Nanofibers in Tissue Engineering & Oral Implantology - A Review

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Abstract
Osseointegration in titanium implants is determined by surface topography. Modification of implant surface could be physicochemical, morphological or biochemical. The artificial biodegradable nanofibers produced by electrospinning process mimics extra cellular matrix. This 3D scaffold enhances neo tissue-genesis due to its high surface area, porosity and biodegradability. They are capable of forming networks of highly porous mesh with remarkable interconnectivity between their pores, making them an attractive choice for a host of advanced applications. In fact, the significant impact of nanofiber technology can be traced from the wide range of fundamental materials that can be used for the synthesis of nanofibers. These include natural polymers, synthetic polymers, carbon-based materials, semiconducting materials, and composite materials. However, to move beyond the current state of nanofibersynthesis and applications towards realization in commercial and industrial settings, several challenges need to be addressed and overcome. This Review explores the applications of electrospun nanofibers in tissue engineering and oral implantology and the future research directions.

Key Words - tissue engineering, nanofibers, implant dentistry, electrospinning; bone regeneration; electrospun nanofibers

I. Introduction
For over four decades, commercially pure titanium (cpTi) and titanium (Ti) alloys have been used for implant therapy due to their remarkable mechanical properties in loadbearing applications, low density, high corrosion resistance, and biocompatibility. Of note, the basis for using Ti in implant dentistry is predicated on its ability to achieve a direct structural and functional interface with living bone (i.e., osseointegration), which has allowed for the successful restoration of masticatory function in partially and completely edentulous patients. Regrettably, despite accumulating evidence regarding the positive role played by implant surface modification both texture/microstructure and chemistry on bone integration, the risk of infection (peri-implantitis), and thus early implant loss, still embodies a major clinical concern.

Significant advances in nanotechnology have helped to pave the way toward the development of antimicrobial coatings that could be used to avoid implant infection. Meanwhile, recent research highlighted the prospective anabolic effects associated with tetracycline-derivatives (e.g., doxycycline and minocycline) as their use seemed to enhance cell proliferation.

While tissue-engineered bone grafts have been investigated for years, challenges still lie in achieving in vivo mechanical/biological properties and vascularization for the treatment of patients who suffer from degeneration or diseases such as periodontitis, trauma, oral cancer, and anatomical abnormality in nature. Electrospun nanofibers may be one of the ideal solutions due to their ECM similarity, since they provide control over nanoporosities similar to the small blood vessel for the cell survival. Electrospun nanofibers have been studied in a variety of the in vitro and in vivo tests, such as mesenchymal stem cell- (MSC-) seeded implantation into a rat calvarial defect model.

NANOFIBER IN TISSUE ENGINEERING
As an interdisciplinary field combining various Biological and engineering expertise, tissue engineering and regenerative medicine seek to restore or regenerate the normal tissue and organ functions using the three fundamental entities of cells, biomolecules, and biomaterials.[1] As one of the most actively researched biomaterials, nanofiber-based scaffold emerges as a versatile alternative for tissue engineering and regenerative medicine applications.[2,3] With their extremely high surface-to-volume ratio and porosity, nanofibers offer a high loading capacity for biological substances and active species. Furthermore, with their interconnected network of micropores mimicking the native in vivo topographic features of extracellular matrix (ECM), nanofi-brous scaffolds present a favorable avenue for cellular growth, proliferation, and differentiation.

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For the particular application of tissue engineering, biodegradable and biocompatible natural or synthetic polymers are typically used as the nanofiber materials. The specific selection of materials depends very much on the types and properties of the tissues to be regenerated as well as the duration of regeneration. An increasing number of studies on the applications of nanofibrous scaffolds for tissue engineering have been reported lately. Some examples are highlighted here.

First, self-assembled chitin nanofibers were synthesized for the fabrication of biodegradable and flexible substrates micropatterned through replica molding for engineering cell sheets [4]. On the substrates, the seeded fibroblast cells attached and aligned along the primary axis of the micropatterned features, leading to the formation of ultrathin and free-standing ordered cell sheets which were flexible and could be easily controlled for the construction of complex tissue structures. Second, aligned gelatin nanofibers-multiwalled CNT composites were synthesized via electrospinning as the scaffolds for the growth of myoblast, specifically, for an improvement in the formation of aligned myotubes with enhanced contractility. The activation of mechanotransduction-related genes was upregulated and myotube maturation and contractions were improved through the presence of the hybrid scaffolds. Third, electrospun PCL nanofibers were functionalized with adhesive peptides for cardiac tissue engineering application, specifically for improving the adhesion and contraction of cardiomyocytes [4]. Fourth, biodegradable electrospun PCL nanofiber-based scaffolds were coated with platelet-rich plasma (PRP-PCL nanofibers) to enhance the adhesion and proliferation of mesenchymal stem cells (MSCs). Fifth, multifunctional osteoinductive hybrid peptide nanofibers were synthesized based on the self-assembly of three bioactive peptide molecules and then utilized as an implant coating to promote bone-like mineralization on a medical grade titanium substrate surface. The nanofibers were functionalized with osteoinductive collagen I-Asp-Gly-Glu-Ala-pEptide sequence to increase the adhesion, proliferation, and osteogenic differentiation of MSCs into mature osteoblast. Sixth, composite chitosan/silk fibroin nanofibrous membrane scaffolds were synthesized based on electrospinning for bone tissue engineering, in particular, for enhancing the proliferation and osteogenic differentiation of human MSCs. Apart from that of MSCs, nanofibrous scaffolds are also used for supporting the differentiation of neural stem cells (NSCs). For example, collagen nanofibrous scaffolds were prepared for facilitating the presynaptic maturation of NSC-derived neurons towards the formation of neural networks. More recently, a unique hybrid polycaprolactone-graphene oxide (PCL-GO) nanofibrous scaffold has been demonstrated to provide instructive physical cues in guiding the specific differentiation of NSCs into mature oligodendrocytes in the absence of chemical inducers (Fig. 1a). In the study, biocompatible and biodegradable polymeric PCL nanofibers were synthesized through electrospinning, followed by oxygen plasma treatment. GO, the oxygenated derivative of graphene, was then uniformly coated on the hydrophilic surface of PCL nanofibers (Fig. 1b). NSCs cultured on the GO-coated PCL nanofibers exhibited extensive branching characteristic of oligodendrocytes (Fig. 1c). Further gene expression investigations revealed that cells grown on PCL-GO scaffolds exhibited significant increase in their MBP expression (i.e., mature oligodendrocyte marker) and slight increase in the TuJ1 expression (i.e., neuron marker) while their GFAP expression (i.e., astrocyte marker) decreased simultaneously (Fig. 1d). By increasing the concentration of GO, the MBP expression increased proportionately. This highlights the crucial role of GO in modulating oligodendrogenesis as well as the synergistic effect brought about by the hybrid PCL-GO nanofibrous composites in promoting the preferential NSC differentiation towards the oligodendrocyte lineage.

For bone regeneration, Kim’s group has shown several electrospun nanofibrous scaffolds made of synthetic and natural polymers with or without mineral deposition such as gelatin-PCL, silk-fibroin-PCL, PLA, gelatin-apatite-poly(lactide-co-caprolactone), mesoporous bioactive glass-incorporated PCL-gelatin, mesoporous silica-shelled PCL, and magnetic nanoparticle-incorporated PCL nanofibrous scaffolds. In addition, a number of polymeric nanofibers have been revealed and used for a cellular platform for bone, but they lack bioactivity and other biofunctionalities to accelerate bone tissue regeneration. For this, artificial mineralization after fabrication or loading additives (i.e., bioactive nanoparticles and growth factors) to scaffolds during electrospinning process was introduced and resulted in the induction of osteogenesis by accelerating natural mineralization or vascularization [6,7].

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FIG 1 for tissue engineering and regenerative medicine applications. (a) Schematic illustration demonstrating the synthesis and application of the hybrid PCL-GO nanofibrous scaffold in guiding and enhancing the specific differentiation of NSCs into mature oligodendrocyte lineage. (b) FESEM image showing the GO-coated PCL nanofibers using GO solution with a concentration of 1 mg/mL. Scale bar represents 2 _m. (c) FESEM image showing the differentiated NSCs seeded on the hybrid PCL-GO nanofibrous scaffold. Scale bar represents 10 _m. (d) The fold change in the gene expression of the different biomarkers indicative of astrocytes (GFAP), neurons (TuJ1), and oligodendrocytes (MBP), derived from the NSCs cultured on various substrates. The hybrid PCL-GO nanofibrous scaffold demonstrated the highest MBP expression. The *, **, and n.s represent p < 0.05, p < 0.01, and no significance, respectively, evaluated based on the Student’s unpaired t-test, as compared to the PLL-coated glass control or between various substrates. [6]

These nanofibrous scaffolds would be employed as a carrier for bone-associated growth factors due to their 3D networked pores to facilitate control over drug release. Recently, electrospun nanofibrous scaffolds were designed to hold a capacity by loading and releasing dual growth factors for the target of bone regeneration. For example, a core-shell structure of a biopolymer fiber made of polyethylene oxide/PCL was shown to facilitate loading and control releasing properties of these growth factors. To increase cell attachment, biofunctional materials have been used for electrospinning. Silk nanofibers having the Arg-Gly-Asp (RGD) sequence which act as receptors for cell adhesion were shown to accelerate MSC attachment, proliferation, and differentiation into osteoblastic lineage.

Guided tissue Regeneration for Periodontium

One of main advantages of electrospinning is its ability to produce fibers of different orientations and size for fibrous scaffolds for tissue regeneration. Research indicates that these fibers are effective as tissue regenerative scaffolds because of their ability to mimic the fibrous extra-cellular matrix (ECM) of the human tissues such as bone and cartilage. Indeed, it has been observed that a higher degree of fiber-orientation makes it possible to accelerate proliferation of fibroblasts[4,8]. This has been attributed to an increased surface area and porosity of electrospun scaffolds. Furthermore, changing the fiber orientation also makes it possible to “control” the direction of cellular proliferation as it has been that cells tend to proliferate in the direction of the fiber orientation. Many biodegradable materials have been electrospun and revealed the potential to function as GTR scaffolds. Electrospun collagen nanofibers have the potential for GTR scaffolds applications. Additionally, collagen fibers have the potential to allow differentiation of human bone marrow-derived mesenchymal stem cells (MSCs). However, to date, no studies have attempted to ascertain the mechanical properties of electrospun
collagen fibers. Research has also been conducted to produce scaffolds composed of collagen blended with PCL, PEO, PLGA and PLLA. One of the major disadvantages of collagen is that, due to its animal origins, there are ethical issues and concerns of cross-infection. Hence, the use of collagen scaffolds could be limited in quite a few demographics.

During the last few years, the idea of functionally graded membrane (FGM) has emerged. This principle aims to produce a multilayered guided tissue regenerative membrane in which each layer has a specific function and physical properties, very much akin to the natural human tissues.

These layers can contain drugs and various growth factors which are released into the surrounding environment to enhance the regeneration of multiple tissues at the same time. It has been speculated that electrospun fibers can form part of these FGMs. Although electrospinning has added exciting new prospects to the field of guided tissue and bone regeneration, much more needed to be explored to validate the use of electrospun scaffolds in the clinical settings. For instance, more research is required to explore the mechanical properties of these scaffolds. More importantly, an adequate number of randomized clinical trials are required to prove their clinical efficacy.

**NANOFIBER IN ORAL IMPLANTOLGY**

Dental implants have emerged as options for dental prostheses; however, the presence of biofilm can cause periimplantitis and lead to dental implant loss. Scientists have been studying some strategies for creating implants that have an osteointegrative surface while reducing biofilm formation and establishment. PCL/tetracycline nanofibers (5, 10 and 25% wt) were evaluated for their antimicrobial activity against periimplantitis-related microorganisms such as P. gingivalis, F. nucleatum, P. intermedia, and A. actinomycetemcomitans. Nanofibers incorporated with 25% wt tetracycline were responsible for inhibiting 100% of the biofilm of these bacteria. These nanomaterials may emerge as new implant surface treatments in the future. In recent years, electrospinning has been deemed a facile approach to synthesize antibiotic-containing polymer nanofibers with significant antimicrobial properties and ability to prevent bacterial infection. Worth mentioning, electrospinning has demonstrated to be a potential method to modify the surface of titanium implants with nanofibers as a coating material, contributing to potentially minimize early implant loss, especially in those patients who are at high risk of periodontal disease.

It has been known that upon implantation, a competition exists between implant integration and bacterial adhesion to the biomaterial surface. Notably, a6 h postimplantation period has been deemed crucial to the long-term success of an implantable device. Collectively, the microbiological data (i.e., TCH-incorporated mats and TCH-incorporated fibers-modified Ti disks) demonstrated significant antimicrobial properties against periimplantitis-related pathogens.

Marco C. Bottino, et al. investigated the antimicrobial and osteogenic properties of titanium (Ti) disks superficially modified with tetracycline (TCH)-incorporated polymer nanofibers cell viability data revealed that the TCH amounts released by the electrospun mats were not cytotoxic.

The experiments were carried out in two phases. The first phase dealt with the synthesis and characterization (i.e., morphology, mechanical strength, drug release, antimicrobial activity, and cytocompatibility) of TCH-incorporated fibers. The second phase was dedicated to evaluating both the antimicrobial and murine-derived osteoprogenitor cell (MC3T3-E1) response of Ti-modified with TCH-incorporated fibers. TCH was successfully incorporated into the submicron-sized and cytocompatible fibers. All TCH-incorporated mats presented significant antimicrobial activity against periodontal pathogens.

The antimicrobial potential of the TCH-incorporated fibers-modified Ti was influenced by both the TCH concentration and bacteria tested. At days 5 and 7, a significant increase in MC3T3-E1 cell number was observed for TCH-incorporated fibers-modified Ti disks when compared to that of TCH-free nanofibers-modified Ti disks and bare Ti. A significant increase in alkaline phosphatase (ALP) levels on the Ti disks modified with TCH-incorporated nanofiber on days 7 and 14 was seen, suggesting that the proposed surface promotes earl osteogenic differentiation. Collectively, the data suggest that TCH-incorporated nanofibers could function as an antimicrobial surface modifier and osteogenic inducer for Ti dental implants.

Adaptation migration and differentiation of mesenchymal stem cells is essential for regeneration of large bone defects. To achieve this, modern graft materials are becoming increasingly important. Among them, electrospun nanofiber scaffolds are promising due to their high physical porosity and potential to mimic the extracellular matrix (ECM). Markus D. Schofer et al. investigated the impact of electrospun PLLA nanofiber scaffolds on bone formation in vivo, using a critical size rat calvarial defect model. In addition, the data analyzed whether direct incorporation of bone morphogenetic protein 2 (BMP-2) into nanofibers could enhance the osteoinductivity of the scaffolds.
Two critical size calvarial defects (5 mm) were created in the parietal bones of adult male Sprague-Dawley rats. Defects were either (1) left unfilled, or treated with (2) bovine spongiosa, (3) PLLA scaffolds alone or (4) PLLA/BMP-2 scaffolds. Cranial CT scans were taken at fixed intervals in vivo. Specimens obtained after euthanasia were processed for histology, histomorphometry and immunostaining (Osteocalcin, BMP-2 and Smad5).[4,5,14]

PLLA scaffolds were well colonized with cells after implantation, but only showed marginal ossification. PLLA/BMP-2 scaffolds showed much better bone regeneration and several ossification foci were observed throughout the defect. PLLA/BMP-2 scaffolds also stimulated significantly faster bone regeneration during the first eight weeks compared to bovine spongiosa. However, no significant differences between these two scaffolds could be observed after twelve weeks.

Expression of osteogenic marker proteins in PLLA/BMP-2 scaffolds continuously increased throughout the observation period. After twelve weeks osteocalcin, BMP-2 and Smad5 were all significantly higher in the PLLA-BMP-2 group than in all other groups.[15,16,17]

They concluded that PLLA nanofiber scaffolds were shown to facilitate cell immigration and thus to achieve high cell densities. However theylacked adequate osteogenic stimuli to allow further differentiation of those cells. The incorporation of rhBMP-2 into PLLA nanofibers could overcome this problem. Hence PLLA/BMP-2 implants were able to close critical size calvarial defects within 8 weeks. Increased expression of osteocalcin, BMP-2 and Smad5 suggests a subsequent activation of the osteoblast lineage. Therefore PLLA/BMP-2 nanofiber scaffolds combine a suitable matrix for cell migration with an osteoinductive stimulus.[18,19,20]

**Electrospun fibers as implant interface layer**

Electrospun nanofibers are known to facilitate cell adhesion and proliferation in numerous in vitro tests. In vivo studies has also shown low inflammatory reactions. Due to the varied demands of implantables, it is not possible to use electrospun nanofibers in all situations. However, the advantages of electrospun fibers may be employed to facilitate integration between implants and surrounding host tissues. They may also be used to reduce infection through incorporation of drugs and to reduce immune response on the implant.

**II. Conclusion & Future research directions**

Academic studies and research on one-dimensional nanofibers are moving ahead at an incredibly fast pace. Novel synthesis tech-niques and applications of nanofibers are being reported in the literature at an ever increasing rate and there is no sign of slow-ing down. However, to move beyond the current state of nanofibersynthesis and applications towards realization in commercial and industrial settings, several challenges need to be addressed and overcome. None of these challenges are trivial, but they are not insurmountable.

Nanofibrous scaffolds serve as one of the mostexciting alternatives for facilitating the regeneration of many types of cells and tissues. Although the relationships between a widerange of nanofibrous scaffolds and cells have been investigated, themajority of these studies are still limited to qualitative proof-of-concept investigations of the cyto compatibility of the nanofibrous scaffolds in terms of cellular adhesion, proliferation, and differ-entiation. Consequently, more attentions should be focused on the quantitative analyses of the changes in cellular functions as influenced by the topographical cues provided by the nanofibrous scaffolds. At the same time, we note that most of the preliminary studies were still conducted in vitro and nanofiber technologies yet to make a real impact in in vivo applications. Encouraging results have been demonstrated in numerous in vitro assays and a small number of in vivo studies, particularly on animal mod-els, with different categories and degrees of tissue injuries. Clearly, more extensive in vivo studies, possibly on human, and clinical trials are needed to evaluate the real impacts and significance of nanofiber technology in healthcare and biomedical engineering.

For the reason, the clinical practice of nanofibrous scaffolds is still scarce. In addition, since dental tissue degeneration may come from biological disorders, further studies of biological interplay between electrospun nanofibers and compromised dental tissue derived cells are essential. These studies will be expected to help understand the biological effect of nanofibers. Conclusively, further elaborated techniques to customize nanofiber scaffolds are imperative, and clinical defects must be categorized into several groups for their customization.

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