Review Article

Electrospinning approaches for periodontal regeneration: A review

Ghsaq M. Alhamdani*, Bassam Ali Al-Turaihi, Azal Hadi Al-Masoody

ABSTRACT

Periodontal disorders are globally prevalent, destructive inflammatory conditions that affect the tooth-supporting tissues, and they are a precursor of premature tooth loss. Tissue engineering (TE) using scaffolds is a promising approach to repair alveolar bone defects resulting from aggressive periodontal lesions. In this field, among numerous methods to produce nanofibers, electrospinning has gained an emergent popularity in the research community due to its remarkable features such as cost-effectiveness, versatility, and amendable operation parameters. Periodontal regeneration was one of the most extensively studied applications for this impressive technique. Major progresses have been achieved in terms of adopting new materials or technique modifications to expand its capabilities. However, there are still some limitations that are hindering the progress of this technique for biomedical applications. Hence, this article will discuss the general concept of electrospinning and surveys the current state of the art in the field of electrospinning to regenerate the periodontal system. In the field of periodontal regeneration and TE, composite, functionalized scaffolds, cell sheets technique, and incorporating additive manufacturing techniques were suggested to overcome these limitations. The review concluded that there still a noticeable paucity in the clinically proven studies that can amplify the hopeful results reported by contemporary literature.

KEY WORDS: Electrospinning, Periodontal tissue regeneration, Tissue engineering

INTRODUCTION

Periodontitis is a multifactorial, highly prevalent chronic inflammatory condition that affects the periodontium causing an irreversible loss of tooth attachment. If not treated, the disease can lead to severe destruction of periodontal ligaments and supporting alveolar bone. This can engender esthetic and functional deteriorations, with premature tooth loss as a final outcome. The ramifications of untreated periodontal disease have a wide range of implications for the individual's quality of life, health providing professionals, and economic system. Adding to this, some life-changing systemic disorders such as diabetes and cardiovascular diseases were recently connected with untreated periodontal infections.[1,2] Thus, the final goal of periodontal rehabilitation is to maintain and/or regenerate the architecture of the tooth-supporting system to revive its function. A wide variety of treatment modalities has been suggested to treat different stages of the disease starting with a simple gum recession and ending with an aggressive periodontal infection associated with alveolar bone degradation. Depending on the severity of the disease, treatment starts with oral hygiene instructions, modifying the risk factors, topical interventions including scaling and root planning with or without adjunctive therapeutics. However, in some clinical scenarios, surgical intervention with adjunctive probiotics proved superior efficiency.[3,4]

All these approaches have been, to some extent, verified to help regaining the lost attachment without reconstructing the structure of the periodontal components and commonly associated with long junctional epithelium formation. In this context, guided tissue/bone regeneration (GTR) was introduced as a new strategy based on using a physical barrier to prevent the invasion of gingival cells.[5] GTR implies complete reconstitution of the injured tissue using synthetic barrier to prevent epithelial overgrowth, allowing proper healing space for the bone and periodontal ligaments.[6] Bioresorbable membranes are preferred candidates considering lack of need for secondary surgery and lower chances of infections. However, to the date, only the fraction of the original periodontal attachment can be restored by the currently provided periodontal therapies.[7] Nevertheless, some persistent

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College of Dentistry, University of Alkafeel, Kufa, Iraq

***Corresponding author:** Ghsaq M. Alhamdani, College of Dentistry, University of Alkafeel, Kufa, Iraq. E-mail: Ghasaq.mhmood@alkafeel.edu.iq

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infections might impair the clinical outcomes of GTR treatment.[8] Lately, better understanding of the natural periodontal apparatus alongside with the recent advances in technology and material sciences has open the scope for more creative mainframes. In the early 2000s, tissue engineering (TE) was introduced as a revolutionary therapeutic approach for periodontal diseases. In this context, electrospinning was introduced as a controlled and reliable method to design and fabricate TE scaffolds. Since then, plentiful advancements had been achieved including the process of electrospinning setup, adopting high performing materials, and the ever-growing applications. This paper will present a briefed review of the current status of electrospinning aided regenerative therapies for periodontal defects.

TE

Since it was first presented by Langer and Vacanti in the 1990s, TE has opened a new scope for thorough, well-designed, and functional regeneration.[9] Bartold describes the principle of TE by incorporating a scaffold with living cells or biologically active particles to design a TE device [Figure 1] that encourages tissue renovation.[10,11] Thus, progenitor mesenchymal cells from periodontal and non-periodontal resources have been employed with encouraging outcomes.[12] Complete reconstruction of dental cementum, alveolar bone, and healthy periodontal ligaments connecting them is the main goal of TE paradigm.[13,14]

This approach aims to initiate new tissue formation on the scaffold as it degrades overtime. To minimize the need for surgical procedures and patient hospitalization, alternatively, *in vitro* implanted scaffolds have been delivered into the defect site to stimulate and guide the new tissue formation *in situ*.[15] Considering the extremely sensitive and complicated environment of the human body, determining the requirements of materials and the scaffold design to be used in TE is highly challenging. It is well recognized in literature that the natural extracellular matrix is nanofibrous proteins that enclose tissue cells and support their functions.[16] These features can be imitated with nanoscaled polymeric fibers to support specific cell line's adhesion, proliferation, and differentiation.[17] Hence, selecting the most appropriate scaffold material, method of fabrication, and the geographical design plays a primary role in the TE outcomes.

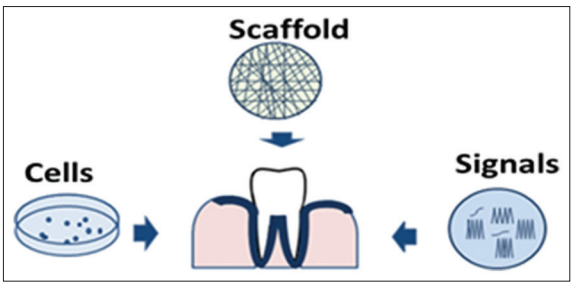

Figure 1: The concept of electrospinning adapted from Bottino *et al*.[6]

MANUFACTURING TECHNIQUES OF MICRO-/NANO-FIBERS

In scientific literature, fibers of diameter range (1–100 nm) are referred as nanofibers. Variable manufacturing techniques have been applied in the fabrication of nanostructured membranes for the management of bone defects triggered by periodontal infections [Table 1].

All the previously stated methods (except electrospinning) were diagnosed with some shortcomings such as follows: Only single polymer can be applied, lack of accuracy, and control of pore dimensions. Meanwhile, electrospinning proposes an enhanced control of the scaffold features by producing a non-woven mesh of randomly oriented fibers with high surface-to-volume ratios providing the required support for cells. Moreover, it maintains a supportive level of interconnectivity for medium access, cell proliferation, and angiogenesis that are critical for the newly formed tissue survival. Electrospinning is favored over solvent casting technique and phase separation considering the high surface area to volume and the high porosity of the produced fibers.[24] Various natural or synthetic-based materials have been employed to manufacture monolayered or three-dimensional scaffolds to grow different types of cells, for example, polysaccharides, polyesters, hydrogels, and thermoplastic elastomers.[15] Bioactive ceramics such as bioactive glass, calcium phosphate, and glass ceramics have also been proven to be promising in this field.[25] As mentioned above, the human body is a very harsh and sensitive environment for any material. Therefore, combining different materials with variable properties are likely to be a sensible method to fulfill most of the scaffold material requirement.

THE CONCEPT OF ELECTROSPINNING

Historically, electrospinning was derived from the process of electrostatic spinning and first identified by Rayleigh, in 1897.[26] Since then, this technology was described in several patents for different applications mainly textile industry. However, the significance of electrospinning in producing nanofibers for wide variety of applications was not recognized until the early nineties.[27] Ultrafine polymeric fibers are generated by applying high voltage to a syringe loaded with polymeric solution or melt. As the repulsive forces resulted from the DC electric source exceed the effect of surface tension, an elongated jet stream of polymer will

Table 1: Methods used to fabricate micro-/nano-fibers

Fabrication technique	Features
Drawing technique	Micro- and nano-scaled fibers can be produced by drawing and solidification of a viscous polymeric solution Disadvantages: Discontinuity and lack of control over fibers diameter[18]
Template synthesis	Advantages: Simplicity and cost-effectiveness Produce nanofibers at low temperature Enable superior control of fiber measurements[19] Disadvantage: Not scalable[20]
Temperature-induced phase separation	A homogenous polymeric solution is being separated (due to thermal changes) into polymer-rich and polymer poor solutions Utilize non-mixable solvent or cooling the solution below the bimodal solubility curve[21] Advantages: Improved control of pore size and mechanical properties Disadvantages: Only applicable with specific polymers[20]
Molecular self-assembly	Application: Materials based on peptides Advantages: Delivers a very distinct nanostructure using weak bonds such as hydrogen bonds, Van der Waals and ionic and bonds[22] Drawbacks: Complexity and lack of control of nanofiber diameter[20]
Melt-blown technique	The polymer melt is extruded through an orifice die The extrudate is drawing down with a jet of hot air (typically at the same temperature as the molten polymer)[23] Advantages: Environmentally benign, no hazardous vapors Drawback: Hard to obtain submicron fibers
Electrospinning	Advantages: Multiscale fibers are producible Natural and synthetic polymers can be utilized Simple and cost-effectiveness The structure of electrospun nanofibers resembles the natural nanoscaled environment of the extracellular matrix[20] Generate interrelated porosity, which is a critical requirement for tissue integration

be created. The organic solvent will vaporize, leaving charged polymeric ultrafine fibers to be suspended on an earthed collector, creating a non-woven mat.[26]

Many types of electrospinning for different applications were thoroughly discussed in literature.[29] The interest with electrospinning for periodontal regeneration was derived from two facts; first, this technology can produce membranes with high porosity and the ability to combine different materials. Flexibility of the technic is another attraction, by manipulating the parameters might produce different properties.

PARAMETERS AFFECTING THE ELECTROSPINNING PROCESS

To achieve the preferred structures for particular applications, the electrospun fiber characteristics such as morphology, dimensions, and organization can be modified by manipulating several elements. The operating parameters are generally classified either as electrospinning, environmental, or solution parameters. Table 2 summarizes these parameters and their effect on the quality of the electrospun fibers.

ELECTROSPUN SCAFFOLDS WITH INTERNAL COMPLEXITY

Various studies have revealed that the type of collecting surface employed to collect the jet stream determines fiber orientation. Allocating a static collector as shown in Figure 2 will produce randomly dispensed fibers. In contrast, a rolling collector (mandrel) as shown in Figure 3 will produce well-aligned fibers. The direction of fiber alignment was proved to affect the cellular behavior; therefore, it became a critical variable in the electrospinning process. Jahani *et al*. pointed that the mesenchymal cells proliferation was remarkably improved when they were attached to randomly deposited polycaprolactone (PCL) nanofibers.[42] In addition, cells were oriented randomly around the fibers compared to the longitudinal pattern when attached to aligned fibers. This behavior was attributed to the surface roughness and the high intensity of interconnected pores.

So as to recreate the original structure and functionality of the damaged periodontal ligaments, recent studies have emphasized mimicking their natural alignment. An experiment conducted by Vaquette and Cooper. (2011)[43] tested the properties of a multilayered scaffold of polyethylene glycol and PCL. The results stated improved quantity and quality of highly aligned collagen fibers against the root surface. In addition, increased periodontal ligament gene expression and enhanced organization for the renewed fibers resulted in promoting perpendicular insertion on tooth surface. The depth of cellular infiltration within the electrospun meshes is a major concern in biomedical applications. The ultrafine fiber's diameter is usually

Table 2: Summary of electrospinning parameters and their effect on electrospun material

Parameter	Effect	Reference
Electrospinning parameters		
↑Applied voltage (beyond critical limit)	Beaded nanofibers	[30]
	Increased fiber diameter	[31]
↑Flow rate	Beaded nanofibers	
	Unspun droplets (ribbon deformities)	[32]
↑Needle to collector distance	Decrease the fiber diameter	[33]
	Increase solvent evaporation	[34]
Solution parameters		
↑Polymer concentration	Prevent polymeric chain entanglement	[35]
↓Polymer concentration	Blocking the needle tip	
	Polymer chain breakage (beading)	[36]
Solution conductivity	High solution conductivity (to the critical limits) will encourage Taylor cone formation	[37]
	Thinner electrospun fibers will be formed	
Type of solvent	The solvent should provide complete polymer dissolution	[38]
	Reasonable boiling point to evaporate as the polymeric jet flies from the needle tip toward the collector	[39]
	Using two different solvents to dissolve the polymer can also increase the degree of the electrospun mat porosity by initiating a phase of separation	
Environmental parameters		
↑Humidity	Thinner filaments	[40]
↑Temperature	Low diameter fibers	[41]
	Higher evaporation rate of the solvent	
	Lower solution viscosity	

Table 3: Examples of polymers and biocomposites used for periodontal regeneration

Scaffold	Type of seeded cells	Reference
Chitosan/collagen	Human PDL cells	[39]
Chitosan	MG63, hES-MP cells	[65]
Gelatin	PDL cells	[66]
Chitosan/coral	Human PDL cells	[67]
PCL/gelatin	-	[68]
Chitosan/freeze-dried sponge	Fatal rat calvarial osteoblastic cells	[69]
Collagen/polycaprolactone/nHA	Human PDL cells	[70]
Collagen/poly d, l–lactide coglycolide/nHA	hMSCs	[71]
PCL/Caco3	Human osteoblasts (hFOB)	[58]
PCL	MSCs	[72]
PVA/type I collagen/nHA	-	[73]
Polyurethane	Human ligament fibroblast	[74]
Polylactic coglycolic acid	PDL cells	[75]
PCL/polyglycolic acid PGE/chitosan	Rat BMCs	[76]
PLGA/calcium phosphate	MC3T3-E1 cells	[57]
Poly (L-lactic acid PLLA/collage/HA	Human osteoblasts (hFOB)	[60]
PLGA/HA	-	[77]
PLLA	*In vivo*	[78]
PLLA/HA	Human cord blood-derived somatic stem cells (unrestricted somatic stem cell)	[79]
PCL/β-TCP	Pre-osteoblast cells MC3T3-E1	[80]
PLCL: PLA: GEL	-	[81]
PLGA/HA	hMScs	[56]
PLA/demineralized bone powder	hMSCs	[59]
PLA-grafted HA	-	[82]
PCL/cap coating	-	[83]
PCL/chitosan	Human osteosarcoma cells (MG63)	[84]
Chitosan/alginate/nHA	MC3T3-E1 cells	[85]
Alginate/HA	-	[86]
PLLA/HA	hMSCs	[87]
PCL/chitosan/n HA	Human periodontal ligament fibroblast (hPLFs)	[88]
PCL/chitosan/n bioactive glass	Osteoblast-like cells (MG-63 cell lines)	
PCL/F18 bioactive glass	MG-63 osteoblast-like cell	[89]
PCL/strontium substituted bioactive glass	MSCs	[90]
PCL/ibuprofen	Epithelial cells, fibroblasts	[63]
PCL/metronidazole	Fibroblast cells L929, human periodontal ligament fibroblasts (hPDLFs)	[62]
PCL/gelatin/n zinc oxide	-	[64]

PCL: Polycaprolactone, HA: Hydroxyapatite, PDLs: Periodontal ligament cells, PLCL: Poly (DL-lactide-co-ε- caprolactone, PLGA: Poly (lactic-co-glycolic acid), PLLA: Poly-L-lactic acid, PLA: Poly (lactide) acid

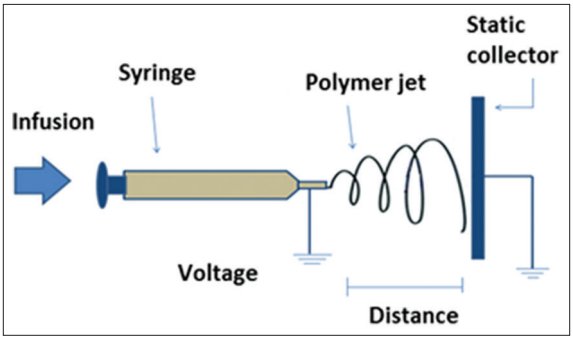

Figure 2: Typical electrospinning set up. Adapted and reconstructed from Pham *et al*.[28]

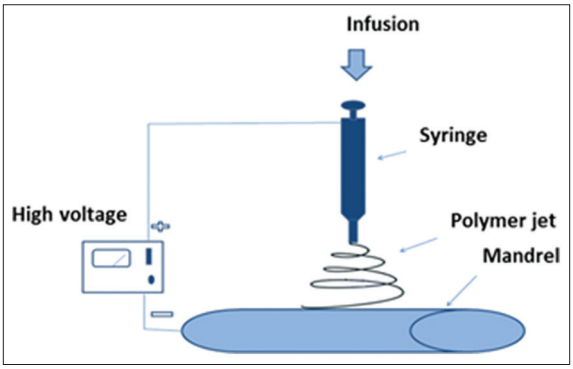

Figure 3: Schematic figure of electrospinning set up with rolling collector (mandrel)

associated with ultrasmall pore size, which will inhibit cellular migration through the depth of the scaffold. Hence, using structured collectors are a simple way to increase the pores size and distribution and, eventually, encourages cellular infiltration and three-dimensional (3D) migrations up to 6-fold.[43] Patterned templates with variable designs and dimensions were used to collect electrospun fibers with specific topographies.[43-45]

Lately, additive manufacturing (AM) techniques were employed to introduce internal complexity to the electrospun membranes by altering the design of the collector. This will grant deposition of fibers with variable diameters, alignment, and porosity, opening the door to manufacture a new generation of scaffolds with different levels of complexity. In this context, Paterson *et al*. described microfabricated electrospun membranes using collectors with synthetic microenvironments called niches. These microscaled structures were introduced by selective laser melting, an AM technique. As the electrospun fibers deposited on the collector, they will reproduce the morphology, distribution, and depth of these niches. The microfabricated membranes demonstrated improved penetration of the MSCs at the niche-like structures.[46] Microfabricated electrospun scaffolds offer larger surface area per unit volume granted by the three-dimensional organized fibers to reinforce cell adhesion and support.[47] Micropockets (niches) are small-sized cavities consisting of areas with low fibers density and a degree of alignment. Stem cells seeded on a ring-shaped microfabricated scaffold were noticed to follow the fibers alignment to guide their migration.[48]

ELECTROSPUN MATERIALS FOR PERIODONTAL REGENERATION

In general, the choice of material used for TE is highly depending on the application and the viability of electrospinning.[49] Table 3 examples of polymers and biocomposites used for periodontal regeneration. Regarding periodontal apparatus, it is necessary to point the anatomical complexity of having both hard (periodontal bone and dental cementum) and soft (connective tissues of periodontal ligaments). Therefore, when designing a scaffold for this purpose, material selection is of high priority. Polymers were very alluring candidates as they have high processing capabilities, biocompatibility, and biodegradation.[17,50] Both naturally derived and synthetic polymers were heavily studied in this area. Natural polymers such as gelatin, collagen, chitin, and chitosan have attracted special attention as permanent bone substitutes. Their biocompatibility and the fact that their chemical and physiological properties resemble those of the natural extracellular matrix have supported cellular attachment, proliferation, and differentiation. However, many natural polymers exhibited non-favorable mechanical integrity, high viscosity, and hydrophilicity. In contrast, synthetic polymers show enhanced strength and tunable electrospinning properties but minimal biological interaction with the target tissues. Hence, the idea of cospinning of two or more types of polymers has grown to overcome these obstacles.

To enhance the final outcomes and exclude the second surgical procedure for removal, biocompatible and biodegradable materials are by large preferred over customary inert implants of the GTR.[51] Considering the fact that periodontal bone is basically a load-bearing construction, biochemical and biomechanical properties of the implanted material are crucial for successful management. Hence, employing electrospun nano-/micro-scaled fibers in tissue building is increasing continuously[37] due to their special nature and capacity to supply the target cells/tissues with a local environment by imitating the extracellular lattice. The ability of producing fibers of different sizes and orientations is one of the main gains of electrospinning technique by which pace and direction of fibroblasts proliferation can be controlled.[52,53]

COMPOSITE MATERIALS FOR PERIODONTAL REGENERATION

In the field of osseous TE, the cells demand precise structures for support and proliferation. High porosity is critical to simulate the natural properties of the bone extracellular matrix, providing an encouraging regeneration environment. This feature is essential to obtain an effective bone regeneration process. Nevertheless, it is a very challenging task to provide an enhanced cellular support and proliferation in one construct because it is essential to achieve a proper balance between the material physicochemical properties and cellular response.[54] To incorporate bioactivity into the scaffold, a new concept proposes combining bioactive molecules with polymeric substrates to interlace the positive virtues of both materials. Calcium phosphate crystals are the natural mineral within the bone matrix, which resembles the hydroxyapatite (HA). Therefore, bioceramics are frequently combined with natural and synthetic polymers either before or after the electrospinning process. For example, nanoparticles of HA were developed to regenerate intrabony periodontal defects and proven to have slightly enhanced clinical outcomes in comparison with traditional β-tricalcium phosphate.[55] HA had also been successfully incorporated with several polymers using simple stirring or ultrasonic stirring[56,57] matrices without affecting the fibrous morphology and porosity of the electrospun membrane. Enhanced alkaline phosphatase activity, osteocalcin expression, and bone sialoprotein in human mesenchymal cells were observed when cultured on HA composite nanofibers. Fujihara *et al*. prepared composite ultrafine fibers from PCL/CaCO3; *in vitro* study demonstrated the scaffold to encourage osteoblast attachment.[58] Nanosized demineralized bone powder also was homogenously electrospun with polymeric solution and demonstrated remarkable enhancement in the scaffold osteoconductivity and mesenchymal cells mineralization.[59] A biocomposite of PLLA/collagen/HA nanofibrous scaffold was introduced by Prabhakaran *et al*. who could improve the proliferation and mineralization of osteoblasts, resulting in favorable bone healing.[60] Since it was first introduced by Larry Hench, bioactive glass was increasingly investigated for its potential regenerative characteristics. The original formula of 45S5 has the ability to enhance the direct bonding between the natural hard and soft tissues by forming a hydroxyl carbonate apatite layer. In addition, it can stimulate the osteoprogenitor cells to form new bone. Researchers investigated new specifications derived from the 45S5 bioglass as potential bone regeneration membranes. When incorporated within polymeric matrix, they presented encouraging mechanical and biological behavior.[61] To overcome the implant-associated infection, there is a growing interest to develop biomaterials with antibacterial performance. Hence, several antimicrobial and nonsteroidal anti-inflammatory medications were assimilated within the regenerative membranes to provide adequate drug concentrations. Metronidazole,[62] ibuprofen,[63] and zinc oxide nanoparticles[64] were successfully integrated and electrospun with polymeric solutions without affecting the structural integrity or the function of the electrospun membranes.

COMBINING ELECTROSPINNING AND AM (BIMODAL SCAFFOLDS)

Despite the fact that electrospinning is a fascinating technique to create tissue regeneration devices, it has some limitations such as raising toxicity concerns regarding organic solvents[91,26] and limited thickness of the scaffold.[92] In addition, controlling the spatial distribution of the pores is a challenging job due to the random pattern of fibers layering.[93] AM has been proposed to tackle these limitations with the aid of computer design to construct the scaffold layer by layer.[94] Naturally, the periodontal apparatus is coming with a sophisticated composition of hard and soft components with variable geometrical properties for each component. Therefore, electrospinning has been combined with different AM methods to prepare multiphasic scaffolds to accommodate the complexity of the native tissue. As electrospinning (melt or solution) is used to fabricate small-scale elements and then combined with an AM approach such as fused deposition modeling (FDM),[95] inject printing,[96] and selected laser sintering.[97,98]

However, AM techniques have their own shortfalls, providing the minimum limits of fabrication resolution. Thus, conjoining the two fabrication methods was aiming to design and fabricate a new generation of TE scaffolds that are called bimodal scaffolds. The resulting constructs incorporated both micro- and nano-scaled elements at different levels. The electrospun fibers are typically supporting cellular adhesion and penetration, while the fused construct is providing the required mechanical support. This promoted cell penetration while retaining nanofibrous elements. Three research groups led by Moroni, Kim, and Park introduced the concept of bimodal scaffolds in 2008 by depositing electrospun meshes in between layers of an FDM constructed scaffold.[92-103]

Another prominent technology based on AM is the 3D printing (3DP), an efficient method to fabricate complex TE scaffolds from natural or synthetic polymers. On the other hand, 3DP lacks the ability to mimic the fiber component of the biological tissues. A combination of electrospinning and 3DP was applied to fabricate multilayered scaffold with maximum functionality to regenerate tissues with complicated

structures such as cornea, muscles, and tendons.[92-103] Furthermore, Park *et al*. successfully fabricated a 3D wax template that is later on used to prepare a biphasic scaffold that can imitate the hierarchical structure of the periodontium.[104] In 2105, Rasperini *et al*. reported the first clinical scenario of human periodontal defect that is treated by a custom-made biodegradable scaffold fabricated with 3DP.[105] AM techniques have opened the field to wider regenerative applications with vast variety of materials including polymers, hydrogels, and ceramics.

MULTIPHASIC SCAFFOLDS AND CELL SHEETS FOR PERIODONTAL TE

Considering the complex architecture of the periodontium and the necessity for a coordinated wound healing process, advanced scaffold designs with the ability to guide the periodontal regeneration were employed to promote therapeutic effects. Multiphasic scaffolds have recently arisen in the arena of periodontal regeneration with huge potentials to empower clinical results. The variations within the architecture and the chemical composition of the multiphasic scaffold are originally aimed to recapitulate the structural alignment and the biochemical configuration of the innate tissue. Here, a TE scaffold of multiple layers that are solidified to each other is used to deliver the cellular component into the periodontal defect. Such a design is highly desirable to impart the compartmentalization properties of the periodontal apparatus. The recent periodontal TE strategies have shifted focus toward assisting normal tissue formation and maturation. Thus, it is essential to establish scaffolds that maintain their integrity to support the newly generated tissue maturity within the interconnected, highly porous multiphasic scaffold architecture, allowing degradation only after the regenerated tissue is completely remodeled.[106] A major drawback of TE is the lack of ability to transport the cells to their specific locations; hence, it does not meet the demands for an effective regeneration. In this context, the use of cell sheets enables more precise and targeted cell provision within the periodontal defect.[107] An innovative non-enzymatic approach to harvest the progenitor cells utilizing cellular sensitivity to temperature changes to isolate them in the form of intact sheets.[108] Animal studies have shown encouraging regenerative potentials of periodontal ligament cells (PDLs) cultivated through this technique.[109-112] However, it was difficult to achieve biomechanical fixation for the cells.[112] From here, the contemporary use of a multiphasic scaffold can afford the essential support for the periodontal cell sheet in addition to create the room required for the newly formed bone within the periodontal defect. Vanquette prepared a polycaprolactone biphasic scaffold for the delivery of PDL cells and osteoblasts to regenerate the periodontal system.[113] The scaffold part that had been employed for bone regeneration was fabricated by FDM. While the periodontal part consisted of an electrospun membrane to facilitate the delivery of PDL cell sheets. Enhanced cellular stability and better adhesion of cell sheets were demonstrated in this study. Melt electrospun scaffolds were produced later by the same group to overcome the stiffness of the FDM fabricated bone compartment and to evoke the favorable porosity offered by electrospinning. A 3D electrospun PCL scaffold seeded with osteoblasts promoted new bone formation in rat models.[114] Further, modifications were demonstrated by covering the bone facing part with a layer of calcium phosphate to enhance the regenerative effect.[115] Soon after, Lee *et al*. presented a triphasic scaffold with region-specific microstructures based on polycaprolactone and HA.[116] However, there is still scarcity of *in vivo* studies to evaluate the limitations and success rates in clinical scenarios.

SUMMARY AND CONCLUSION

It is unquestionable that electrospinning is an impressive technique for fabricating nanoscaled constructs for TE applications. The past few decades witnessed several breakthroughs in this field based on the growing understanding of the spinning fundamentals, parameters that control the fibber's properties. Furthermore, a wide range of polymers and bioactive particles was successfully used with this technique. On the other hand, there are still numerous limitations that need to be overcome. For example, it is challenging to reproduce identical fibers in the presence of numerous factors controlling the process of electrospinning. This can affect the predictability of the fiber's diameter, pore size, and distribution which can directly alter cellular response. Deeper understanding of the natural structure of the periodontal system played a great role in modifying the basic concept of TE scaffold manufacturing to accommodate its complexity. A wide variety of combinations of scaffolds/cells was examined for their biocompatibility, bioactivity, and regenerative potentials; many of them were with very encouraging outcomes. Yet, the fact that the periodontal apparatus containing hard and soft components (with specific requirements for each component) has triggered numerous research groups to optimize their scaffolds to control the pace and direction/spatial drive of the regenerated tissues. Multiphasic scaffolds allowed compartmentalization of the periodontal tissue during the regeneration period by providing the required mechanical support for the hard component of the periodontal system. Nevertheless, regarding the soft component, recreating well-aligned fibers with

perpendicular insertions on the hard components are still an obstacle. Huge steps have been taken to the date to expand the electrospinning applications in the field of TE, and yet, more efforts needed to be focused toward clinical trials.

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