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Review

Electrospun Nanofibrous Membranes for Preventing Tendon Adhesion

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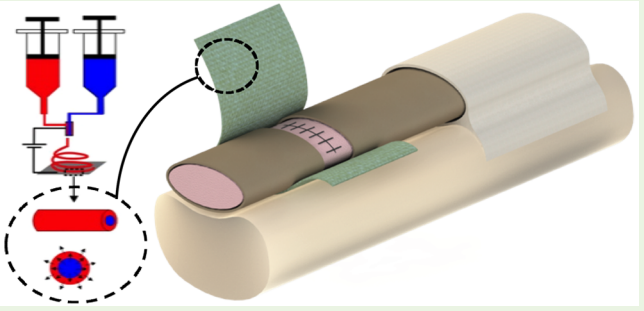
ABSTRACT: Tendon injuries are frequent, and surgical interventions toward their treatment might result in significant clinical complications. Pretendinous adhesion results in the disruption of the normal gliding mechanism of a damaged tendon, painful movements, and an increased chance of rerupture in the future. To alleviate postsurgical tendon-sheath adhesions, many investigations have been directed toward the development of repair approaches using electrospun nanofiber scaffolds. Such methods mainly take advantage of nanofibrous membranes (NFMs) as physical barriers to prevent or minimize adhesion of a repaired tendon to its surrounding sheath. In addition, these nanofibers can also locally deliver antiadhesion and anti-inflammatory agents to reduce the risk of tendon adhesion. This article reviews recent advances in the design, fabrication, and characterization of nanofibrous membranes developed to serve as (i) biomimetic tendon sheaths and (ii) physical barriers. Various features of the membranes are discussed to present insights for further development of repair methods suitable for clinical practice.



KEYWORDS: *tendon injury, adhesion, electrospun nanofibers, physical barrier*

INTRODUCTION

Tendons are dense connective tissues responsible for transferring forces from muscles to bones.[1] They can store elastic energy and endure large tensile forces resulting from locomotion.[1] Tendon injuries may occur through sudden tearing and lacerations, overloading, or aging.[2] Although there have been major advances in surgical methods and rehabilitation techniques over the past decades, tendon repair may still encounter postsurgical unsatisfactory outcomes. A major complication following tendon surgery is peritendinous adhesion formation within the healing zone in which the adjacent cells and tissues adhere to the injured tendon, consequently limiting the tendon gliding during flexion.[3−5] Specific areas of tendons are covered by sheath membranes to facilitate gliding. A tendon sheath has a fibrous outer layer and an inner synovial layer containing peritendinous fluid with the main inclusion of hyaluronic acid (HA) for lubrication purposes and prevention of fibroblast adhesion.[6] Tendon adhesion mainly occurs due to disruption of the sheath, thus allowing invasion of fibroblasts and tenocytes to the repair site.[7] Cell adhesion is a complicated process depending on environmental factors and surface physicochemical parameters of the tissue.[8]

To alleviate postsurgical tissue adhesion at the site of injury, several approaches have been recently developed employing tissue engineering principles (Table 1). These strategies concentrate on modulating the host response to injury by the activation and enhancement of the tendon's own repair system as well as preventing pathophysiological processes. These have been achieved by developing biomaterials either alone as biomimetic physical barriers or as tendon synovial sheaths for systemic and localized delivery of pharmaceutics, stimulatory factors, cells, and genes.[9,10] Recently, nanofibrous membranes (NFMs) have been recognized as promising carriers for the delivery of pharmaceutical agents due to their high functional characteristics.[11] In addition, nanofibers could also be employed as antiadhesion barriers in the site of tendon injury. To manufacture such micro-/nanofibers, different methods have been employed such as self-assembly, phase separation, and electrospinning.[11] Electrospinning is a robust and convenient technique, which is popular for production of the polymeric micro and nanofibers for different biomedical applications.[8,12] Electrospun scaffolds have gained popularity

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Table 1. Various Approaches Developed for the Fabrication and Characterization of Electrospun Nanofibers for Prevention of Tendon Adhesion and Promotion of Tendon Healing

			physical/mechanical properties			in vitro study			in vivo study	
						drug release (%)		cell culture		
ref	material	content load	method	fiber diameter (μm)	tensile strength (MPa)	burst release	sustained release	type of cell	animal model	adhesion severity
50	PELLA solved in DCM and acetone	•IBU 0%[a]	blending	1.45 ± 0.71	3.72 ± 0.32			L929 mouse fibroblasts	Leghorn chicken	moderate[b]
		•IBU 2%		1.40 ± 0.52	3.42 ± 0.36	38 (in 2 d)	52 (in 18 d)			
		•IBU 6%[a]		1.32 ± 0.67	3.13 ± 0.38	47 (in 2 d)	48 (in 18 d)			mild[c]
		•IBU 10%		1.25 ± 0.59	2.89 ± 0.31	62 (in 2 d)	36 (in 18 d)			
80	PLLA solved in DCM	•no load[a]	blending	0.8	4.41 ± 0.26			×	Leghorn chicken	moderate
		•IBU[a]			4.45 ± 0.31	46 (in 12 h)	54 (in 20 d)			moderate
		•MMS−IBU[a]			4.84 ± 0.34	6 (in 12 h)	85 (in 100 d)			mild
86	PLLA solved in DCM and DFM	•no load[a]	core−shell	0.86 ± 0.24	4.38 ± 0.33			C3H10T1/2	Sprague−Dawley rat	mild
		•bFGF[a]		0.81 ± 0.22	4.14 ± 0.22	20 (in 2 d)	80 (in 16 d)			mild
		•bFGF/DGNs[a]		0.77 ± 0.21	3.54 ± 0.25	20 (in 2 d)	80 (in 28 d)			mild
92	PLGA (85:15) solved in DCM and DFM	•HBDS: PDGF-BB growth factor ASC stem cell	blending and sequential process	0.4−0.7	N.A.	30 (in 2 d)	60 (in 10 d)	adipose-derived mesenchymal stem cells	mongrel dog	mild
52	PCL solved in THF, H_2O, and HFIP	•HA 0%[a]	emulsion and sequential process	PCL layer was 3.66 ± 0.57	2.13 ± 0.25			C3H10T1/2	Leghorn chicken	moderate
		•HA 4%[a]		PCL-HA was 125% 2.86 ± 0.71	1.91 ± 0.21	65.8 (in 4 d)	32.7 (in 10 d)			
		•HA 8%[a]			1.77 ± 0.18	79.6 (in 4 d)	18.9 (in 10 d)			
		•HA 12%[a]			1.55 ± 0.21	88.6 (in 4 d)	9.9 (in 10 d)			mild
67	PELA solved in DCM and DFM	•celecoxib 0%[a]	blending	1.82 ± 0.43	3.04 ± 0.32			tenocytes and dermal fibroblast	New Zealand white rabbit	moderate
		•celecoxib 2%[a]		1.76 ± 0.51	2.87 ± 0.27	43 (in 4 d)	32 (in 16 d)			
		•celecoxib 6%[a]		1.53 ± 0.57	2.77 ± 0.34	55 (in 4 d)	40 (in 16 d)			mild
		•celecoxib 10%		1.27 ± 0.42	2.72 ± 0.31	67 (in 4 d)	33 (in 16 d)			
6	PELA solved in DCM and DFM	•no load	emulsion, blending and sequential process	N.A.	N.A.	N.A.		×	New Zealand white rabbit	moderate
		•celecoxib in outer layer, HA gel as middle layer								mild
51	PCL solved in MC and DFM	•no load	emulsion and core−shell process	0.432 ± 0.123	N.A.	90% HA (in 10 d)	10% HA (in 40 d)	human foreskin fibroblasts (Hs68)	New Zealand white rabbit	moderate
		•PCL sheath with HA core		0.328 ± 0.107		100% Ag (in 2 d)				mild
		•PCL sheath containing silver nitrate with HA core		0.344 ± 0.92						mild
3	PLLA solved in chloroform and methanol	1 layer CS.Col[e] U[e]	single fiber	0.700 ± 0.250	1.3 ± 0.1	N.A.		tenocytes	N.A.	
		2 layer CS.Col U			1.9 ± 0.15					
		3 layer CS.Col U			5.6 ± 1.1					
		1 layer CS.Col C[f]			1.5 ± 0.1					
		2 layer CS.Col C			1.8 ± 0.05					
		3 layer CS.Col C			3.8 ± 0.7					
58	PCL solved in MC and DFM	•no load	surface modification	0.432 ± 0.123	1.36 ± 0.06	N.A.		human foreskin fibroblasts (Hs68)	New Zealand white rabbit	moderate
		•HA grafted		0.673 ± 0.172	1.64 ± 0.18					no adhesion
60	PCL solved in MC and DFM	•no load	surface modification	0.432 ± 0.123	1.4 ± 0.1	N.A.		human foreskin fibroblasts (Hs68)	New Zealand white rabbit	moderate
		•chitosan grafted		0.481 ± 0.157	2.2 ± 0.5					no adhesion

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Table 1. continued

			physical/mechanical properties			in vitro study			in vivo study	
						drug release (%)		cell culture		
ref	material	content load	method	fiber diameter (μm)	tensile strength (MPa)	burst release	sustained release	type of cell	animal model	adhesion severity
63	PCL[a]	none	blending	0.475 ± 0.128	1.35 ± 0.07	N.A.		human foreskin fibroblasts (Hs68)	New Zealand white rabbit	mild
	PCL+25%PEG			0.621 ± 0.161	2.67 ± 0.71					no adhesion
	PCL+50%PEG			0.540 ± 0.163	1.36 ± 0.09					
	PCL+75%PEG[a] solved in MC and DMF			0.446 ± 0.211	1.27 ± 0.11					
39	PELA solved in DCM, acetone and THF	•0[a]	emulsion, blending and sequential process	outer layer: 3.52 ± 0.63, 3.46 ± 0.72, 3.32 ± 0.69, 3.21 ± 0.75	2.92 ± 0.29			×	Leghorn chicken	mild
		•2		inner layer: 2.93 ± 0.73	2.84 ± 0.31	43.5 (in 4 d)	21.3 (in 16 d)			
		•6[a]			2.81 ± 0.34	55.8 (in 4 d)	35.2 (in 16 d)			mild
		•10% celecoxib loaded in outer layer HA loaded in an inner layer			2.76 ± 0.39	65.3 (in 4 d)	30.4 (in 16 d)			
56	PLLA solved in DCM and DMF	•Ag 0%	blending	0.85 ± 0.21	N.A			C3H10T1/2 mouse fibroblasts	N.A.	N.A.
		•Ag 4%		0.89 ± 0.38		35(in 6 d)	23 (in 18 d)			
		•Ag 8%		0.95 ± 0.42		55 (in 6 d)	27 (in 18 d)			
		•Ag12%		1.08 ± 0.32		80 (in 6 d)	15 (in 18 d)			
85	HA solved in formic acid	•IBU 0%[a]	blending	N.A	N.A			3T3 fibroblast cell	New Zealand white rabbits	mild
		•IBU 20%		0.52 ± 0.16	0.63 ± 0.53	58 (in 24 h)	72 (in 20 d)			
		•IBU 30%[a]		0.58 ± 0.17	0.94 ± 0.89	52 (in 24 h)	62 (in 20 d)			no adhesion
		•IBU 40%		0.63 ± 0.21	1.43 ± 0.13	41 (in 24 h)	60 (in 20 d)			
93	PLA and PCL	HA grafted	blending	contains two layer	N.A			L-929 cells	New Zealand white rabbits	moderate
				micro fiber layer: 2.0 ± 0.59				MSC		
				Nano fiber layer: 0.6 ± 0.18						
69	PEG and PCL solved in DCM and DMF	PEG/PCL containing Ag as sheath with HA containing	blending and core–shell process	N.A	N.A	50% IBU (in 8 h) (irrespective of its content)	50% IBU (in 21 d) (irrespective of its content)	3T3 fibroblast cell	new zealand white rabbits	mild
		•IBU 0%[a]				80% HA (in 4 d)	80% HA (in 17 d)			
		•IBU 10%				78–81% Ag (in 4 d)	19–22% Ag (in 17 d)			no adhesion
		•IBU 30%[a]								
		•IBU 50% as core								
71	PCL solved in HFIP	three layer containing: PCL layer, amniotic layer, PCL layer	single fiber	0.475 ± 0.147	12.8 ± 0.22			tenocytes and fibroblasts	New Zealand white rabbits	no adhesion
66	PLA and PCL solved in Chloroform	none	single fiber	4.3 ± 0.9	N.A			L929	SD rat	no adhesion
				8.2 ± 1.1						no adhesion
95	PLLA/HA	no load	core–shell	1.62 ± 0.34	2.56 ± 0.25			chicken embryonic fibroblasts	Leghorn chickens	mild
		siNC		1.97 ± 0.31	2.62 ± 0.29	N.A.	N.A.			
		siRNA		1.92 ± 0.41	2.68 ± 0.32	9 (in 2 d)	17 (in 15 d)			mild
		siNC/PDA		2.02 ± 0.32	2.52 ± 0.23	N.A.	N.A.			
		siRNA/PDA		2.07 ± 0.33	2.59 ± 0.24	17 (in 2 d)	78 (in 30 d)			no adhesion

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Table 1. continued

[a]Shows the selected group for in vivo analysis. [b]Moderate (33−66% of the tendon surface). [c]Mild (less than 33% of the tendon surface). [d]Collagen. [e]Uncoated scaffold [f]Gel coated scaffold.

due to their similar characteristics to the natural extracellular matrix (ECM) because of their large specific surface area, high porosity, and connected pores.[13,14] Importantly, physical and architectural properties of electrospun scaffolds, as physical barriers, could be easily modulated to reduce the fiber diameter,[15,16] increase the rotation speed of the drum[17] and surface modification of the fibers,[18] and tune the fiber alignment.[19] Furthermore, other fiber properties could be modulated by controlling electrospinning parameters such as applied voltage,[20] flow rate of the polymer,[21] and the distance between the capillary and collector,[22] along with the solution parameters including viscosity, electrical conductivity, and solvent volatility.[23,24]

Electrospun NFMs, designed as drug carrier systems, should protect the activity of their payload and enable the delivery of drugs or biomolecules in desired patterns for specific applications. In such systems, the drug delivery kinetics could be controlled by the drug-loading technique, the materials' composition, and the architecture.

Generally, electrospun fibers are fabricated in two architectures including single fibers and core−shell structured fibers. In order to encapsulate pharmaceutical agents into nanofibers, various drug-loading techniques have been employed such as surface modification (or surface functionalization), blending, and coaxial and emulsion electrospinning, Figure 1.[25] The surface modification refers to a process in

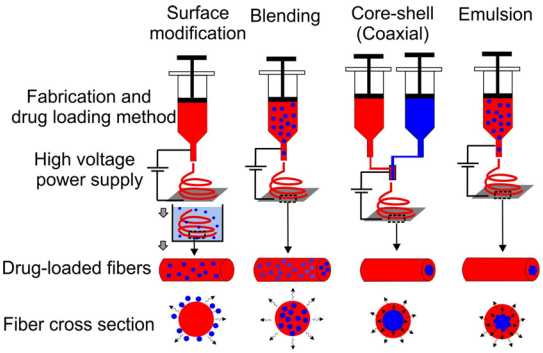

Figure 1. Different drug loading techniques in polymeric electrospun nanofibers. The red color represents polymer. The blue color is for drugs, and the black arrows indicate the drug release direction. Reproduced with permission from ref 25. Copyright 2020 Elsevier.

which the surface of nanofibers is physically and chemically altered with various bioactive molecules.[26] This technique is usually applied to fragile molecules like nucleic acids and proteins, which is likely to have rapid degradation and to lose biofunctionality during the electrospinning process.[13] A rich variety of therapeutic molecules such as growth factors,[27,28] proteins,[29] and polysaccharides[30,31] have been physically or chemically formulated on the surface of electrospun nanofibers to achieve controlled topical release within a defined period of time. Although a surface modified scaffold could provide a robust delivery pattern thanks to a large surface area to volume ratio of nanofibers, the delivery period may not last long enough for some applications.[25] To accomplish release of therapeutic agents on a longer term, blending, coaxial, and emulsion electrospinning methods were established. In blending techniques, the molecules or the drugs are dissolved directly in a polymeric solution prior to electrospinning.[25] In this method, compatibility of polymer solutions and the drug

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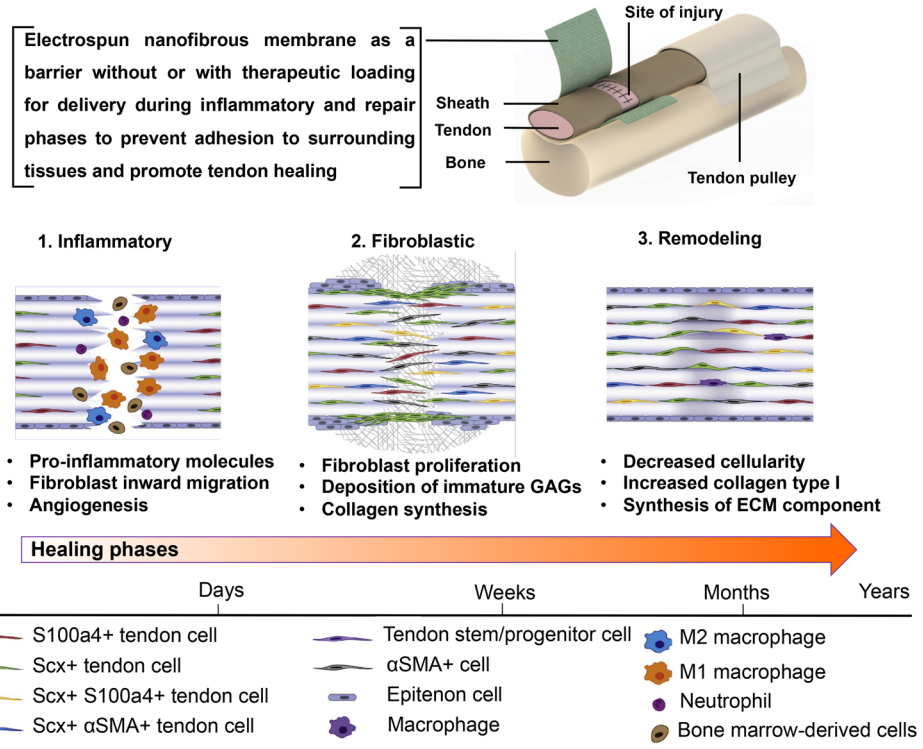

Figure 2. Healing phases of tendon with various cell populations involved in healing phases. Tendon has various cell types during hemostasis that are categorized by different transcription factors. Tendon contain scleraxis $(Scx)^+$ cells, $S100a4^+$ cells, $Scx^+S100a4^+$ cells, and Scx^+ alpha smooth muscle actin $(aSMA)^+$ cells. Reproduced and modified with permission from refs 43 and 48. Copyright 2020 Elsevier.

physical properties is vital in terms of wettability for an appropriate drug solubility and distribution within the polymer solution.[26] In addition, a common solvent is required to be used for the preparation of drug and polymer solutions.

Coaxial electrospinning is a modification of the conventional electrospinning process that employs two nozzles with concentric arrangement to fabricate fibers with core−shell configuration.[26] This technique provides a structure for which biomolecules could be protected from environmental hazards through locating in the core. Coaxial electrospinning could extend the drug release period due to the prolonged route of drug diffusion in comparison with blending electrospinning.[25,32,33] In emulsion electrospinning, bioactive molecules which are dissolved in aqueous solution are dispersed in a polymer solution (with an organic solvent).[34] This technique requires the same basic setup of blending electrospinning. However, in emulsion electrospinning, a similar solvent for the drug and the polymer is not necessary.[35] Additionally, in this technique, an organic solvent rapidly evaporates and viscosity increases. Therefore, the viscosity gradient results in the migration of the aqueous phase towrd the center of the jet.[36] This quasi core−shell fiber structure enables drug release for an extended period of time.[37]

Long-term release could also be achieved by making multilayer scaffolds such as layer-by-layer electrospun structures. In this way, mechanical aspects of the scaffold such as strength along with the required properties for producing a variety of drug release patterns could be modulated in a flexible manner.[38] To this end, a sequential electrospinning process has been widely employed to produce scaffolds that may consist of two or more layers depending on the application and requirements for the drug release profile.[39,40] Such scaffolds could be formed using the aforementioned electrospinning techniques.[40,41]

This article reviews the design of various biomimetic tendon sheaths and physical barriers with antiadhesion delivery systems fabricated using electrospinning methods. Various features of these systems were analyzed to present insights for use in clinical practice.

1. THE PATHOLOGICAL MECHANISMS LEADING TO TENDON ADHESION

Natural healing of an injured tendon occurs in three main phases through distinctive molecular and cellular cascades.[1] The three overlapping phases include (i) inflammation, (ii) fibroblastic/proliferation, and (iii) remodeling, Figure 2. After injury, the initial inflammatory phase begins following extensive cell death. Subsequently, inflammatory cells such as neutrophils, monocytes, and macrophages infiltrate the injured area.[1,42] Next, components of the ECM, predominantly collagen type III, are synthesized by recruited fibroblasts.[1] The Proliferative phase involves cell migration to the injury site, proliferation and deposition of collagen fibrils, followed by the remodeling phase.[42]

Tendon healing is a complex process that involves contributions of both intrinsic and extrinsic mechanisms.[43] The intrinsic mechanism is due to the cell populations that originate from tendon parenchayma, epitenon, and/or endotenon. The extrinsic mechanism occurs because of the extrinsic cells recruited from the outside of an injured tendon to the injury site. Extrinsic mechanisms involve cell populations migrating from blood and the surrounding tissues such as paratenon and synovial sheath.[43]

It is now accepted that these two mechanisms function simultaneously. The hypothesis is that the invasion of

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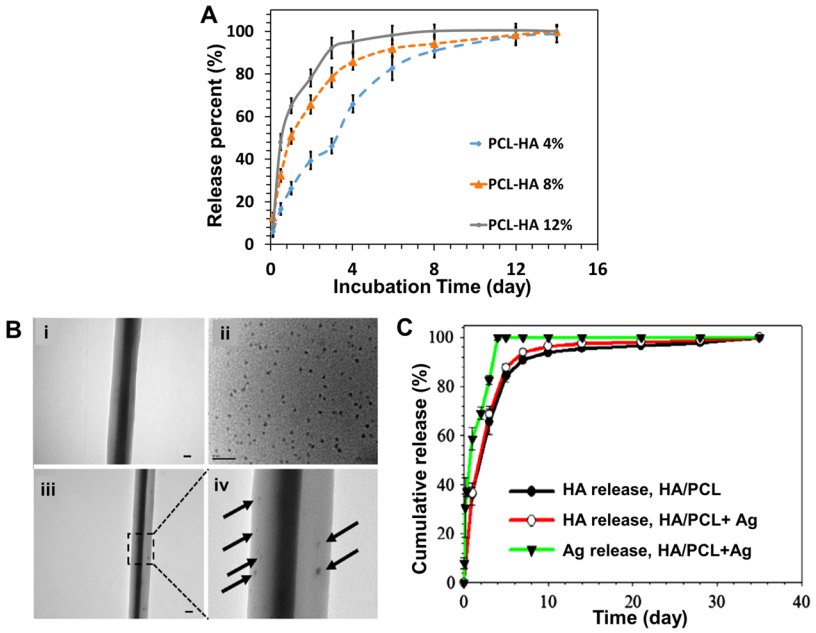

Figure 3. (A) *In vitro* quantification of HA release from different PCL-HA fibers after incubation in the release medium, reproduced with permission from ref 52. Copyright 2020 American Chemical Society. (B) (i) TEM micrographs of HA/PCL, (ii) HA/PCL + Ag, in higher magnification (iii) nanofibers (all scale bar = 100 nm and Ag nanoparticles in the PCL sheath are shown by arrows), and (iv) the TEM micrograph of a casted PCL film obtained from the UV-irradiated (3 h) sheath solution (bar = 50 nm). (C) Cumulative drug releases during incubation time of various membranes. Adapted with permission from ref 51. Copyright 2020 Elsevier.

fibroblasts and inflammatory cells from the tendon periphery, blood vessels, and circulation to the injured site results in adhesions. Subsequently, intrinsic cells from the endotenon are activated as they migrate and proliferate to the injury site. This leads to reorganization of the ECM and inferring the mechanical properties of the tendon.[1,44,45] Overall, conditions that favor the extrinsic mechanism may result in a higher level of scar tissue and adhesion formation in comparison with those that facilitate the contribution from intrinsic cell populations.[43] Any method that hinders extrinsic repair and/or promotes intrinsic repair can impede adhesion formation, facilitate tendon gliding, and increase the strength of the repair.[46] An anti-adhesion membrane could provide multiple functions to lubricate and reduce attachment and infiltration of fibroblasts while preventing postsurgical infection and inflammation.[47] In the following sections, nanofibrous membranes are reported and discussed in two categories of biomimetic tendon sheaths and physical barriers. In fact, all the reported membranes act as a barrier aiming to reduce postsurgical adhesions; however, the structure of biomimetic ones resembles the synovial sheath surrounding a tendon.

2. NANOFIBROUS MEMBRANES AS BIOMIMETIC TENDON SHEATH

Tendon-sheath adhesion could be reduced mainly through the inhibition of fibroblast proliferation and inflammatory responses of the tendon's surrounding tissues. The tendon sheath is a membrane that can only be found in specific areas of hand and foot tendons where a change of direction and increase in friction necessitate very efficient lubrication.[49] Moreover, a tendon sheath performs the function of a biological barrier, not allowing the invasion of surrounding fibrous tissue as well as promoting the healing process of the tendon.[6]

Tendon injury typically results in tearing of the tendon sheath compromising its function. The overexpression of proinflammatory cytokines post injury combined with the lack of presence of an effective barrier lead to excessive proliferation of fibroblasts migrating to the injury site and creating a dense fibrous layer. This common complication causes pain and functional obstruction, which eventually may need a surgical intervention.[50] Thus, it is required to develop and employ an alternative structure that can provide a role for the tendon sheath to reduce adhesion and facilitate tendon repair.[6] Use of electrospun fibrous membranes presents a promising strategy to restore the dual functions of the tendon sheath.[51] To this end, a biomimetic nanofiber-based sheath should have desired mechanical properties with the ability to prevent the invasion of peripheral fibrous tissue.[52] HA is one of the most widely used agents in the development of biomimetic tendon sheaths due to having the features of synovial fluid.[53] HA not only reduces the postsurgical adhesion but also promotes tendon healing.[54,55] In the following sections, various fiber-based membranes without and with therapeutic agents are discussed.

2.1. Biomimetic Tendon Sheaths without Therapeutic Agents. A biomimetic tendon sheath should have a bilayer structure, in which the inner layer could promote gliding and the outer layer could impede cell attachment and penetration. Liu et al.[52] fabricated an electrospun bilayer membrane consisting of an inner layer, which was formed from an HA-loaded poly(ε-caprolactone) (HA/PCL) membrane coated by a PCL fibrous membrane. The bilayer membrane was fabricated through the combined methods of sequential and microgel electrospinning. Four different scenarios including PCL-HA0%, PCL-HA4%, PCL-HA8%, and PCL-HA12% were tested. *In vitro*, an HA release test revealed a pronounced burst release phase, followed by a gradual release to the end of the test. The electrospun PCL-HA4% composite had lower initial burst release (65.8% release in the first 4 days) in comparison

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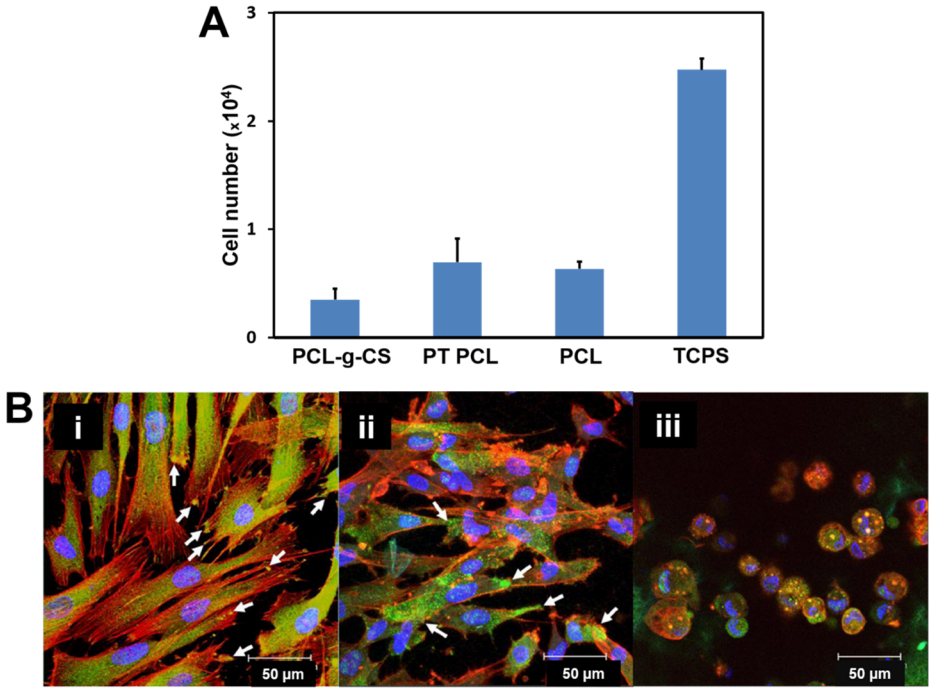

Figure 4. (A) MTS assay results present fibroblasts attachment to TCPS, PCL, plasma-treated PCL (PT PCL), and PCL-*g*-CS nanofibers after 24 h. (B) The arrangement of cytoskeleton and expression of fibroblasts' focal adhesion protein was observed by confocal microscopy on different nanofibrous membranes including (i) TCPS, (ii) PCL, and (iii) PCL-*g*-CS. Analyses have been carried out using actin cytoskeleton and vinculin staining ($p < 0.05$). Vinculin focal adhesion, actin cytoskeleton, and cell nucleus are indicated in green, red, and blue, respectively. The vinculin focal adhesion expression is shown with arrows. Adapted with permission from ref 60. Copyright 2020 Elsevier.

with that of electrospun PCL-HA12% membranes (88.6% release in the first 4 days). Figure 3A demonstrates *in vitro* HA release from the membranes with drug loadings of 4, 8, and 12%. Animal studies showed that the outer sheath membrane obstructed the adhesion to surrounding tissues, whereas the inner layer of HA-loaded PCL promoted gliding as well as the healing process of the tendon.

The majority of the developed fiber-based polymeric tendon sheaths have revolved around the reduction of tendon adhesion. Bacterial infection is another complication as a result of tendon sheath rupture. Thus, an ideal design of tendon sheaths should have dual functions to release antiadhesion and antibacterial agents during healing. Silver has been considered for its protective function against a broad range of microorganisms. In particular, silver nanoparticles have shown strong antibacterial properties.[51] Chen et al.[51] employed a coaxial electrospinning method for the fabrication of a dual-functional core−shell NFM with an HA-loaded core and Ag nanoparticle-embedded PCL shell, Figure 3B. HA was used to mimic the biological role of HA in synovial fluid for effective lubrication to enhance tendon gliding, while Ag provided antibacterial activity. They conducted *in vitro* and *in vivo* investigations on rabbit flexor tendons to evaluate three different membranes, including PCL, HA/PCL, and HA/PCL +Ag. According to *in vitro* results, a steady release of HA from both HA/PCL and HA/PCL+Ag NFMs was obtained for 21 days, while 90% of the cargo was released by day 10 (Figure 3C). Furthermore, the gradual release of Ag lasted for 4 days, which was adequate to inhibit bacterial infection throughout the early stage after surgery with a high risk of infection. In contrast with the control group with major adhesion in the middle of the repaired tendons and the granulating tissue, only few adhesion sites were found in the tendons treated with the PCL or HA/PCL NFMs. The efficacy of the HA/PCL+Ag NFM was also investigated, and the result indicated better adhesion prevention in comparison with PCL or HA/PCL NFMs.

In another study, Liu et al.[56] investigated the performance of AgNP-loaded poly(L-lactide) (PLLA) electrospun scaffolds for hindering of the adhesion and infection. The PLLA/AgNP blend solutions for electrospinning were prepared in the forms of PLLA/AgNP 4%, PLLA/AgNP 8%, and PLLA/AgNP 12%. The cell proliferation over the PLLA nanofibrous membrane with or without AgNP content was studied for 4 days. It was observed that the increase of AgNPs content resulted in a decrease of live cell count over the AgNPs-loaded PLLA fibrous membranes. It was observed that the cell growth after 4 days and 1 day showed a similar trend on different surfaces. *In vitro* tests indicated burst release of Ag ions from the PLLA/AgNP fibers in all samples during the first 2 days. The results indicated that the AgNP-loaded PLLA fibrous membrane had a better antibacterial ability compared to the membranes made of only PLLA. Overall, the AgNP-loaded PLLA fibrous membranes were capable of alleviating the post-injury infection and formation of adhesion. In a study conducted by Deepthi et al.,[3] aligned poly(l-lactic acid) (PLLA) membranes were fabricated, mimicking the aligned collagen bundles of tendon. For tendon regeneration, the glycol aminoglycans of the sheath ECM were mimicked through layered fabrication of chitosan-collagen hydrogel and PLLA fibers. At the final stage, the construct was coated with alginate (Alg) gel to form a bilayer tendon sheath mimicking structure, which can be used as an effective copolymer to prevent peritendinous adhesion.[57] It was observed that the tensile strength of the engineered constructs was adequate for regenerating the immobilized flexor tendon. The protein adsorption test indicated Alg-coated

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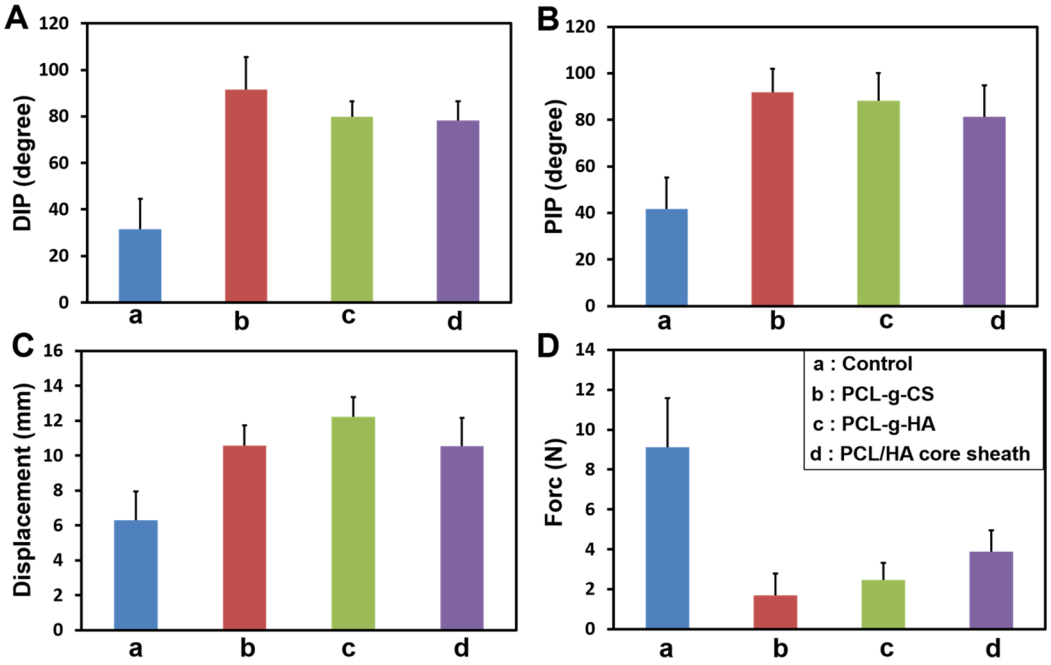

Figure 5. Examination of peritendinous adhesions 3 weeks after surgery using different parameters. (A) Angle of DIP joint flexion, (B) angle of PIP joint flexion, (c) displacement of gliding tendon, and (D) pull-out force. (A) Control, (B) PCL-*g*-CS [47], (C) PCL-*g*-HA,[58] and (D) PCL/HA core sheath[65] (each group, n = 8). Reproduced with permission from refs 51, 58, and 60. Copyright 2020 Elsevier.

scaffolds have lower protein adsorption resulting from the absence of binding sites of protein in Alg. Thus, it may facilitate the decrease of peritendinous adhesion.

It has been studied that NFMs fabricated based on polymer blends or copolymers are more successful to prevent peritendinous adhesion than single-component NFM.[24] However, it is necessary to take extreme care in regulating the electrospining procedure, and complex copolymerization phases need to be carried out. To address these complications, Chen et al.[58] grafted the molecules of HA to the surface of PCL nanofibers after the electrospinning step. This alternative method improves the flexibility of selection of grafted macromolecules and the electrospinning circumstances compared with making a composite NFM. They compared the performance of electrospun PCL and HA-grafted PCL with the Seprafilm *in vivo*. Seprafilm is formed of sodium hyaluronate and carboxymethyl cellulose, presenting a hydrophilic property.[59] The thickness of the membrane was measured as 200 ± 50 μm. The peritendinous adhesions were histologically examined at 2 and 8 weeks postoperatively by gross evaluation. In Seprafilm and the PCL NFM samples, a weak bonding was formed between the tendon and the surrounding tissue due to small bundles of fibrous tissues. In contrast, they claim that there was no trace of adhesion in the group with repaired tendons using the PCL-*g*-HA NFM. The evaluation of tendon healing level was performed by calculating the mechanical strength of a treated tendon at 2 weeks postoperatively. Comparing all groups, a negligible difference was observed in breaking force. Therefore, all antiadhesion barriers had an equal healing rate.

In other research carried out by Chen et al.,[60] chitosan-grafted polycaprolactone (PCL-*g*-CS) NFMs was fabricated to examine its features for the prevention of peritendinous adhesions. The graft of the PCL membrane and chitosan molecules was performed covalently using the coupling agent of carbodiimide. Chitosan is a natural amino polysaccharide. It carries various features including excellent biocompatibility and biodegradability, hemostatic activity, nontoxicity, and antibacterial activity with poor cell adhesion properties.[24] Many studies have shown that chitosan can reduce postoperative adhesion formation. The *in vitro* and *in vivo* experiments demonstrated that PCL-*g*-CS has improved efficacy over those of PCL NFM and Seprafilm in prevention of peritendinous adhesions.

Figure 4A shows cell attachment on PCL, plasma-treated PCL, PCL-*g*-CS NFMs, and tissue-culture polystyrene (TCPS) surfaces. The number of cells attached to the TCPS group was significantly higher than for the others. In addition to the morphology, the distribution of cytoskeletal actin and expression of focal adhesion protein of fibroblasts were displayed for these samples, Figure 4B. The PCL NFM group presented better cell distribution, increased vinculin expression, and importantly a very good arrangement of fibrous F-actin cytoskeleton. A positively charged surface of CS, similar to amino groups, may result in electrostatic repulsion and poor cell attachment on the CS membrane. They evaluated the peritendinous adhesion by direct observation at 2, 4, and 8 weeks after the operation. The gross evaluation of the untreated control group showed dense adhesion formations in the middle of the tendon and the vascular granulating tissue around the tendon. The *in vivo* results showed that tendons wrapped with Seprafilm and the PCL NFM contain small fibrous tissue bundles that make a connection between the tendon and its surrounding tissues. It was concluded that PCL-*g*-CS NFM had a remarkable potential to prevent tendon adhesion.

We compared the level of postsurgery peritendinous adhesion for three investigations performed by the Chen group[51,58,60] in Figure 5. Three criteria were considered as follows: (i) the proximal phalangeal (PIP) and distal phalangeal (DIP) joint range of motion, (ii) tendon gliding displacement, and (iii) the pull-out force. The angle between

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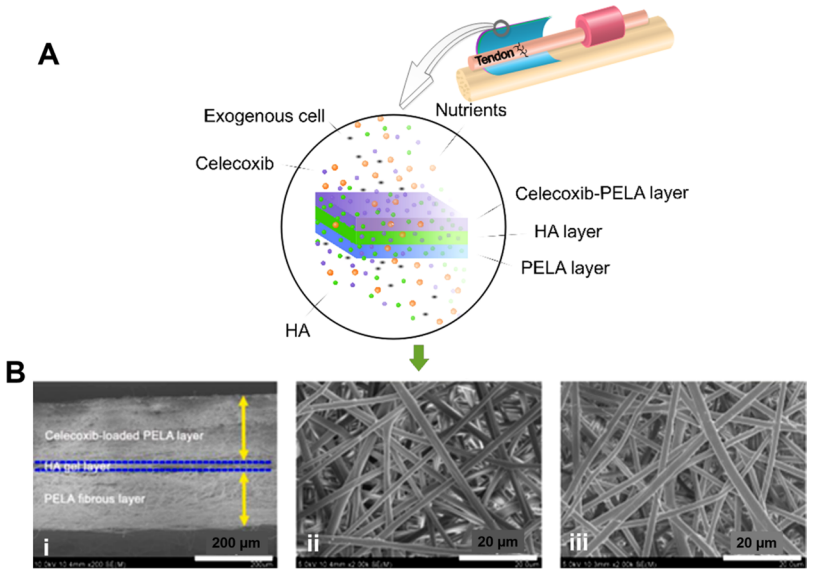

Figure 6. (A) Schematic representation of multiple layer scaffold, functioning as a physical barrier to prevent tendon adhesions. Reproduced with permission from ref 6. Copyright 2020 MDPI. (B) SEM images of (i) cross-sectional characteristics of the multiple layer scaffold, (ii) surface morphology of the PELA electrospun fibers, and (iii) celecoxib-loaded PELA electrospun fibers. Adapted with permission from ref 6. Copyright 2020 MDPI.

the DIP and the middle phalangeal as well as the angle between the middle phalangeal and the PIP were considered since they are more clinically relevant. To evaluate tendon gliding displacement, the constant force that leads to separation of the tendon from its sheath and their distance after dragging were measured. The maximum applied force to completely detach the tendon from the tendon sheath was also introduced as the pull-out force. Figure 5 shows quantitative evaluation of the range of motion, gliding displacement, and the pull-out force of the selected samples for the four selected NFMs including control group, PCL-grafted-HA, PCL-grafted-CS, and HA/PCL+Ag treatments at 3 weeks, postoperatively. It is clear that postsurgery adhesion of control samples (the ones received conventional treatment) were much higher than the other samples. However, there was no significant difference between the selected samples of different treatments in peritendinous adhesion criteria. An increase in motion angles, especially in DIP, was observed for PCL-grafted-CS sample, showing a better antiadhesion effect of chitosan in comparison to HA and HA/Ag. Figures 5A,B and 6C show a slightly higher amount of gliding displacement of the PCL-grafted-HA sample than PCL-grafted-CS one, which might be associated with a more lubricating property of HA. In addition, the PCL-grafted-CS sample required the least pull-out force for full removal of the tendon from its sheath, Figure 5D. It demonstrated that chitosan grafted to the PCL membrane had the lowest degree of adhesion among the three treatments at 3 weeks after surgery. Overall, HA, HA/Ag, and chitosan grafted to PCL membranes could provide lubrication and antibacterial activity. However, chitosan was the most effective material to prevent postsurgery tendon adhesion to its surrounding tissues due to its strong anti-inflammatory property.

PCL is a widely used biodegradable polyester in medical devices. It has unique features including stability under ambient conditions, low cost, and sufficient mechanical properties that make it popular for drug delivery applications and in tissue engineering.[61] However, its use as an antiadhesion barrier film might be limited due to its stiffness and hydrophobicity. To tackle this issue, PCL could be mixed or modified with other polymers to obtain appropriate properties for adhesion prevention. Poly(ethylene glycol) (PEG) has been suggested as a suitable copolymer for PCL due to its hydrophilic nature and high biocompatibility.[62] Chen et al.[63] examined different combinations of blended PEG/PCL NFMs (0% PEG, 25% PEG, 50% PEG, and 75% PEG) under *in vitro* conditions to recognize the best PEG/PCL ratio. It was shown that the combination of PEG, as a hydrophilic polymer, with PCL could reduce the hydrophobic property of a pristine PCL membrane. Also, the increase of PEG concentration resulted in enhanced surface hydrophilicity. The rabbit flexor digitorum profundus (FDP) tendon model was employed to investigate the antiadhesion efficacy of the composite NFMs. Four study groups were considered as untreated controls; NFM included 75% PEG, PCL NFM, and Seprafilm. Gross evaluation, microscopic investigation, and functional assays indicated that the composite NFM had proper biodegradability and biocompatibility with a higher prevention effect on peritendinous adhesion over Seprafilm and PCL NFM.

The degradation pattern of micro/nanofibers influences the performance of the nanofibrous membrane in preventing peritendinous adhesion and drug release kinetics during the whole release period.[64] Drug release kinetics for non-biodegradable polymeric matrices depend on the concentration gradient, diffusivity of the substance in the polymer structure, and diffusion length.[26,65] However, for a biodegradable carrier system, drug release is also affected by the enhanced diffusion through the pores generated during the polymer degradation. In these systems, the release of drugs entrapped within the polymer matrix is facilitated through the degradation process.[25]

To compare two common synthetic polymers with different degradation kinetics as a biomimetic tendon sheath, Song et al.[66] fabricated MFMs of polylactic acid (PLA) and PCL. The morphology, degradation rate, cell adhesion, antiadhesion effects, and biocompatibility were studied both in vitro and in

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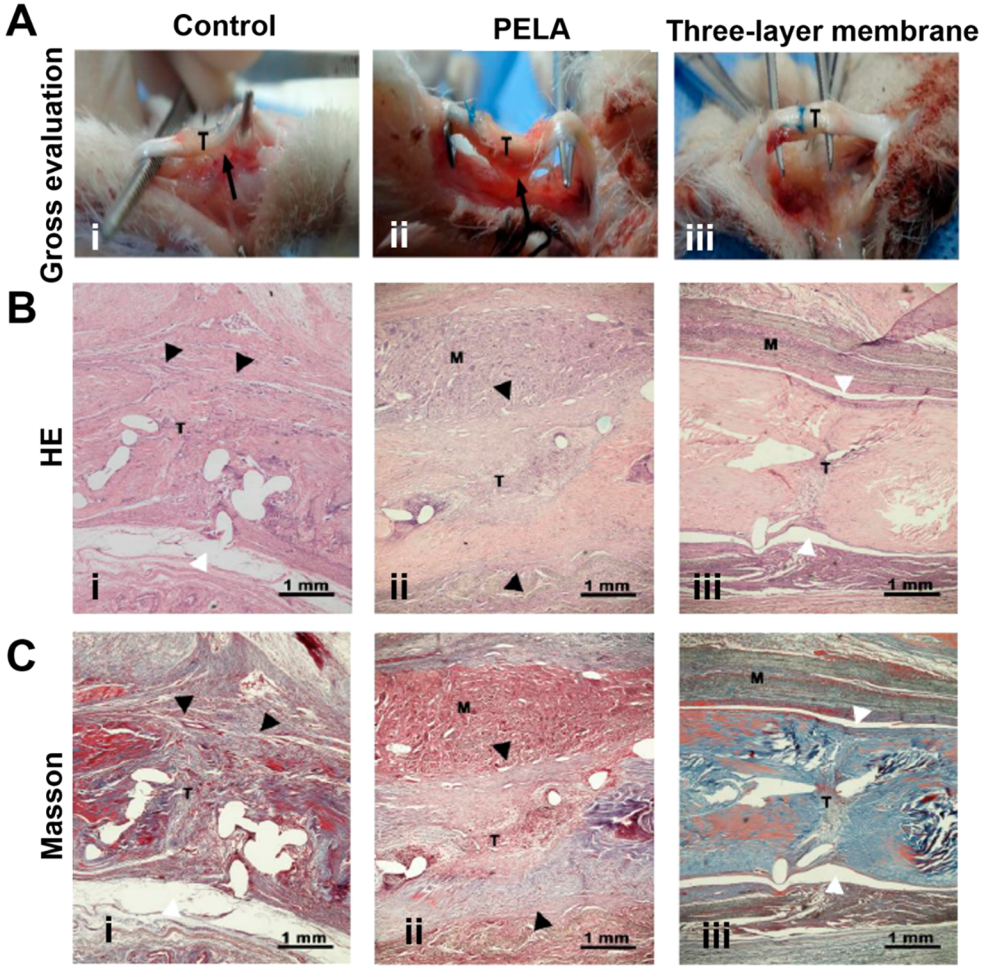

Figure 7. (A) Gross examination of adhesion in an *in vivo* rabbit model of FDP tendon. (B) HE and (C) Masson staining in (i) the control group without treatment, (ii) PELA scaffold, and (iii) multilayer PELA/HA scaffold. (Black arrowheads refer to peritendinous adhesions between the membrane (M) and tendon (T).) Adapted with permission from ref 6. Copyright 2020 MDPI.

vivo. PLA and PCL NFMs showed different degradation behaviors in three kinds of medium including PBS, PBS with α-chymotrypsin, and PBS with elastase. The mass loss process showed that the degradation of the PLA membrane in the presence of elastase was faster than the rate in the PBS with α-chymotrypsin. PLA film in PBS presented the slowest degradation rate. The PCL membrane showed slow degradation in the three media with a maximum 5 wt % mass loss with or without enzymes. An *in vivo* study exhibited a more effective function of the PLA membrane in terms of adhesion reduction and promoting functional recovery than the PCL sample. Masson's and HE staining micrographs represented the density and distribution of collagen formation after the repair phase. The electrospun polyester films had considerable antiadhesion capabilities compared to the control group. Moreover, the dense and ordered arrangement of tendon cells and collagen fibers in PLA and PCL membranes exhibited the potential of these NFMs in improving tendon healing. In addition, no visible difference was observed in stained areas of the repaired and normal positions.

2.2. Biomimetic Tendon Sheaths with Therapeutic Agents. Despite extensive studies, the mechanism of adhesion formation is not thoroughly clear. The fibroblast proliferation and synthesis of excessive collagen that cause vascularization, an inflammatory response, new ECM, and the growth of blood vessels might be the probable reasons for peritendinous adhesions.[4,7] Thus, inhibition of fibroblast proliferation and synthesis of excessive collagen could prohibit adhesion formation, in which ERK1/2 and SMAD2/3 contribute significantly.[67] Jiang et al.[67] carried out *in vitro* and *in vivo* investigations to examine the incorporation of celecoxib in electrospun poly(L-lactic acid)-polyethylene glycol (PELA) diblock copolymer membranes. Celecoxib is recognized as a nonsteroidal anti-inflammatory drug (NSAID) that can prohibit ERK1/2 and SMAD2/3 phosphorylation to repress fibroblast proliferation and collagen synthesis. Some studies showed that COX-2 inhibitors reduce intra-abdominal adhesions.[68] Accordingly, celecoxib could be employed to hinder tendon adhesions. An *in vitro* drug release test was performed for three different concentrations of celecoxib loaded into the PELA electrospun membrane (PELA-2%, PELA-6%, and PELA-10% membranes). The results showed that as celecoxib concentration increased, the average fibers' diameter declined. The increase of celecoxib content led to greater stiffness of the fibrous matrix. *In vitro* drug release indicated that celecoxib burst releases throughout the first 2 days from PELA-2%, PELA-6%, and PELA-10% membranes were 32.8%, 40.7%, and 47.3%, respectively. Also, it was revealed that almost 100% of the drug was released from the fibrous scaffold of PELA-6% and PELA-10% after 20 days. The

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cell study showed that with increasing celecoxib concentration, viability, tenocytes, and dermal fibroblast counts reduced over those of the membranes. This might prove the anti-inflammation and antiadhesion effects of celecoxib; however, it has the potential to impair intrinsic healing of the tendon. Thus, PELA-6% NFMs were selected as a treatment sheath to wrap around the repair site of the rabbit tendon to compare with the unloaded PELA group and the group that received only conventional treatment. The results from the animal model indicated that the PELA scaffolds carrying celecoxib decreased peritendinous adhesions and inflammatory reactions. This might be due to the suppression of COX-2 expression and fibroblast proliferation. However, from the results of maximum tensile strength and the histological analysis of tendons, it could be concluded that celecoxib diminished the healing process of tendons compared to the tendons treated with PELA membranes with no celecoxib loading.

To address this issue, Jiang et al.[6] produced a biomimetic three-layer electrospun tendon sheath using the sequential electrospinning method. The biomimetic tendon sheath scaffold consisted of outer, middle, and inner layers made of celecoxib-loaded PELA (E/L = 10:90) membrane, HA gel, and PELA membrane, respectively, Figure 6A. The middle HA gel acts as a barrier to prevent celecoxib penetration toward the tendon, which impairs the tendon healing. Celecoxib was loaded in the outer layer to avoid its negative effects on tendon healing, taking advantage of its ability to prevent fibroblast proliferation. Moreover, HA gel combined with a high hydrophobic PELA layer can provide smooth tendon gliding. The thickness of the PELA layer and the celecoxib-loaded PELA layer were reported to be 130 and 170 μm, respectively, Figure 6B. Results of the *in vivo* animal study showed that the membrane could enhance the antiadhesive function by the celecoxib-loaded PELA as the outer layer. Celecoxib could not only play the role of the native fibrous sheath by preventing cell invasion but also significantly suppress fibroblast proliferation and collagen synthesis. Maximal tensile strength and work of flexion were also studied to assess peritendinous adhesion and the healing process of tendons. Maximum tensile strength of the groups had a negligible discrepancy. The three-layer membrane group showed a considerably lower work of flexion than those of the control and PELA fibrous membrane groups.

Figure 7A shows gross examination of the treated rabbit FDP tendon in the cases with conventional treatment, PELA, and three-layer scaffold implanted. Figure 7B and C represent HE and Masson staining of the untreated control group, PELA scaffold, and the three-layer scaffold. Clear peritendinous adhesions around the repair site was observed in the control group (the group received only suture-based repair). The three-layer scaffold showed fewer adhesion sites than the unloaded PELA scaffold. Moreover, it was observed that tendon surface was smooth and collagen bundles were well organized indicating better tendon healing compared to other cases. In a similar study carried out by Li et al.,[39] the tendon sheath was mimicked to enhance tendon healing and its gliding. They implemented a mimetic bilayer sheath membrane formed from a PELA/HA fibrous membrane and electrospun celecoxib-PELA membrane to represent the synovial layer of a native sheath and its outer layer, respectively. For *in vitro* investigations, celecoxib in four concentrations (0%, 2%, 6%, and 10% w/w = celecoxib/PELA) were incorporated in the outer layer. The characterization of NFMs showed that the increase of celecoxib concentration could increase the fiber diameter. In addition, contact angle measurements revealed that HA increased the hydrophilicity of the PELA electrospun fibrous membrane. However, celecoxib increased the hydrophobicity of the PELA membrane. An *in vitro* drug release test suggested a burst release during the first 4 days, and the rest was released up to 3 weeks. *In vivo* tests showed the celecoxib-loaded outer layer, functioning as a physical barrier, provided a therapeutic opportunity to reduce inflammation and adhesion to surrounding tissues. Prevention of tendon healing is a major concern in relation to the incorporation of NSAIDs into a physical barrier. This challenge was addressed by loading celecoxib in an outer layer to improve the antiadhesion effect without interrupting the intrinsic healing. In addition, HA release from the inner part of the bilayer tendon sheath could enhance the healing process of the tendon and its gliding.

Within the past few years, some investigations have been directed toward the development of complex and multipurpose membranes to carry various functions for the simultaneous reduction of inflammation and bacterial infection and to promote tendon gliding with antiadhesion properties.

In one study, Shalumon et al.[69] carried out *in vitro* and *in vivo* studies on core−shell NFMs with embedded silver nanoparticles in poly(ethylene glycol)/poly(caprolactone) in the shell and HA/ibuprofen in the core. Despite the core−shell structure of fabricated nanofibers, ibuprofen (IBU) had rapid release kinetics within the first 8 h where about 50% of the total drug was released regardless of its content in the membrane. The HA and Ag nanoparticles had slower release rates compared to IBU. On the basis of the results of *in vitro* studies, Shalumon and colleagues suggested that cell viability in IBU-loaded membranes directly depends on dosage, while higher concentrations could result in considerable cytotoxicity; this was also suggested by other research.[70] Therefore, this endorsed that NFMs are only cytocompatible up to an optimum drug concentration.

Liu et al.[71] employed amnion to develop an NFM capable of sustained delivery of multiple growth factors to promote tendon healing and prevent tendon adhesion. Amnion, as a human-derived tissue, inherently contains multiple growth factors that could be used as a release source to promote tendon healing and prevent adhesion.[71] In this way, fresh amnion was initially treated by freeze and vacuum drying. Then, two surfaces of freeze-dried amnions were covered with electrospun PCL nanofibers to form a multilayer composite membrane to mimic tendon sheath function. The human amniotic membrane contains various growth factors including transforming growth factor (TGF-β1) and fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF). The growth factors could diffuse into the repair site through the porous structure of the PCL membrane and improve the proliferation of tenocytes and collagen synthesis. Mechanical characterizations revealed that the fabricated composite membrane exhibited significantly higher mechanical properties in comparison with the freeze-dried amnion. The results of histological analysis of the rabbit tendon repair model indicated that the only use of amnion could not successfully prevent tendon adhesion, and loose fiber bundles were observed in the surrounding tissue. However, it had a positive effect on tendon healing and accelerated the healing procedure. The use of a composite nanofibrous membrane had more promising impacts on the prevention of tendon adhesion;

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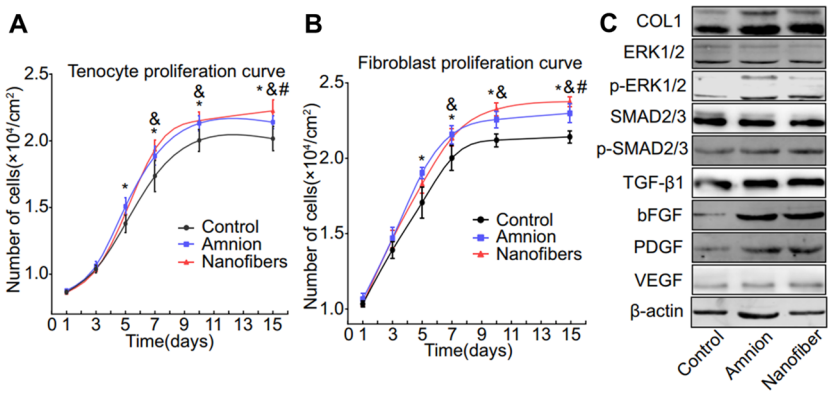

Figure 8. (A, B) Adhesion and proliferation of tenocytes and fibroblasts on the surface of the control samples, freeze-dried amnion, and nanofiber membranes. (C) The expression levels of TGF-β1, bFGF, VEGF, PDGF, COL1, SMAD2/3, p-SMAD2/3, ERK1/2, and P-ERK1/2 proteins in the tested groups, on the seventh day of cell culture, adapted with permission from ref 71. Copyright 2020 PMC.

however, no significant difference on tendon healing between the two groups of amnion and composite membrane was observed. The results of the Western blot analysis represented that gradual release of growth factors resulting in regulation of the ERK1/2 and SMAD2/3 pathways. Consequently, the adhesion and proliferation of tenocytes and fibroblasts were promoted, and collagen synthesis was improved. However, the specific mechanism of how the ERK and SMAD signaling pathways interact with each other remains unclear. These two pathways are primarily involved in regulation of the pathological process of tendon healing and adhesion formation. The results of adhesion and proliferation of tenocytes and fibroblasts as well as Western blot analysis are shown in Figure 8.

Overall, engineering a tendon sheath mimicking the architecture and mechanical properties of the injured tissue has been pursued as a logical strategy for preventing adhesion postsurgical interventions. In this approach, NFMs are typically coated by a lubricating material to facilitate the sliding of the tendon. In addition to lubrication, the exterior layer is expected to prevent excessive inflammation and prevent the adhesion of invading cell populations such as fibroblasts. While the strategy seems reasonable in theory, there are significant challenges associated with the correct NFM composition and the coating material. One of the key challenges is the longevity of the utilized materials. While some of the studies have reported successful results in terms of prevention of the adhesion upon acute injury, they have not been followed for a long time. For example, as most of the used hydrogel coatings are PEG- and chitosan-based, it is not clear if they can support the formation or repair of the natural sheath to replace the temporary system. Thus, once the coating layer is worn down, the exposure of the inner layers of the tendon or NFMs can activate a cascade of inflammatory response leading to adhesion. Another solution that has been explored extensively is the localized release of drugs or biological factors to modulate the environment. However, the similarity of the cells responsible for tendon healing and the migratory cells causing adhesion has created a challenge on how to separate the two layers. Thus, the use of more specific therapeutics or better targeted delivery is essential to preventing fibrosis without hindering healing.

3. NANOFIBROUS MEMBRANES AS PHYSICAL BARRIERS

Electrospun fibrous membranes are potentially useful for the prevention of postsurgical peritendinous adhesions with minimal side effects to tendon healing.[72] Various therapeutic agents could be also loaded in such fibers such as NSAIDs,[73] fibrinolytic agents,[74,75] antibiotics,[76] vitamins,[77] and growth factors[78] to enhance tendon healing or provide more efficacious inhibition of fibroblast proliferation and reduction of inflammation responses. In the other words, physical barriers loaded with drug cargos can provide dual functions including adhesion formation prevention and healing promotion. However, potential issues such as the inflammatory response of such barriers should be investigated before transition to the clinic.[60]

Delivery of Pharmaceutical Agents and Nanoparticles. NSAIDs are very applicable for injured tendons.[48] The injection of NSAIDs is done a few days after the injury time but may lead to extensively different patient reactions.[48] Despite decreasing tendon adhesions with NSAIDs like ibuprofen through targeting the inflammatory phase of healing, adhesion formation cannot be entirely avoided via using them alone. NSAIDs also have rapid clearance and a damaging influence on cell proliferation and prostaglandins(PG) synthesis.[48] Therefore, NSAID application is beneficial if the dose, timing, and mode of delivery is adjusted to relieve pain with no effect on tissue repair.[79]

Owing to their unique features, electrospun fibrous membranes present flexible delivery platforms for long-term sustained release of anti-inflammation and antiadhesion agents to the tendon.[50,80] The diameter of electrospun nanofibers could be tuned in a large range from 2 nm to several micrometers.[24] They also provide a large ratio of surface area-to-volume with a large porosity of up to 91.63% along with very small pore size ranging from 2 to 465 μm.[24,50] Furthermore, there are other advantages that present electrospun fibers as an enabling drug delivery method, including high drug loading (up to 60% wt. drug/wt. polymer) and high encapsulation efficiency (up to 100%) that is defined as the ratio of actual drug mass in fibers to the expected drug mass in fibers.

There are various polymers with a wide spectrum of chemical properties that could be electrospun in an easy and low cost fashion.[81] Taking advantage of the electrospun fibers, Liu et al.[50] performed *in vitro* and *in vivo* analyses to determine

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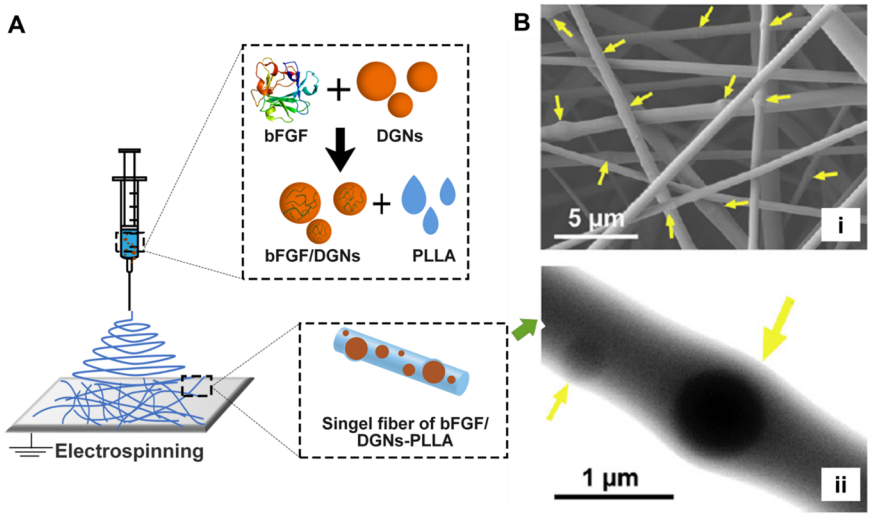

Figure 9. (A) Schematic illustration for loading bFGF into DGNs using a freeze-induced procedure for particle formation and electrospinning of bFGF/DGNs-loaded PLLA to produce a membrane capable of sustained release of bFGF and protection of the biological activity of bFGF. Reproduced with permission from ref 86. Copyright 2020 Elsevier. (B) SEM (i) and TEM (ii) images of electrospun bFGF/DGNs-PLLA. Adapted with permission from ref 86. Copyright 2020 Elsevier.

the capacity of electrospun ibuprofen-loaded PLLA and PEG−PELA nanofibers to avoid adhesion formation and decrease inflammation. Incorporation of PEG could enhance the PLLA hydrophilicity and the flexibility of the electrospun membranes for easy handling and placement. However, a high content of PEG (40% or 50%) may suppress the intrinsic healing of a tendon due to the closure of the interconnected pores required for the transport of cytokines and growth factors to the site of healing. Thus, PELA fibers containing 10% PEG were selected for animal studies that can provide effective blocking of cell adhesion/proliferation. For the *in vivo* trials, Leghorn chickens were randomly examined as the treatment group. To this end, a piece of PELA membrane loaded with ibuprofen or without ibuprofen was used to cover the injury spot, and the control group only underwent conventional treatment.

Results of the *in vitro* tests for drug release showed that throughout the first 2 days, the burst release of ibuprofen from the electrospun fibers was 38% (PELA-2%), 47% (PELA-6%), and 62% (PELA-10%). Over the next 10 days, a sustained release was achieved. Kinetics of drug release were mostly dependent on drug diffusion and degradation of the polymer matrix; thus, the drug release rate was improved as the drug content increased. Evaluation of tissue adhesion was carried out after 21 days postoperatively. Results revealed that the ibuprofen-loaded PELA membranes could alleviate peritendinous adhesions. Moreover, inflammatory cell infiltration was significantly decreased without interfering with the tendon healing.

Liu et al.[80] also fabricated composite systems with the purpose of achieving a long-term drug release for a minimum duration of 100 days and the reduction of postsurgical peritendinous adhesion and inflammation. Three different scaffolds were fabricated including electrospun PLLA, PLLA−IBU, and drug-loaded modified mesoporous silica nanoparticles (MMSs) in PLLA (PLLA−MMS−IBU). MMSs were employed as proper carriers for controlled drug delivery due to their unique properties such as biodegradability, blood compatibility, and the ability to sustain the release of therapeutic agents.[82,83] IBU-loaded MMS was encapsulated within the PLLA nanofibers through a cosolvent-based electrospinning process. *In vitro* release examinations demonstrated that the burst release of electrospun PLLA−MMS−IBU fibrous membranes was considerably slower, i.e., 6% release in the first 12 h, in comparison with electrospun PLLA−IBU membranes with a 46% release in the first 12 h. Moreover, the sustained release from PLLA−MMS−IBU was 100 days, which was considerably longer than PLLA−IBU within 20 days. Such characteristics are mainly associated with drug diffusion through two layers. The entrapped drug in the porous structure of MMSs should initially diffuse from the nanoparticles into the PLLA fibers with subsequent diffusion from the nanofibers in their surrounding material.[84] *In vivo* animal studies, performed on a chicken model, revealed that 4 weeks after implantation, anti-adhesion and anti-inflammatory properties were enhanced using both PLLA−IBU and PLLA−MMS−IBU compared with using only the PLLA fibrous membrane. Eight weeks postoperatively, histological evaluation indicated that a higher number of cells in inflammatory phase was observed in the PLLA group in comparison with the control group (the group received conventional treatment), due to the acidic environment created by the fibers' degradation. However, PLLA−MMS−IBU indicated low adhesion formation and inflammation and better tendon healing even 8 weeks postsurgery.

Indeed, nanofibrous membranes as antiadhesion barriers can simultaneously serve as drug delivery systems for tendon regeneration. In an example, Chen et al.[85] designed a drug carrier system based on HA to deliver ibuprofen to reduce the inflammation response and prevent fibroblast attachment and penetration at the site of injury. To overcome the poor mechanical properties and fast degradation rate of HA, they prepared a dual cross-linked electrospun membrane using $FeCl_3$ and 1,4-butanediol diglycidyl ether as cross-linking agents. However, the mechanical properties of fabricated membranes in this study were significantly lower than those membranes designed based on synthetic polymers with the same drug contents. They concluded that the increase of drug load in the membrane enhanced ultimate stress and Young's modulus. The *in vivo* tests revealed that the drug loaded nanofibrous membrane presented better outcomes in inhib-

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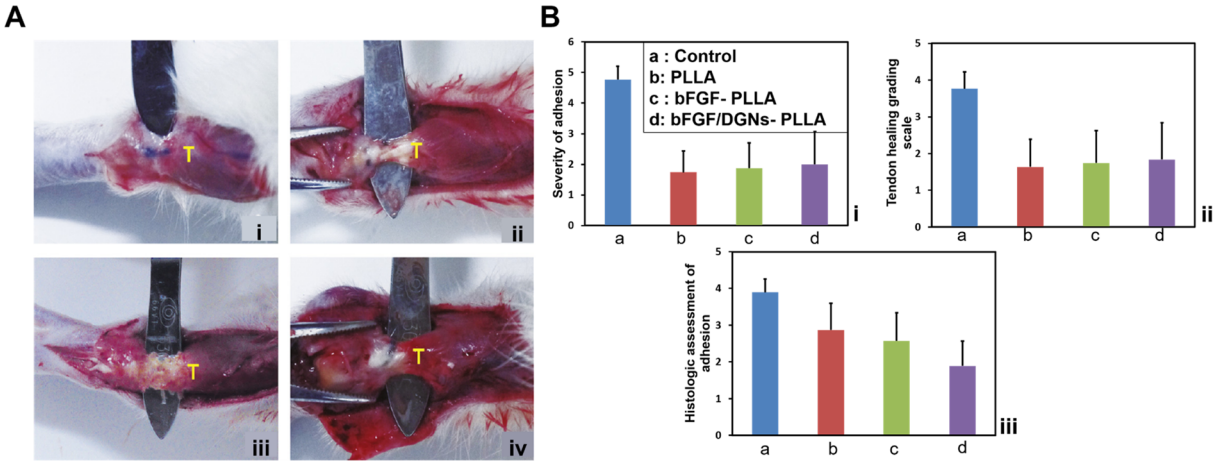

Figure 10. (A) Gross examination of an *in vivo* model of rat for Achilles tendon surgery after 21 days. (i) The control group underwent conventional treatment; (ii) group with the PLLA scaffold treatment; (iii) group with the bFGF-PLLA scaffold treatment; (iv) group with the bFGF/DGNs-PLLA scaffold treatment (in the images, T represents Tendon). Adapted with permission from ref 86. Copyright 2020 Elsevier. (B) Parameter study for peritendinous adhesions: (i) severity level of adhesion, (ii) histologic quantification of tendon healing quality 21 days after surgery, (iii) histologic assessment of tendon adhesions. Reproduced with permission from ref 86. Copyright 2020 Elsevier.

ition of tendon adhesion compared to Seprafilm and a nonloaded membrane in the inhibition of tendon adhesion.

Delivery of Biologics. Fabrication of ideal scaffolds for the repair and regeneration of injured tissues with a thorough recovery of functions of the original tissue have had great significance in tissue engineering. The scaffolds fabricated based on biomaterials may not enable proper regulation of cellular behaviors for tissue assembly.[86] Thus, development of scaffolds made of biomaterials with incorporated biologics including cells and growth factors has gained significant attention. Growth factors, recognized as a group of bioactive proteins, have been widely investigated as natural functional molecules for cell recruitment and ECM synthesis at the site of injury.[48] The incorporation of growth factors into ECM-mimicking nanofibers regulates proliferation through enhanced signal transfer between cells and their surrounding ECM.[87] However, uncontrolled release of growth factors to the surrounding tissues could affect neighboring cells and result in side effects such as adhesion formation.[88] Different growth factors have been studied for tendon healing. VEGF enhances revascularization of repairing tendon and healing process.[5] It has been observed that PDGF not only benefits the repairing function of tendon tissue in the canine model but also improves tendon glide.[48] Moreover, basic fibroblast growth factor (bFGF) is another type of growth factor that enhances proliferation and differentiation of mesenchymal stem cells (MSC) and differentiation toward tenogenic lineage. In this way, the expression of tendon ECM proteins as well as cellular collagen production could be increased.[48]

Although growth factors are used to regulate cellular behavior, they could be easily degraded under *in vivo* conditions. This results in the loss of their biological activities. Electrospun scaffolds provide porous media to preserve the bioactivity of growth factors for their sustained release.[89] However, biological functionality of growth factors could be damaged during the preparation of electrospinning solutions.[86,90,91] To enhance the stability of growth factors under *in vivo* conditions, Liu et al.[86] fabricated electrospun poly L-lactic acid (PLLA) copolymer nanofibers containing preformulated dextran glassy nanoparticles (DGNs) with loadings of bFGF, Figure 9A. In this study, the bioactivity of bFGF during the sustained drug release was aimed to be preserved in order to reach simultaneous healing and peritendinous adhesion prevention. Figure 9B represents the SEM and TEM images of electrospun bFGF/DGNs-PLLA. The effect of employing PLLA, bFGF-PLLA, and bFGF/DGN-loaded PLLA scaffolds on cell proliferation, adhesion, and tendon healing was investigated. In addition, the incorporation of bFGF and DGNs enhanced the hydrophilicity of the scaffold. Their results also revealed a decline of the maximal tensile strength (3.54 ± 0.25 MPa) and elongation (48%) for the bFGF/DGNs-PLLA scaffold.

The *in vitro* study revealed no burst release for bFGF/DGNs-PLLA scaffolds, and a sustained release for 30 days was achieved. In this fiber configuration, bFGF was protected by dextran. The bFGF/DGNs-PLLA scaffold showed an efficiency of 49 ± 13% for protein encapsulation. A rapid burst release behavior was also obtained for the bFGF-PLLA scaffold with 20 days of release. The efficiency of protein encapsulation was reported to be 25 ± 16% for the bFGF-PLLA scaffold as the control sample prepared by the emulsion electrospinning method. Moreover, the total bFGF content of the bFGF/DGNs-PLLA scaffold was higher than double that in the bFGF-PLLA scaffold, which proves the preservation of bFGF activities in dextran. The *in vitro* results also showed that cells had higher adherence and proliferation on the bFGF/DGNs-PLLA fibrous scaffold compared to the bFGF-PLLA scaffold. It shows that the bFGF bioactivity could be well preserved using preformulated DGNs in the fibrous scaffolds.

In vivo studies in a rat model demonstrated that the bFGF/DGNs-PLLA scaffold enhanced the healing process and produced oriented collagen bundles in tendons. More capillaries and a higher expression of type I collagen was also observed for the cases using bFGF/DGNs-PLLA fibrous scaffolds.[4] The *in vivo* results of the four test groups were summarized in Figure 10.

Manning et al.[92] developed a surgical repair strategy to combine growth factor delivery with autologous stem cells. Their scaffold was comprised of a heparin/fibrin-based delivery system (HBDS) with the structural integrity of poly lactic-*co*-glycolic acid (PLGA) electrospun nanofibers. The layered design provided modularity to customize the size of the

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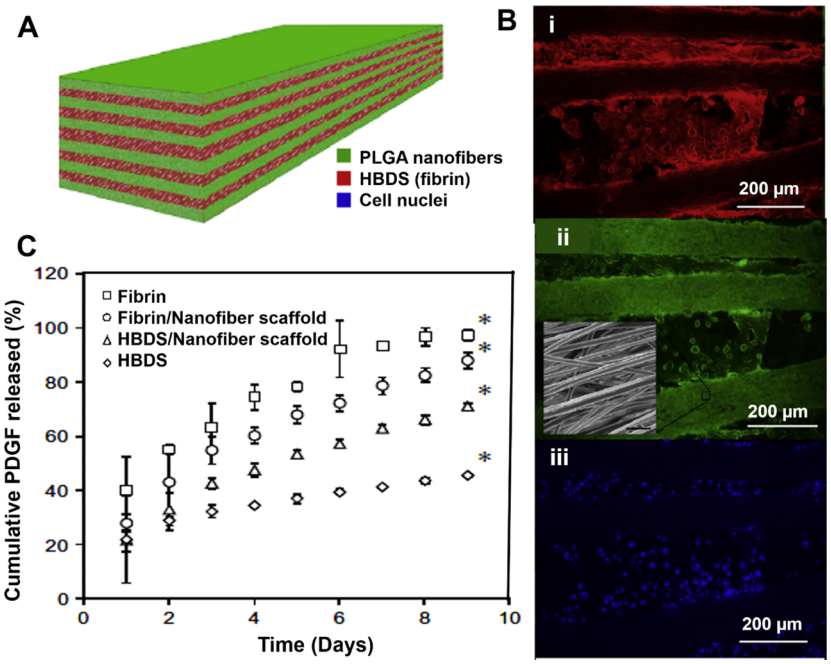

Figure 11. (A) Schematic representation of HBDS/nanofiber scaffold including 11 layers of aligned electrospun PLGA nanofibers and HBDS. (B) *In vitro* micrograph demonstration of the HBDS/nanofiber scaffold. (i) The HBDS was labeled in red, (ii) the PLGA was labeled in green showing SEM image of fibers, and (iii) the ASC nuclei is in blue color. (C) PDGF-BB release kinetics from the fibrin alone, the fibrin/nanofiber, the HBDS/nanofiber, and the HBDS alone. The difference of the groups was considerable in 9 days ($p < 0.05$ at 9 days according to a multifactor ANOVA; $n = 4–6$). Adapted with permission from ref 41. Copyright 2020 Elsevier.

scaffold according to the size of the healing site with controlled delivery of the cell and growth factor. The HBDS/nanofiber scaffold is comprised of 11 alternating layers including six layers of aligned electrospun PLGA nanofibrous membranes and five layers of fibrin, Figures 11A and B.

Figure 11C indicates the release kinetics of PDGF for three different study groups including the fibrin, fibrin/nanofiber, HBDS, and HBDS/nanofiber scaffolds. On the first day, the initial burst release of growth factor for the HBDS/nanofiber scaffold was 22% of the entire dosage, while over the next 8 days, the remaining growth factor was released gradually, with an entire release of 71% until day 9. The fibrin/nanofiber scaffold without HBDS had a quite similar initial burst release of growth factor (28%); however, an entire release of 88% by day 9 occurred. The fibrin alone had a maximum amount of initial burst (40%), and almost the whole loaded growth factor (97%) was released in 9 days. Remarkably, the HBDS alone presented the minimum growth factor release (46%) throughout the experiment. The faster release kinetics of the HBDS/nanofiber scaffold compared with HBDS alone might be associated with the larger surface of the thin rectangular HBDS layers within the HBDS/nanofiber scaffold in comparison with the bulk HBDS. Scaffold biocompatibility for tendon repair and cell viability after implantation were studied in an *in vivo* dog model.[92]

Liao et al.[93] also performed a study to incorporate mesenchymal stem cells into multiscale nanofibrous membranes. The fabricated electrospun membranes were fabricated using a copolymer of L-lactide and ε-caprolactone (PLCL) with hierarchical microscale to nanoscale topographic arrangement. To enhance the functionality of the designed membrane, they grafted the top microfiber layer with HA molecules, which has anti-inflammatory characteristics and can elevate gliding due to its lubricating action. PLCL, heat-treated PLCL at 125 °C for 2 h, and PLCL-HA grafted membranes were evaluated *in vitro*. The annealing process resulted in the reduction of the mechanical properties of PLCL postheat treatment compared to the non-heat-treated PLCL. The Young's modulus of PLCL increased about 1.5 times after surface modification with HA. The intrinsic hydrating characteristic of HA also increases the permeability and porosity of scaffold, which play a critical role in determining the scaffold efficiency through affecting cell penetration, and nutrient and waste transfer. The bilayer multiscale electrospun scaffold was implanted in the site of injury so that the top layer made of microfibers faced the tendon sheath to inhibit cell adhesion while still allowing for supplying the membrane with high permeability. On the other hand, the layer with nanofibers faced the tendon to promote cell proliferation. The results of *in vivo* experiments indicated that, despite the delivery of MSC, there was no desired outcome in tendon healing. Moreover, the analysis of range of motion, which was employed as a parameter to quantify the adhesion, indicated no decrease in adhesion formation.

In addition to cell-based therapeutics and delivery of growth factors for tendon regeneration, another emerging approach is the sustained delivery of cytokines.[94] Inflammation is the result of cell proliferation or change in the cellular behavior. Cytokines as hormone-like proteins modulate cell behavior and are divided into two categories: pro-inflammatory and anti-inflammatory. Interleukin-1 (I-1), IL-12, IL-18, IFN-γ, and tumor necrosis factor (TNF-α) are pro-inflammatory cytokines. Some other cytokines including IL-4, IL-10, IL-13, interferon α (IFN-α), and IFN-β are anti-inflammatory cytokines, which can prevent cell proliferation and decrease inflammatory response.[46] Fatemi et al.[46] for the first time investigated the effects of two anti-inflammatory cytokines including IFN-α and IFN-β, on the formation of tendon adhesion and tensile strength of the flexor tendon post-surgery

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using a rabbit model. There were four groups of rabbits in which interferon (IFN)-α, 5-fluorouracil (5-FU), normal saline, and IFN-β were locally applied to the repaired sites, respectively. Three weeks later, tensometric and histopathologic evaluations were performed.[46] The findings revealed that local application of 5-FU significantly decreased peritendinous adhesion, while local IFN-α and IFN-β had no major antiadhesion effect. The required force for detaching the tendon from the sheath was not different among the groups; however, the required time for detachment was considerably shorter in the 5-FU group.[46]

Delivery of Genes. Recent efforts on the use of electrospun fibrous scaffolds and scaffolds with pharmacological agents have not been able to completely prevent adhesion tissue formation.[95] Given the fact that adhesion can be effectively inhibited by targeting a crucial cellular signal that regulates the mechanism of adhesion and related downstream signal pathways in formation of adhesions, gene-based therapeutics have been developed for peritendinous antiadhesion.[95] The goal of gene delivery is introducing exogenous genetic materials into cells to alter the DNA and subsequently induce, silence, upregulate, or downregulate the expression profile and secretion of proteins. The benefit of gene delivery is natural synthesis of proteins by host cell mechanisms. This prevents the reduced bioactivity and activation of an immune response, which often occurs throughout the delivery of exogenous biomolecules.[10] A variety of *in vitro* and *in vivo* gene transfer methods have been studied for acceleration of the tendon repair to induce local production of growth factors such as GDF-7 or PDGF. The gene delivery carriers include two groups: (i) viral vectors such as adenovirus and retrovirus or (ii) nonviral vectors mainly including liposomes, cationic polymers, and peptide conjugation.[1,8]

Small interfering RNA (siRNA)-mediated gene silencing has been developed for the downregulation of specific genes in recent years.[95] Several studies have focused on sustained delivery of siRNA using nanofiber-mediated delivery systems.[95] Among these systems, nanoparticles, formed by the electrostatic self-assembly of siRNA and loaded in electrospun membranes, could be a promising method for sustainable gene silencing. Despite that, there are several challenges involved in these systems including low efficiency, loss of functional biological activity, toxicity concerns, and complex electrospinning techniques.[95]

The pathology of peritendinous adhesion formation is associated with fibroblast proliferation and collagen type III (Col III) deposition.[95] It was investigated that downregulation of extracellular signal-regulated kinase (ERK) 2 and SMAD2/3 could prevent fibroblast proliferation and reduce Col III deposition.[67,96]

In this context, Liu et al.[95] developed a multifunctional, cationic 2,6-pyridinedicarboxaldehyde-polyethylenimine (PDA)-mediated extracellular ERK2-siRNA delivery system, in the form of electrospun poly l-lactic acid/hyaluronan scaffolds (P/H). This polymeric system that functions as a physical barrier enables controlled release of bioactive siRNA for long-term inhibition of peritendinous adhesions and ERK2 silencing.[95] In this study, the function of the siRNA+PDA+P/H membrane was compared with control membranes including P/H, small interfering negative control (siNC)+P/H, siRNA+P/H, and siNC+PDA+P/H. The results of *in vitro* analysis revealed that ERK2-siRNA release from the siRNA+PDA+P/H scaffold within nearly 30 days was twice as long as that of the siRNA+P/H scaffold. *In vitro* evaluation of cell adhesion and proliferation rate after 4 days of culture indicated that less transfected chicken embryonic fibroblasts (UMNSAH/DF-1) adhered to and proliferated on the siRNA+PDA+P/H membrane compared with the P/H, siNC+P/H, siRNA+P/H, and siNC+PDA+P/H membranes. This occurs as a result of the highly bioactive transfection of ERK2-siRNA from the siRNA+PDA+P/H membrane. The *in vivo* evaluation on a chicken model of peritendinous adhesion showed that Col III density in the group treated with siRNA+PDA+P/H reduced compared with P/H and siRNA+P/H groups via downregulation of the expression of ERK2 and SMAD3 genes and that adhesion formation could be inhibited.[95]

Overall, NFMs as physical barriers for the directed tissue growth have been attractive in different areas of regenerative medicine.[97] As a pure physical barrier, the pore size distribution is the key important architectural feature that should prevent cellular migration until the tendon healing completes and the natural barrier and sheath is formed. However, since most of the used materials degrade and slowly adsorb proteins in the environment, cells can anchor on them and eventually migrate into them; this can cause adhesion to the implanted NFM. To modulate this challenge, the material composition and surface chemistry can be tailored. In addition, therapeutics can be delivered. One important strategy is to reprogram the cells interfacing with the NFMs. In the latter case, the cells can be transfected by siRNAs or plasmids such that phenotype change can be achieved, and their response would be modulated. Similar to a tissue engineered tendon sheath, the longevity of these strategies should be further explored. One limitation of NFMs in comparison to tissue engineered tendon sheaths is the lack of a lubricating layer which facilitates the function of the repaired tendon, which is the cost for their simplicity.

4. CONCLUSIONS AND FUTURE DIRECTIONS

This article reviewed the electrospun nanofiber-based membranes and novel membranes loaded with therapeutic agents to enhance postsurgical tendon repair and inhibit adhesion formation. The developed membranes were categorized into (i) biomimetic tendon sheaths as well as (ii) physical barriers to avoid the adhesion of a tendon to its surrounding sheath or tissue. To this end, both categories of electrospun membranes are required to mimic structural, biomechanical, and biochemical features of a native tendon-sheath structure and provide various features desired for clinical translation. These features include biocompatibility of an electrospun membrane that enables cells to grow, infiltrate, and proliferate on or into nanofibers and to prevent and minimize inflammation. Biodegradability of the membrane is another critical feature. Electrospun scaffolds should degrade progressively, which allows cells to reproduce the natural collagen. Mechanical properties of electrospun scaffolds should mimic physiological stiffness and strength of the native tendon. Membranes also should have proper porosity for cell infiltration and release of pharmacological or biological agents for antiadhesion.

Importantly, tendon injury is mostly accompanied by the damage of the tendon sheath. Nanofibrous physical barriers, designed to avoid tendon adhesion to the tendon sheath due to scar formation, should have desirable mechanical properties with the effective ability to prevent invasion of peripheral fibrous tissue as well as promote tendon gliding. Synthetic polymers have presented unique features to form the base

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material for biomimetic tendon sheaths owning to their durability and excellent mechanical properties. However, the hydrophobic nature of synthetic polymers and their slow biodegradable rate make them imperative to association with natural polymers, which have a faster degradation rate. The hydrophilic nature could maintain the structural integrity of the membrane with controlled drug release during tendon regeneration. Among the natural biopolymers, the use of HA has been showed to be effective for tissue integration and prevention of adhesions to surrounding tissues. The selection of an appropriate combination of synthetic and natural polymers to fabricate multilayer drug-loaded fibrous membranes has created a promising path for future studies.

Importantly, in delivery of biologics using biomaterials, differentiation of stem cells and macrophages could be directed into desired or undesired phenotypes due to material cues such as elasticity, topography, and surface chemistry.[98] In this way, tendon regeneration may be either promoted or impeded. Material cues also could affect the release of bioactive agents from stem cells. Given this fact, investigation on the effect of material cures along with other biochemical cues on cell-secreted factors such as exosomes may promote new therapeutic pathways.

In particular, animal models with similar human tendon-sheath physiology should be performed to evaluate a series of standard parameters of tendon repair including inflammation, biomechanical strength, and adhesion level. A common achievement among the studies that developed nanofibrous membranes loaded with pharmaceutics and nanoparticles was long-term and sustained drug release followed by overall inhibition of inflammation and reduction of adhesion formation at the site of injury. However, they might have detrimental effects on tendon healing through the reduction of cell proliferation. Given that no adequately effective pharmaceutical-based therapy has been presented, the concept of biological therapy has been proposed. Since tendon cells have poor regenerative potential, biologics-based therapy enables the acceleration of the body's own intrinsic capacity to regenerate and heal the injured tendon. To this end, most recent studies reviewed in this paper investigated the flat and multilayered electrospun membranes as a delivery system for the combination of different biologic-based agents, which is obviously more functional than delivery of single or dual biological molecules and may have more potential for commercialization. These studies incorporated growth factors as natural functional molecules at the repair site, and it has been realized that tendon healing could be accelerated due to the promotion of mitogenesis and angiogenesis. Similar to the delivery of pharmaceutics, outcomes for growth factory delivery are highly dependent on release profile and delivered dosage. Future membranes should allow for customization of loading content, mechanical properties, and the dimensions according to patient needs and the healing site.

For clinical applications, evaluation of human body response including inflammatory and immune reactions to the implanted membranes with therapeutic agents is of the utmost importance. A better understanding of the role of various biomaterial and biochemical cures on immune mechanisms that influence the host response is required to improve clinical outcomes. Owing to this point, gene-based therapeutics for tendon healing that carry high potential to induce host inflammatory and immune responses require precise study considerations and might be difficult for clinical translation.

Another important area that has been less explored is the method of the delivery of these sheath-like barriers. Nanofibrous barriers could not be fixed over tendons using sutures or staples during the surgical procedures. Sutures or staples create abrupt changes in the physical and architectural features of the barrier and surrounding tissue including vasculature inducing adverse inflammation. In addition, sutures and staples as well as thick membranes may impede tendon gliding through tendon pulleys. In addition, since there is a patient-to-patient variability in tendon and defect sizes, the membranes should be cut and adjusted to fit the injury site during the operation. If the membrane is improperly sized, it can affect the host response. The use of *in situ* fabrication devices that can directly fabricate barriers within the patient's body could be an important solution to this pressing need.[99] There has been a trend for the use of *in situ* printers, electrospinning, and blow spinning devices, and it is expected that they can be used to solve the adhesion problem post-tendon-surgeries. Sterilization is also another key factor that should be considered during fabrication and handling of the membranes. Membranes loaded with biologics may require fabrication in a clean room environment, as they cannot be sterilized through the conventional sterilization methods such as irradiation or ethylene oxide exposure.

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Notes

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■ ABBREVIATION

PCL, poly(ε-caprolactone)
DCM, dichloromethane
PLA, polylactic acid
DMF, dimethylformamide
PLLA, poly(L-lactic acid)
MC, methylene chloride
PEG, polyethylene glycol
PDGF, platelet-derived growth factor
PLGA, poly lactic-*co*-glycolic acid
VEGF, vascular endothelial growth factor
PELA, poly(L-lactic acid)-polyethylene glycol
bFGF, basic fibroblast growth factor
HA, hyaluronic acid
MSC, mesenchymal stem cells
CS, chitosan
FDP, flexor digitorum profundus
IBU, ibuprofen
PG, prostaglandins
NSAIDs, nonsteroidal anti-inflammatory drugs
ECM, extracellular matrix
MMS, mesoporous silica nanoparticles
NFM, nanofibrous membrane
DGNs, dextran glassy nanoparticles
T, tendon
AgNPs, silver nanoparticles
AT, Achilles tendon
HFIP, hexa fluoro isopropanol
siRNA, small interfering RNA
Col III, collagen type III
siNC, small interfering negative control
PDA, cationic 2,6-pridinedicarbo xaldehyde-polyethylenimine
ERK, extracellular signal-regulated kinase

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