control. In addition, this process cannot be used to create scalable, block scaffolds with an interconnected porous structure. It is obviously that a simple and one-step real-time technology for the preparation of controllable porous NF matrix is urgently needed for the application of tissue engineering of different tissues and/or organs.

The Technique of 3D NFs Collector (Mechanism, Device, Physiochemical Properties of NFs, and Cellular Behavior)

The working mechanism of electrospinning is that driven by high voltage, a charged polymer jet overcomes its surface tension and deposits onto low potential targets in the form of numerous NFs. Commonly, the NFs collected on the flat surface with equipotential density. We designed a NF collector mounted with multiple movable sharp and electric conductive needles. The corona discharge effect leads to continuous deposition of 3D NF matrices on the surface of the NF collector [114]. As a result, the local electric field around the needle tip creates strength much higher than the surrounding conductor, resulting in an acceleration of free electrons to a high velocity, which ionizes neutral air molecules [114]. Thus, the charged polymer jet prefers to deposit onto the sharp tip of the needle during the electrospinning (Fig. 1a) [114]. According to this mechanism, we have designed a 3D NF collector with numerous movable needles where electrospun NFs are gradually deposited to form 3D architectures (Fig. 1) [114]. Unlike conventional electrospinning that lays down a uniform deposition, the electric field vectors in the vicinity of the collector majorly target two fractions—the projecting points of needles (A) and the edging corner of the platform (B/C), which enforces the deposition of spinning nanofibers along the alignment of B-A-C and allows a triangle-shaped fiber sheet formation as shown in Fig. 1b-1 [114]. When two points are more prominent on the surface of collector such as points D and E, the spinning fibers are deposited to these points giving a wave-shaped fiber sheet formation (Fig. 1b) [114]. When the collector was fully covered by a deposited fiber sheet, the needles' positions were re-adjusted by gradually pushing those pierced needles forward. At the same time, a new fiber sheet would start depositing on the tips. After several rounds, 3D NFs architectures were gradually built on the surface of the collector by stacking multilayers of fiber sheet into bulk (Fig. 1). Thus, using the coronal charge effect provides a simple and one-step approach to develop the 3D nanofibers. In comparison to the 2D PCL nanofibers, the 3D PCL nanofibers have a looser microstructure and larger pore sizes via scanning electron microscopy (SEM) [114]. The pore sizes of 2D NFs was in the range of 0–1 μm^2; the

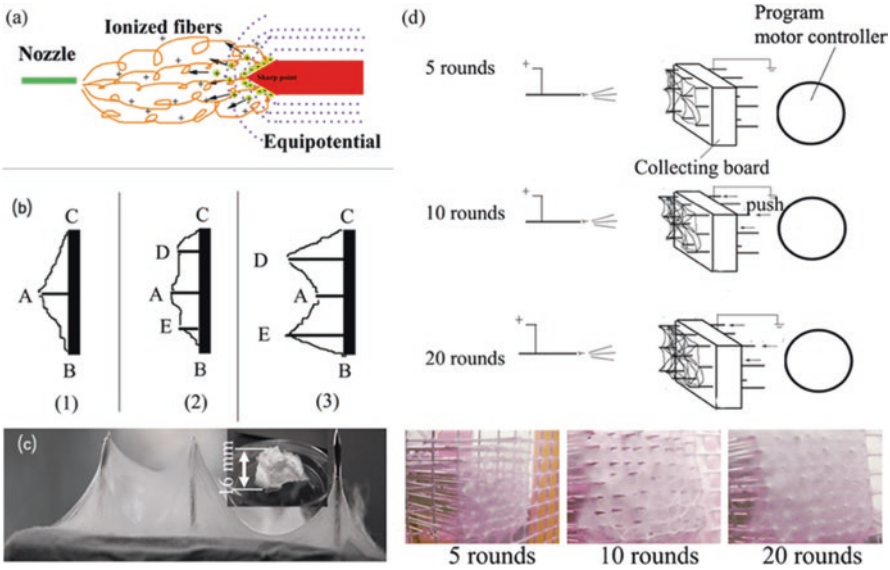


Fig. 1 The fabrication of PCL 3D nanofibers (NFs). (**a**) A diagram of the mechanism of NFs depositing on the needle tip by coronal discharge effect. (**b**) Illustration of a cross-sectional view of electrospun fibers built-up between the spinneret and needle-collector. (**c**) Photograph of collected fibers deposited along needles and platform during electrospinning. (**d**) Multiple rounds (5, 10, and 20) to form 3D nanofibers on needle collectors [114]

3D NFs had larger pores size were mainly in the range of 0.1–10 μm^2. In addition, 3D NFs with looser structure stimulated the infiltration, proliferation, and differentiation of murine pre-osteoblastic MC3T3-E1 cells [114]. The pre-osteoblast cells infiltrated the entire 3D PCL nanofibers, while they only spread on the surface of 2D nanofibers after 7 days culture. A significantly higher cellular proliferation was also discovered on 3D NFs at 7-day culture than that on the 2D NFs ($p < 0.01$). The looser structure further increased the differentiation level of the cells, which had a significantly higher alkaline phosphatase (ALP) concentration on 3D NFs ($p < 0.01$) [114]. However, one of the key limitations is that the microstructure and shape of the formed 3D NFs based on the corona discharge mechanism are neither controllable nor reproducible because of the manual movement of the mounted needles. Since both the macrostructure and microstructure of the NFs affect cell behavior, establishing reference NF scaffolds with well-characterized cell response is critical to advancing their use in the tissue engineering field. Further development of this novel coronal discharge-based porous NF fabrication technique requires standardization of the electrospinning process and characterization methods. Therefore, we developed a programmed electrospun 3D NF collector that can be used to fabricate 3D NFs with desired microstructure, such as pore size and porosity, by precisely controlling the moving speed of NF collector during electrospinning [115]. This device can be used to precisely control the needle collectors constantly moving forward via different

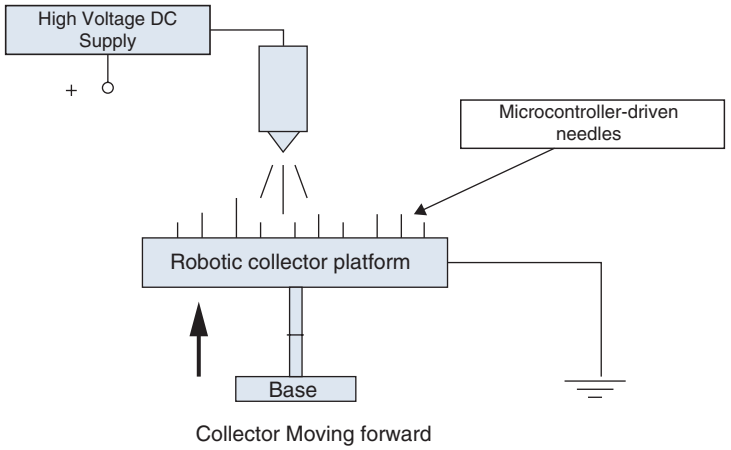


Fig. 2 Illustration of automatic 3D nanofibers collector (unpublished data)

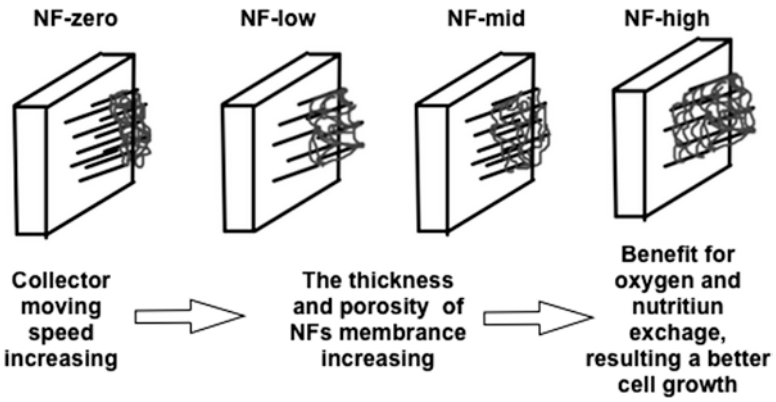
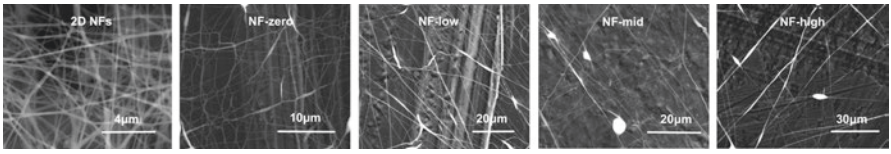


Fig. 3 Illustration of four types of NFs formed on collector with different moving speeds

moving speeds (0–0.232 mm/min) (Fig. 2). Four types of polycaprolactone (PCL) 3D NF matrices with different microstructures can be obtained concurrently on the NF collector surface by setting different forward moving speed of the NF collector device (from low to high) (Fig. 3). A linear increase in the NF sheet thickness was recorded with increasing NF collector moving speed with 1-h electrospinning. Scanning electron microscopy (SEM) measurement showed a looser microstructure and an increase of porosity with the increase of collector moving speed (Fig. 4). NFs prepared at high collector speed showed enhanced cell proliferation and differentiation (ALP expression) of pre-osteoblastic MC3T3 cells compared to NFs collected on a static collector. A programmable NF collector permits the fabrication of reproducible 3D NF scaffolds of variable size and adjustable microstructure. This simple, controllable, one-step 3D electrospun NFs fabrication may help move forwarding the clinical translation of electrospun NFs in regenerative medicine.



	2D-NFs	NF-zero	NF-low	NF-mid	NF-high
Porosity (%)	65.39±1.58	70.14±2.7	76.11±2.02	84.63±3.2	88.88±1.82

Fig. 4 The morphology of five types PCL NFs. Porosity was calculated by SEM images and Image J software. Experiments repeated three times with triplicate. 2D NF: NFs were collected in a flat collector surface

Nanofibers Coating as Drug-Eluting Device to Enhance Osteointegration and Treat Periprosthetic Infection (PJI)

Coaxial Nanofibrous Coating as a Controlled Drug-Eluting Device (Current Technology Development Status)

Electrospun NF can be used as a drug-eluting device by embedding drugs into the polymer solution before electrospinning. The drug release kinetics is determined by NFs structure and the degradation rate of NFs. Polyvinyl alcohol (PVA) has been extensively used in electrospinning because of its excellent biocompatibility and good electrospun NF-forming capability [116]. It has been reported that the morphology and chemical composition of electrospun PVA/HA NFs were similar to the basic architecture of bone [117]. We [116] developed electrospun PVA/Collagen/HA NFs and found that the inclusion of HA and collagen in the PVA NFs significantly increased the fiber stability and the mechanical strength. The encapsulated nano-HA crystals and collagen also enhanced the adhesion and proliferation of osteoblastic MC3T3 cells in vitro. However, these blended PVA/Collagen/HA NFs cannot be used as a desired drug release device because of their fast degradation rate (~10 days) [116].

Coaxial electrospinning has been used to prepare coaxial core-sheath NFs that can be used to control and extend the embedded drug release. We have used this technology to prepare coaxial NFs scaffolds as a local drug-eluting device to enhance osseointegration and prevent infection both in vitro and in vivo [89, 118]. During coaxial electrospinning, a spinneret is employed to trap a secondary fluid layer (containing labile drugs) within the core of the forming NFs [89]. The sheath solution acts as a guide and surrounds the core material. The sheath structure represents a physical barrier to reduce the initial burst release and protects the drugs in the core fiber. The concentration gradient inside the core fiber is the driving force

for diffusion [89]. In a core-sheath system, a drug release rate is affected by both the concentration gradient and the degradation rate of the sheath barrier. Therefore, control of the drug release rate can be achieved by preparation of various formulations and thicknesses of slow/fast degradation sheath fibers and/or modification of physiochemical properties of NFs [89, 119]. We [89] developed coaxial electrospun PCL/PVA core-sheath NFs blended with both HA nanorods and type-I collagen (PCL^{Col}/PVA^{HA}). The incorporation of collagen into the PCL sheath (PCL^{col}) increased its hydrophilicity and provided numerous binding sites for cell adhesion. The incorporation of HA into the PVA core (PVA^{HA}) increased the surface roughness and mechanical strength of NFs. The PVA^{HA} core was used as a drug reservoir. This hybrid core-sheath NF scaffold takes advantage of the slow degradation nature of PCL and the bioactivity of PVA while minimizing the disadvantages of both. Doxycycline (Doxy) embedded in the PCL^{Col}/PVA^{HA} NFs showed more sustained release (~1 month) compared with the blended NFs (completely released within 48 h). Doxy released is stable and bactericidal as evidenced by a modified *S. aureus* growth inhibition assay [120]. We also found that PCL^{col}/PVA^{HA} NF coating enhanced osseointegration in vivo.

In the next section, we would like to introduce our two recent studies using implants with coaxial nanofiber coating from the aspects of osseointegration enhancement and infection inhibition.

Sustained Strontium Release from Coaxial NFs to Enhance Osseointegration

The use of NF coating needs careful understanding and coordination of its rate of degradation with the physiology of osseointegration. An early and sufficient osseointegration resulting in "the formation of a direct interface between an implant and bone without intervening soft tissue" is critical for the early implant stability (~1 month). Obviously PCL is not an appropriate NF material because of its much slower degradation rate both in vivo and in vitro [121] that has been verified in our previous pilot study. Therefore, we added PLGA to PCL as the sheath fiber in this study because of its faster and controllable degradation rate (~1 month) comparable to that of osseointegration physiology [122]. Another benefit of PLGA is its stronger binding to the Ti surface [123, 124] than that of PCL [89]. One reason for this is that PLGA has much higher ratio of oxygen atoms in its molecular structure than that of PCL, thus providing more electrostatic interaction on the Ti surface [125].

Strontium (Sr^{2+}) is a minor element that can be found in our body and daily diet [126]. Nearly 99% of Sr^{2+} ions are deposited in bone [126]. The Sr^{2+} ion has the similar cellular transport pathway as calcium ions, and has strong affinity for the incorporation in the bone matrix during mineralization [126, 127]. Sr^{2+} enhances bone formation and strength through the inhibition of osteoclasts and activation of

osteoblasts [128–131]. There are few studies that investigated the role(s) of Sr^{2+} in the field of implant osseointegration [132, 133]. Park et al. found that Sr^{2+}-embedded Ti implants significantly enhanced implant osseointegration as compared with the control Ti implants in a rabbit tibia implantation model [134].

We [135] have developed a Sr^{2+}-doped coaxial PCL/PLGA-PVA NF coating to enhance the osseointegration. The sheath fiber formula PCL/PLGA (1:1, v/v) was optimized to match the NFs degradation rate to the implant osseointegration physiology, which is about 1 month. Although an initial Sr^{2+} burst release was observed, a sustained release of Sr^{2+} from the PCL/PLGA (1:1, v/v)-PVA coaxial NFs was detected for over 2 months. The Sr^{2+}-doped PCL/PLGA-PVA coaxial NFs were biocompatible and significantly enhanced the differentiation of murine pre-osteoblast MC3T3-E1 cells using both indirect and direct contact approaches in vitro. Taken together, Sr^{2+}-doped PCL/PLGA-PVA coaxial NFs are promising nanofabricated implant coatings to promote earlier and sufficient implant osseointegration.

Sustained Release of Doxycycline from Coaxial NFs to Prevent and Treat PJI (In Vitro and In Vivo Study)

For the prevention and treatment of PJI, we have developed a Doxy-doped coaxial electrospun PCL/PVA NFs as the Ti pin coating [118]. This hybrid core-sheath NF scaffold takes advantage of the slow degradation nature of PCL and the bioactivity of PVA while minimizing the disadvantages of both. The slow degradation of the PCL concomitantly reduced the Doxy diffusion from the PVA (core materials). Doxy embedded in the PCL/PVA NFs showed more sustained release (~1 month) compared with the blended NFs (completely released within 48 h). The Doxy-doped coaxial PCL/PVA NFs were directly deposited on the Ti pin surface during electrospinning with the aim to improve osseointegration and inhibition infection. The bone–implant surface (%) in the NFs-coated Ti pin groups was significantly higher ($p < 0.05$) than the non-coating groups after implantation 2, 4, and 8 weeks in a *S. aureus* infected rat tibia implantation model [118]. In addition, the Doxy-loaded NFs inhibited bacterial growth up to 8 weeks in vivo [118]. However, the bacteria grown back and formed biofilm after 16 weeks implantation. These two studies showed the great potential of NFs coating for the enhancement of implant osseointegration and infection inhibition. The results of the in vivo study revealed the two challenges in the application of nanofibers as implant coating. The first problem is the weak bonding of the nanofibers with the implant surface. The nanofibers may be separated from the Ti pin surface during implantation, which may cause larger gaps between the implant and the surrounding tissue. We have blended PLGA with the NFs to increase the bonding strength to Ti pin surface. More strategies could be developed to strengthen adhesion of nanofibers coatings to different metal implant surfaces. The second problem is the lack of a long-term infection inhibition.

Nanofiber is an ideal matrix for host cells and bacteria adhesion and growth. If any bacterial residues are left in the matrix, they may slowly colonize, and form a biofilm when the antibiotic dose was low or missing. Thus, the antibiotic dose should be high enough to kill the majority of bacteria without toxicity to the host tissue within 48 h after implantation. In addition, a sustained and controllable antibiotic dose at a sufficient level is needed to prevent the recurrence of infection. Multiple antibacterial drugs can be embedded within the same NF coating to increase the antibacterial efficiency, especially those that inhibit later infection or disrupt biofilm formation.

Summary and Conclusions

Orthopedic implants have been widely and successfully applied in TJA worldwide. As for the duration of the implants, although 10- to 15-year survival rate is higher than 90%, the amount of implant failures is a clinical challenge. It lays huge burden on patients, physically and mentally. This chapter generally introduced the two leading complications of TJA, that is, insufficient osseointegration and infection. The implant surface is the "racing arena" for host cells and bacteria after implantation. An ideal implant surface should benefit host cell growth and inhibit bacteria adhesion. For this reason, implant coatings have become a potential solution to promote TJA success. It has drawn much interest to enhance osseointegration and inhibit infection. Various current coating strategies for osseointegration and infection prevention are compared and summarized in the chapter. At the end, we discussed the possibility of using nanofibers as implant coating and briefly introduced our researches about nanofibers Ti implant coating. More efforts are needed to develop advanced implant coating technologies that are more "bone-like" and multifunctional to both enhance osseointegration and prevent infection.

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