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Biomaterials for Modulating Lymphatic Function in Immunoengineering

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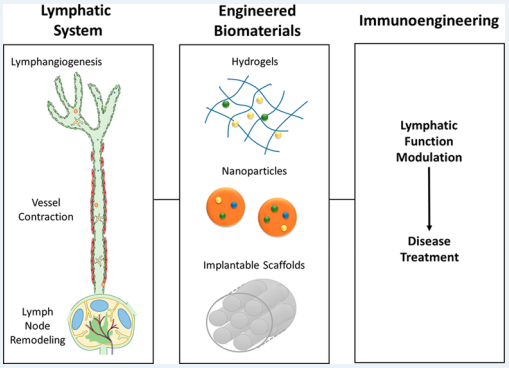
ABSTRACT: Immunoengineering is a rapidly growing and interdisciplinary field focused on developing tools to study and understand the immune system, then employing that knowledge to modulate immune response for the treatment of disease. Because of its roles in housing a substantial fraction of the body's lymphocytes, in facilitating immune cell trafficking, and direct immune modulatory functions, among others, the lymphatic system plays multifaceted roles in immune regulation. In this review, the potential for biomaterials to be applied to regulate the lymphatic system and its functions to achieve immunomodulation and the treatment of disease are described. Three related processes—lymphangiogenesis, lymphatic vessel contraction, and lymph node remodeling—are specifically explored. The molecular regulation of each process and their roles in pathologies are briefly outlined, with putative therapeutic targets and the lymphatic remodeling that can result from disease highlighted. Applications of biomaterials that harness these pathways for the treatment of disease via immunomodulation are discussed.



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The lymphatic system (Figure 1) is a network of vasculature and immune organs that plays critical roles in fluid balance, lipid transport, and in immune cell trafficking and the immune response. While lymph drainage is critical in removing excess fluid from the interstitial space, relieving edema, and maintaining fluid homeostasis, that fluid also contains migratory immune cells[1] and soluble antigen that are transported to downstream lymph nodes (LNs), enabling immune cell interactions and the adaptive immune response. This drainage is critical in a normal immune response, and inhibition of lymphatic function can disrupt both healthy immune responses[2] and the very structure of LNs.[3,4] In addition to enabling lymph transport and subsequent immune responses, lymphatic vessels have direct, multifaceted immunomodulatory roles[5−7] through recruitment of leukocytes and facilitation of cell migration,[8] regulation of T cell homeostasis[9,10] or activation,[11] and dendritic cell modulation.[12] These many functions make the lymphatic system a key component of the immune response and a clear target for immunomodulation.

As the lymphatic system is increasingly implicated in playing important immunomodulatory roles and in regulating disease progression and resolution, interest in therapeutic lymphatic modulation increases; just as disease states modulate lymphatic vessels and LN for their benefit, the lymphatic system can be directed to reverse pathologies and promote therapeutic fluid transport and immune responses. Biomaterials have great potential for such *in vivo* lymphatic modulation. As examples, biomaterials possess surface and mechanical properties that regulate lymphangiogenesis *in vitro*[13,14] and *in vivo*;[15] are used to tightly control the delivery and presentation of pro-lymphangiogenic factors *in vivo*;[16,17] and enable lymphatic-targeted delivery of immunomodulatory agents for the induction of desired immune responses.[18,19] Biomaterials are uniquely valuable for modulating lymphatics in that they enable the delivery of instructive signals to specific lymphatic components, such as vessels or lymph nodes, and can control not only the location of agent release, but also the concentration and context of delivery to precisely regulate the desired response by lymphatic vessels and immune cells alike. This versatile and precise control of the lymphatic response is thus a valuable tool in immunoengineering. Herein, the application of biomaterials to the modulation of lymphangiogenesis, the collecting lymphatic vessel function, and lymph node remodeling for immunomodulation applications are considered.

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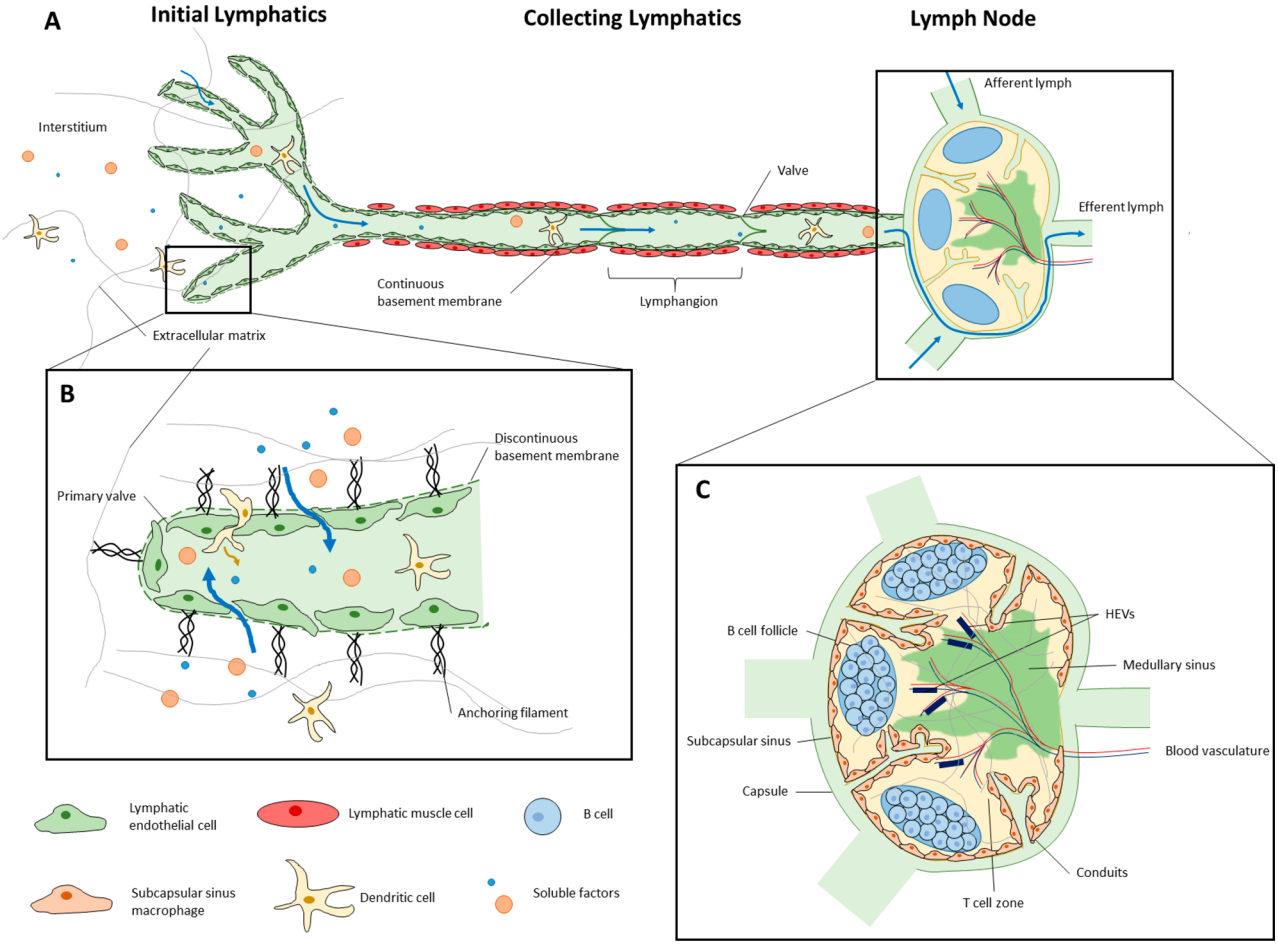

Figure 1. Structure and function of the lymphatic system. (A) Fluid leaves the tissue interstitium and enters initial lymphatic vessels, flowing through larger collecting vessels and the lymph node. Readers are referred to refs 20−23 for an in depth discussion of lymphatic structure and function. (B) Initial lymphatic vessels are composed of overlapping LECs on a discontinuous basement membrane[24] that allow fluid, migratory immune cells, and soluble factors, including nanoparticles in the 20−100 nm size range, to enter the vessels.[25] Lymph is moved away from the periphery through larger collecting lymphatic vessels that are surrounded by a layer of specialized lymphatic muscle cells that produce coordinated contractions to propel lymph downstream. (C) Fluid subsequently flows through lymph nodes (LN), secondary lymphoid organs that house cells of the adaptive immune system and are a critical site of antigen presentation. As lymph flows through the LN to eventually exit via an efferent lymphatic vessel,[22] soluble antigen can be processed by lymph-sampling sinus-lining macrophages. The conduit system allows molecules smaller than 70 kDa to access deeper regions of the LN,[26,27] and LEC-mediated transcytosis facilitates diffusion of antibodies into the LN parenchyma.[28] Antigen-presenting cells (APCs) can carry antigen from the periphery[29] and traffic into the LN to present their antigen to B and T lymphocytes residing in distinct locations within the LN,[22] which then drive the resulting immune response. Cell migration, fluid movement, and antigen transport are all supported by a system of LN stromal cells that provide a scaffold for all critical LN functions to occur.[22,28] The structure of LNs is critical to their function and changes with the immune response.

THE LYMPHATIC SYSTEM IN IMMUNOMODULATION

Lymphangiogenesis. *Molecular Regulators as Therapeutic Targets.* Lymphangiogenesis, or the growth of new lymphatic vessels from preexisting lymphatic vessels, is a tightly regulated process, and an understanding of its regulatory factors is critical to enable directed lymphatic vessel growth. After the generation of the lymphatic system during embryonic development, adult lymphatic vessels are typically quiescent and lymphangiogenesis is primarily observed in disease states such as chronic inflammation,[30] LN remodeling in response to infection or disease, tumor growth and metastasis,[31,32] and wound healing or tissue regeneration.[17] Lymphangiogenesis is primarily regulated by vascular endothelial growth factor receptors and their ligands that also play a critical role in angiogenesis, highlighting the close relationship between the lymphatic and blood vasculature. While angiogenesis, or the growth of new blood vessels, is primarily regulated by VEGFR-1 and VEGFR-2 and their ligand VEGF-A, lymphangiogenesis is primarily regulated by the binding of VEGF-C[33,34] or VEGF-D[35] to VEGFR-3 expressed on LECs. Lymphangiogenesis is also regulated by the epidermal growth factor (EGF) pathway. Epidermal growth factor receptor (EGFR) has been found to be expressed on healthy LECs, where EGF treatment induces lymphatic vessel growth,[36] and on lymphatic vessels in tumor microenvironments where EGF induces lymphangiogenesis and subsequent lymphatic metastasis,[37] making EGF modulation a potentially promising therapeutic for regulating lymphangiogenesis. Other growth factors such as fibroblast growth factors (FGFs),[38] platelet-derived growth factors (PDGFs),[39] and angiopoietin-1[40] have been shown to regulate lymphangiogenesis, and nongrowth factor regulators such as nitric oxide[41] and directional interstitial flow[42] are also important in modulating lymphangiogenesis.

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Table 1. Therapeutic Modulation of Lymphangiogenesis[a]

disease model	mechanism of manipulation	major findings[b]	refs
Inhibition of Pathological Lymphangiogenesis			
corneal transplantation rejection or suture-induced corneal inflammation	VEGFC/D trap	improves graft survival	44−46
heart transplantation rejection	VEGFC/D trap and antibody blockade	inhibits LEC-derived chemokine production and immune cell trafficking; improves allograft survival	47
obliterative bronchiolitis	VEGFC/D trapping	inhibits T cell responses and obliterative bronchiolitis development	48
chemical carcinogenesis in the skin	VEGFC/D trap	fewer tumors and delayed onset; reduced macrophage number and inflammation	49
melanoma and lung, prostate, and bladder cancer xenografts	VEGFC/D trap	suppresses LN and distal metastasis	50−53
tumor xenografts	VEGFC and NRP2 blocking antibody	inhibits distal lymphatic dilation. SMC remodeling, and postsentinel LN metastasis	54
neuroblastoma xenograft	Anti-VEGFD	inhibits lymphatic metastasis	55
breast and gastric cancer	Anti-VEGFR3	suppresses LN and distal metastasis	56−58
orthotopic breast, spontaneous pancreatic cancer	VEGFR3 TK inhibitor	suppresses tumor growth and LN metastasis	59
lung cancer xenograft	TK inhibitor of VEGFR2/3	suppresses tumor growth	60
heterotopic brain cancer and othotopic breast cancer	Anti-NRP2	suppresses metastasis to LNs and lungs	61
breast cancer	Blocking of NRP2-VEGFR3 complex formation	a somatotropin peptide binds to NRP2 and attenuates VEGFR3 signaling	62
pancreatic cancer xenograft	Anti-ephrinB2	suppresses angiogenesis, tumor growth	63
lung cancer xenograft	Anti-ANG2	inhibits tumor growth and LN metastasis	64
breast cancer	SphK1 inhibitor	inhibits tumor growth and LN metastasis	65
breast cancer	Anti-CXCL12	synergistically inhibits metastasis with anti-VEGFC treatment	66
suture-induced inflammatory lymphangiogenesis	recombinant thrombospondin-1	suppresses inflammatory lymphangiogenesis	67
airway inflammation	anti-TNF-α	reduced leukocyte influx, LV remodeling, and LN hypertrophy	68
HNSCC	mTOR inhibition	Suppresses tumor lymphangiogenesis and LN metastasis; improves survival	69
pancreatic cancer	suppresses tumor-derived VEGFC	mTOR inhibitor reduces metastasis	70
breast cancer xenograft	NSAIDs	inhibits VEGFD-induced prostaglandin synthesis, and thereby collecting lymphatic vessel dilation and metastasis	71
melanoma	photodynamic laser therapy	destroys tumor-bearing lymphatic vessels and inhibits metastasis	72
Induction of Therapeutic Lymphangiogenesis			
primary lymphedema	VEGFC application	reduces primary lymphedema in *Chy* mice	73
secondary lymphedema	VEGFC application	reduces surgery-induced lymphedema in rabbits	74
secondary lymphedema	LN transfer and VEGFC application	recovery of lymphatic vessel structure and function	75−78
hypercholesterolemia	VEGFC application	improves RCT	79

[a]Table taken from ref 43 with permission. Copyright 2014 American Society for Clinical Investigation. [b]In addition to inhibition of lymphangiogenesis. HNSCC, head and neck squamous cell carcinoma, TK, tyrosine kinase.

Immunomodulatory Effects of Lymphangiogenesis in Disease. Because of the lymphatic system's critical role in fluid balance, soluble factor transport, and immune cell migration, lymphangiogenesis within peripheral tissues can have significant effects to either help or hinder disease progression depending on context. This makes the growth of lymphatic vessels an enticing target for the treatment of a variety of diseases. See Table 1 for examples of therapeutic modulation of lymphangiogenesis.[43]

Inflammation. Peripheral lymphatic vessels are known to proliferate in chronic inflammatory conditions in a variety of tissues,[30,80] a process called inflammation-associated lymphangiogenesis, and lymphangiogenesis plays a complicated role in inflammatory diseases. Depending on the disease context and timing, lymphangiogenesis can either be critical for the resolution of inflammation or a component of the pathology, as reviewed in-depth elsewhere.[80,81] Along these lines, in some inflammatory disease contexts, lymphangiogenesis enables beneficial immune responses and fluid clearance. In a *Mycoplasma pulmonis* lung epithelium infection model of chronic inflammation, as one example, lymphangiogenesis and lymphatic vessel remodeling at the site of infection are observed alongside LN hypertrophy. Inhibition of tracheal lymphangiogenesis worsened local lymphedema and reduced LN hypertrophy, indicative of an impaired response to the infection, suggesting the therapeutic importance of lymphangiogenesis in this context as it provides a drain for excess fluid and supports increased migration of immune cells to LN in response to the infection.[82] It is important to note that in this model of airway inflammation, LN hypertrophy reduced with antibiotic treatment and inflammation resolution, but newly generated tracheal lymphatic vessels persisted even after the inflammation was resolved.[82] This study thus suggests not only a potential role for lymphangiogenesis enhancement to promote fluid balance and a healthy immune response, but also highlights the importance of treatment timing—in a situation where peripheral lymphangiogenesis is undesirable, preemptive antilymphangiogenic therapy may prevent the growth of new vessels, but those vessels may persist once produced.

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Organ transplantation is an entirely different context in which chronic inflammation-associated lymphangiogenesis is associated with reduced graft survival. In kidney transplantation, early renal lymphangiogenesis has been associated with avoiding acute rejection as it provides early clearance of fluid and immune cells.[83] Chronic lymphangiogenesis, however, appears to worsen outcomes and contribute to the maintenance of inflammation. In studies of organ rejection, lymphangiogenesis has been observed to negatively affect graft survival,[84] primarily by enhancing drainage from the transplant to the draining LN and enabling an immune response; inhibiting lymphangiogenesis has improved outcomes in islet[85] and cornea transplantation[86] and reduced immune cell infiltration in heart transplants,[47] and lymphatic vessels exacerbate inflammation in kidney transplantation.[87] Organ transplantation thus represents a context in which chronic lymphangiogenesis contributes to pathology, and highlights a potential application for antilymphangiogenic therapy.

Cancer and Metastasis. Lymphatic vessels are critical in moving fluid, antigen, and immune cells, but are also employed by cancer cells metastasizing from primary tumors. To take advantage of lymphatic vessels for rapid transport to distant tissues in a low fluid shear stress environment, tumors can invade existing lymphatic vessels or express lymphangiogenic factors, such as VEGF-C[88] or EGF,[37] themselves to promote the formation of tumor-associated lymphatic vessels and increase metastasis.[31] These vessels promote cancer cell transport out of the tumor; interstitial flow can drive cancer cell migration toward lymphatic vessels,[89] and both lymphatic vessels[90] and tumor cells[91] express chemokines that can drive cancer cell chemotaxis into lymphatic vessels and enhance drainage to sentinel LNs, priming them for metastasis and modulating the immune response to tumor growth. This mechanism makes lymphangiogenic pathways a clear target for the prevention of LN metastasis, and interventions have been successfully employed; inhibiting lymphangiogenic molecules, particularly VEGF-C, has been shown to reduce lymphangiogenesis and subsequent LN metastasis by many groups.[51,92]

Though lymphangiogenesis inhibition shows promise in preventing metastasis, the relationship between tumors and their lymphatic vasculature is highly complex. Lymphatic vessels are not merely a route for cancer cell metastasis; in addition, they are responsible for modulation of the tumor immune environment[93] and for the interplay between the tumor and immune system that tumors hijack to bypass the normal immune response. Because this tight relationship between tumors and the immune system is mediated by lymphatic vessels, tumor lymphangiogenesis is actually critical in the efficacy of immunotherapies[94] and inhibiting lymphangiogenesis has been shown to abrogate the tumor response to photodynamic therapy and checkpoint blockade.[95] When targeting tumor lymphangiogenesis, a careful balance between limiting LN metastasis and enabling immunotherapy must be struck, and care taken that lymphatic vasculature alterations induced by treatment do not worsen prognosis by increasing metastasis as has been observed with some chemotherapeutic treatment modalities.[96]

Cardiovascular Disease. The lymphatic system plays a critical role in maintaining the health of the cardiovascular system.[97,98] For example, lymphatic vessel obstruction negatively affects cardiac function, inducing edema and causing altered electrical signaling, ventricular fibrillation, and myofibrillar degeneration.[99−101] Chronic edema can also induce fibrosis in the cardiac interstitium and cause reduced cardiac output,[102] contributing to the risk of heart failure.[97] Modulating lymphangiogenesis to regulate lymph flow and immune cell migration is thus is a potential approach to treat a variety of cardiovascular diseases. For example, lymphatic drainage is critical in mediating reverse cholesterol transport (RCT)[103] in the prevention of atherosclerosis. Enhancing lymphangiogenesis with VEGF-C can improve RCT and reduce cholesterol accumulation,[79] while inhibiting transport via lymphatic disruption impedes clearance of cholesterol-loaded macrophages from tissue.[79,104]

Functional cardiac lymphatic vessels are also critical in recovery from myocardial infarction. Enhanced lymphangiogenesis and remodeling of cardiac lymphatic vasculature has been observed after myocardial infarction,[105] and blocking the lymphangiogenic response through VEGFR3 inhibition impedes cardiac lymphatic transport, resulting in increased inflammation and edema in a postinfarct heart.[16] Enhancing lymphangiogenesis reduces inflammation and fibrosis,[105] and enhancing lymph drainage in the heart after ischemia can reduce tissue damage and prevent infarction development.[106]

Application of Biomaterials for Modulating Lymphangiogenesis. As discussed above, there are a wide variety of approaches to modulating lymphangiogenesis for therapeutic applications (Figure 2). One of the most common approaches

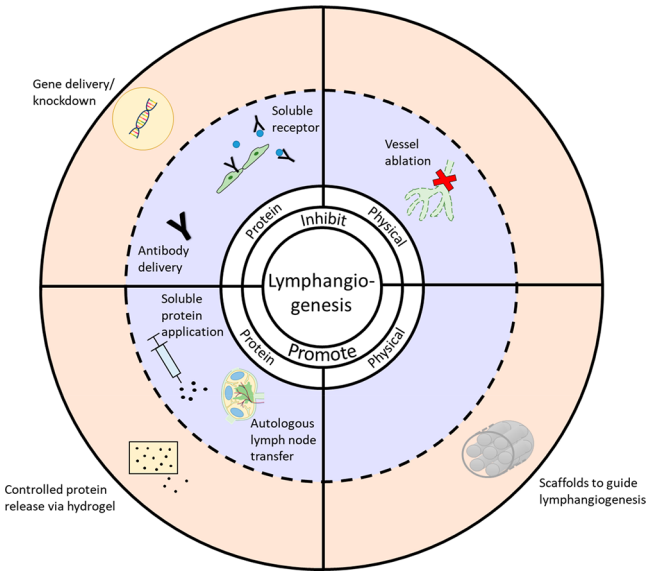

Figure 2. Modulation of lymphangiogenesis. A wide variety of approaches to modulating lymphangiogenesis are in use (blue background) and biomaterials can, in many cases, be used to expand upon and improve those approaches (orange background).

is the regulation of the VEGF-C/VEGFR3 pathway. Lymphangiogenesis can be promoted by the addition of VEGF-C protein itself[79] or by the implantation of autologous, growth factor-producing LNs.[107] Inhibition of this pathway can be achieved through VEGF-C/D trapping through the expression of soluble VEGFR-3 by either adenoviral gene delivery[47] or through delivery of anti-VEGFR-3,[94] VEGF-C neutralizing antibodies, or small molecule pathway inhibitors.[16] Other growth factor pathways can be modulated using similar approaches.[36] Though these options are feasible for research purposes, they have translated poorly to the clinic. Viral gene delivery poses a variety of challenges in patients,[108] and the use

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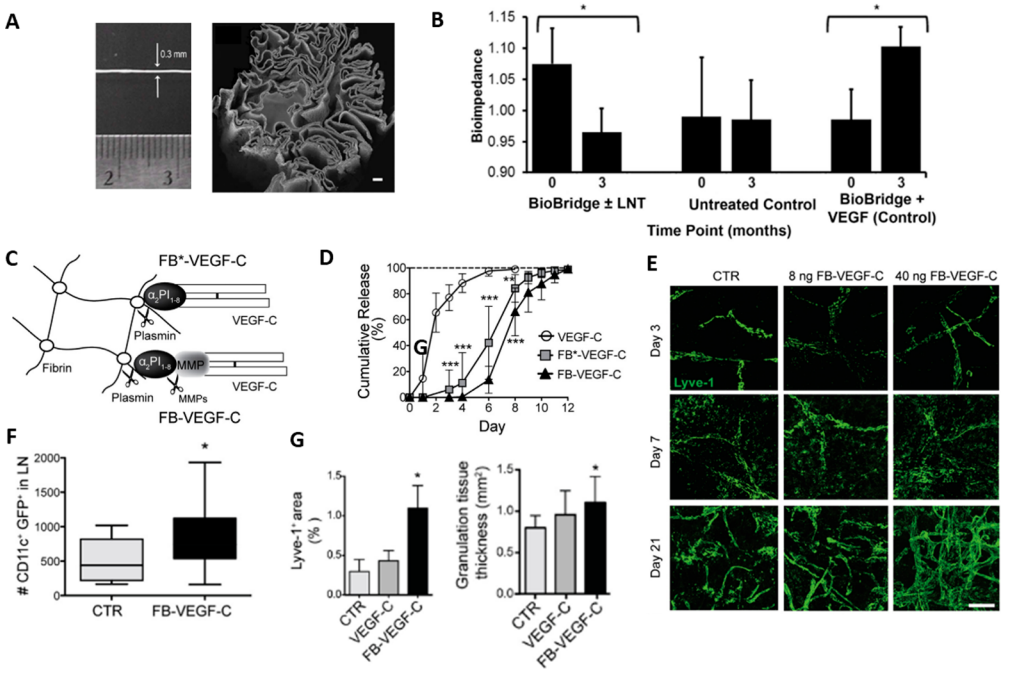

Figure 3. Biomaterials for promoting lymphangiogenesis. (A) Hadamitzky et al. implanted nanofibrillar collagen scaffolds into a porcine model of lymphedema, (B) and observed that only when the scaffolds were delivered with or without exogenous LN transfer was there any measurable reduction in interstitial fluid volume, as measured by bioimpedance. Adding exogenous VEGF-C to the implanted scaffold negated any benefit of the scaffold. (C) Guc et al. developed plasmin-cleavable VEGF-C-releasing hydrogels that (D) release their VEGF-C payload in a controlled fashion, compared to rapid release of free protein, *in vivo*. (E) The hydrogel induces significant expansion of LYVE1+ vessels (green) in a dose-dependent fashion and (F) increases leukocyte trafficking to LNs. (G) In a diabetic wound model, the FB-VEGF-C hydrogels enhanced lymphangiogenesis and improved wound healing, as measured by increased granulation tissue. Panels A and B adapted with permission from ref 15. Copyright 2016 Elsevier. Panels C−G adapted with permission from ref 17, licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Copyright 2017 Elsevier.

of protein or small molecule regulators raises issues with maintaining spatial and temporal control over delivery. Off-target delivery of therapeutics can negatively impact nontarget tissues or related pathways, resulting in side effects,[109,110] and their nontargeted delivery and short half-life can necessitate repeated treatment or increased dosage.

As interest in modulation of lymphangiogenesis grows, the role of biomaterials in overcoming challenges of traditional therapeutic approaches is becoming increasingly evident. There has been significant research into the use of biomaterials to induce lymphangiogenesis in peripheral tissue for the treatment of a wide variety of pathologies that could benefit from improved lymph flow or a resolution of inflammation. While biomaterial surface and mechanical properties alone have been shown to regulate lymphangiogenesis, biomaterials are often combined with cells or pro-lymphangiogenic growth factors to enhance vessel growth *in vivo*. These approaches have been shown to enhance lymphangiogenesis and can be used to treat a variety of pathologies, including lymphedema and chronic wounds.

There is great interest in how surface and physical properties of biomaterials directly (e.g., in the absence of coformulated/delivered growth factors) influence LEC proliferation, sprouting, and orientation. BECs and LECs have been observed to proliferate and form networks with architecture similar to the *in vivo* condition preferentially on different substrates; in hydrogel matrices with cleavable matrix-bound VEGF-C, BECs form vessels preferentially in collagen-containing matrices, while LECs form capillary networks with nearly physiological structure preferentially when implanted in a flexible fibrin matrix.[13] However, LECs can be cultured on a wide variety of hydrogel substrates, including gelatin,[111] matrigel,[112] fibrin,[13,113,114] collagen,[13,15,114] and hyaluronic acid.[115] Hydrogels are not a requirement for LEC growth, however. Rigid polyglycolic acid (PGA) tube scaffolds have been employed for *in vitro* LEC culture, and generated a tubular structure that obtained a lymphatic-like phenotype when transplanted *in vivo*.[116] The surface properties and fiber alignment of electrospun biomaterial scaffolds have also been observed to regulate the migration and alignment of LECs, driving their orientation in the direction of fiber alignment *in vitro* and maximizing migration on fibers of a particular size and density.[14]

These principles have been put into practice in the clinic for the treatment of secondary lymphedema. BioBridge by Fibralign Corporation is a nanofibrillar collagen scaffold, a thread-like structure with aligned layers that provide a large surface area for cell attachment and migration (Figure 3A. These scaffolds were found to guide the cytoskeletal organization of endothelial cells[117] and LECs[15] in the direction of the collagen fibers. When implanted in a porcine lymphedema model, the BioBridge scaffold enhanced lymphangiogenesis and the generation of lymphatic collecting vessels, improving lymphatic function and fluid clearance even without the addition of exogenous mediator VEGF-C (Figure 3B).[15] BioBridge is currently in clinical testing, highlighting the potential for modulating LEC migration, proliferation, and lymphangiogenesis by careful selection of optimal biomaterials, even without the presence of molecular mediators of lymphangiogenesis.

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Table 2. Regulators of Lymphatic Vessel Contraction

regulator	system	effect	refs
NO (iNOS)	mouse hindlimb lymphatics	overwhelms NO gradient, reducing lymphatic contraction strength	133
NO(eNOS)	mouse hindlimb lymphatics	eNOS inhibition or knockout reduces contraction strength and increases frequency	133
	mouse tail lymphatics	inhibition or knockout reduces lymph velocity through effects on collecting lymphatics	136
NO (exogenous)	rat tail lymphatics	addition of GTNO reduces lymph flow through reduced contraction frequency and effective contraction length	134
prostaglandins	rat iliac lymph node afferent vessel	vessel dilation (PGE_2) or vessel constriction	137
	bovine mesenteric vessel	increased contraction frequency and amplitude (PGA_2 and PGB_2) or decreased contraction amplitude (PGE_2; and PGI_2)	138
bradykinin	rat mesentery	increased contraction frequency and pump flow index	139
histamine	guinea pig mesentery	increased contraction frequency and decreased contraction amplitude	130
	guinea pig mesentery	increased contraction frequency and decreased contraction amplitude	140

In addition to the promotion of lymphangiogenesis by material selection alone, significant work has been done to combine biomaterials with additional pro-lymphangiogenic factors, such as cells, growth factors, and small molecule drugs, to further enhance lymphatic vessel growth, and these systems can be applied to a wide variety of diseases. Significant work, for example, has been done in using implanted hydrogels delivering exogenous growth factors, particularly VEGF-C, for the enhancement of lymphatic vessel growth. Such hydrogels provide unique advantages for enhancing peripheral lymphangiogenesis: they are easily modifiable; can contain free or tethered growth factor at controllable concentrations,[118] in controllable locations,[16] and in combination with other factors; have controllable stiffness; are often biodegradable; and can be injectable for ease of delivery.[118]

It has been shown that LEC proliferation and sprouting *in vitro* are most improved when cells are exposed to a constant release of VEGF-C; accordingly, alginate hydrogels that gradually release VEGF-C significantly improve LEC sprouting in a chick chorioallantoic membrane (CAM) assay.[118] Similar VEGF-C-releasing hydrogel systems have been employed to treat a wide variety of pathologies *in vivo*. For example, implantation of a VEGF-C-releasing gel at the site of mouse hindlimb lymphatic injury has been shown to induce lymphangiogenesis and reduce hind paw edema in combination with extracorporeal shock wave therapy.[111] When combined with human adipose-derived stem cells, the gel reduced edema and increased density of LYVE-1 positive lymphatic vessels,[119] making this system a promising approach for the treatment of lymphedema. Similar systems have also been used to enhance wound healing. A fibrin/collagen hydrogel containing fibrin-binding VEGF-C improved wound healing in a diabetic mouse model after subcutaneous implantation; the VEGF-C variant released from the hydrogel by fibrin cleavage by infiltrating immune cells (Figure 3C,D) induced local stimulation of lymphangiogenesis (Figure 3E) and enhanced leukocyte trafficking (Figure 3F that was not observed with free VEGF-C, resulting in increased lymphangiogenesis and granulation tissue production when implanted in diabetic animals (Figure 3G).[17] VEGF-C-releasing hydrogels have also been applied to the heart after myocardial infarction, and found to reduce inflammation, edema, and fibrosis while improving postinfarct function.[16] VEGF-C released from such hydrogels may not only act directly on lymphatic vessels through activation of VEGFR-3 on LECS, but may also promote lymphangiogenesis by acting on myeloid cells, which have been shown to express VEGFR-3 in the context of cancer and inflammation[120] and to incorporate into lymphatic vessels[121] during inflammation-associated lymphangiogenesis.

Biomaterials have also been employed to induce anti-lymphangiogenic effects in the context of graft rejection and cancer, primarily through the use of nanoparticle (NP) gene delivery systems. The delivery of plasmids expressing a VEGF-binding recombinant construct enhanced survival of corneal grafts by reducing angiogenesis and lymphangiogenesis,[122] and the delivery of anti-VEGFR-3 siRNA in polyethylenimine-alginate nanoparticles[123] or anti-VEGF-C siRNA in calcium carbonate nanoparticles[124] has been shown to inhibit lymphangiogenesis and reduce tumor growth and metastasis, respectively. While biomaterials-based approaches to directly delivering molecular regulators of tumor vascularization are currently mostly limited to antiangiogenic regulator delivery, additional nanoparticle systems specifically targeting lymphangiogenesis may be employed since the role of lymphangiogenesis in cancer progression is continually clarified and antilymphangiogenic therapeutics are approved for clinical use.

Lymphatic Vessel Contraction. *Molecular and Cellular Regulation.* The contraction of collecting lymphatic vessels is critical to the function of the lymphatic system, and its natural regulatory mechanisms provide many targets for engineering vessel function. The intrinsic contraction of lymphatic vessels is granted by lymphatic muscle cells (LMCs), which share features with both striated cardiac muscle and vascular smooth muscle.[125] LMC contractions are regulated by the flux of ions across many ion channels[126−128] and by a variety of inflammatory mediators (Table 2), a critical point due to the lymphatic system's important role in the inflammatory response. Histamine, secreted by immune cells during inflammation, drives lymph formation and flow both by increasing capillary permeability and subsequent interstitial pressure[129] and through direct effects on lymphatic muscle cells.[130] Arachidonic acid metabolites are also critically involved in lymphatic pumping regulation; prostaglandins have been observed to induce lymphatic contractions, thromboxane synthesis inhibition suppresses contraction,[131] and leukotriene B_4 antagonism improves lymphatic function and inflammation in a mouse tail lymphedema model.[132]

Perhaps the most well-studied regulator of lymphatic contractions is nitric oxide (NO), a reactive small molecule known for its inflammatory roles and its vasoactive effects in the blood vasculature that plays complex roles in lymphatic pumping. LECs express endothelial nitric oxide synthase (eNOS), producing a basal level of nitric oxide required for normal lymphatic pumping; when eNOS is inhibited[133] or exogenous NO is added,[134] normal pumping function is

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disrupted. NO production is thought to be tightly regulated both spatially and temporally within lymphatic vessels, with the pulsatile flow of lymph in vessels driving transient, cyclical production of NO, especially in the region of secondary valves, that supports coordinated contraction.[135]

Immune Regulation of Vessel Contraction in Disease. Inflammation and Cancer. Lymphatic vessel contraction is regulated not only by lymphatic vessels themselves, but also by immune cells in the context of inflammation, further complicating the relationship between lymphatic function and inflammation resolution. Histamine secreted by mast cells causes increased contraction frequency and decreased amplitude, altering lymphatic pumping.[140] Nitric oxide is also a critical regulator of lymphatic pumping during the inflammatory response, when inducible nitric oxide synthase (iNOS) expressed by $CD11b^{+}Gr\text{-}1^{+}$ myeloid-derived suppressor cells (MDSCs) in inflamed tissue overwhelms the natural NO gradient produced by LEC eNOS, resulting in inhibited lymphatic contraction and reduced antigen delivery to draining LNs from the periphery.[133] Tumors can also regulate collecting lymphatic vessels to their advantage, producing VEGF-D that regulates prostaglandin production and causes dilation of tumor-associated collecting lymphatic vessels, resulting in increased LN metastasis.[71] Normal collecting vessel function is thus hugely important in the immune response, and altered vessel function in the context of inflammation or tumorigenesis can have significant effects.

Lymphedema. Lymphedema occurs when lymphatic drainage is insufficient to maintain physiological interstitial fluid volumes, leading to edema and structural changes in the affected tissue.[141] In primary lymphedema, lymphatic vessels do not properly develop to transport homeostatic levels of fluid, often as a result of mutations in genes critical for lymphatic development,[142] such as VEGFR-3[143] or FOXC2.[144] Secondary lymphedema, the more common manifestation of the disease, occurs due to some postnatal insult, such as surgical LN removal in breast cancer treatment[145] or filariasis.[146] The contraction of lymphatic vessels in patients with lymphedema is often impaired. Lymphedematous legs show irregular contractions too weak to propel lymph,[147] and lymphatic congestion lymphoscintigraphy measurements reveal lymphatic pump failure in patients with arm lymphedema after breast cancer surgery.[148] Inflammation can also disrupt lymphatic vessel contractility, particularly through the production of iNOS by immune cells surrounding vessels,[133] and inflammatory disease is often associated with lymphedema.

Metabolic Disorders. Obesity is a significant risk factor for secondary lymphedema,[149,150] and studies have shown that lymphatic function is impaired in obesity.[151,152] These effects are mediated in part by iNOS-producing inflammatory cells surrounding lymphatic vessels, and lymphatic vessel function can be restored by iNOS inhibition or the suppression of perilymphatic T cells.[153]

The barrier integrity of collecting lymphatic vessels also plays a significant role in the immune response, and abnormal vessel permeability can contribute to disease. Though collecting lymphatic vessels are typically not considered permeable to migrating immune cells, lymphatic vessel permeability allows the leakage of lymph and its components into adipose tissue surrounding lymphatic vessels and enables antigen exposure to the dendritic cells associated with lymphatic collecting vessels.[154] Collecting vessel permeability is also altered in several pathologies, particularly in metabolic diseases. In diabetic mice, for example, collecting vessels are leakier than in wild type vessels, and can be rescued by increasing NO signaling.[155] Similar functional deficiencies have also been implicated in hypercholesterolemia and atherosclerosis, making the barrier function of collecting vessels critical in maintaining normal physiology.

Biomaterials Regulating Collecting Lymphatic Function—Opportunities and Potential. In spite of the clear importance of physiological collecting vessel function in the movement of lymph, avoidance of disease development, and the immune response, the employment of biomaterials to specifically enhance collecting vessel function *in vivo* has remained largely unexplored. This may be significantly due to the growing state of the field—the contractile physiology and regulators of vessel barrier function are poorly understood, and more is learned every day about the tight relationship between lymphatic vessels and cells of the immune system. Because of this, many studies on improving vessel function currently involve molecular mediators delivered through topical application, local injection, or systemic delivery. For example, when NO is used therapeutically to modulate lymphatic vessel function it is often applied directly to the site of action through topical application[134,156] or inhalation;[157] these local delivery approaches, however, may be insufficient for the modulation of vessels that are not superficial and accessible. Additionally, many of the regulators of lymphatic vessel pumping and permeability are small molecules (NO, prostaglandins, histamine, etc.), the biodistribution of which can be very challenging to control due to their blood permeability. These molecules are also often involved in other critical physiological functions,[158,159] especially in the blood vasculature due to physiological similarities between lymphatic and blood vessels, and systemic delivery could result in side effects due to action in off-target tissues. If small molecule mediators of lymphatic vessel contractility are to be employed therapeutically, sufficient concentrations must be achieved in target tissues while off-target delivery should be avoided to limit side effects, a balancing act that is challenging to manage.

To this end, biomaterials could be valuable in solving this problem as more lymphatic-targeted, controlled drug delivery systems are developed. This could be achieved through conjugation to a nanoparticle, or even *in vivo* association with a native protein, such as albumin,[160] that has access to lymph. This approach is fairly common for targeting drugs to LNs, and has recently been explored for the delivery of NO to lymphatic tissues.[161] When NO-loaded nanoparticles are injected in the skin, they create a depot that slowly drains into lymphatic vessels while releasing their drug payload in a lymphatic-targeted fashion that enhances the delivery of NO to lymphatic tissues. While NO accumulation was primarily measured in the LN, nanoparticles are presumably also releasing NO in collecting lymphatic vessels en route to the LN that could modulate lymphatic vessel pumping and lymph flow. This may be true for many drugs delivered in this fashion.

While NP-based systems enable the uptake of a small molecule payload into lymphatic vessels, additional challenges with vessel targeting may arise due to low retention time of NPs within lymphatic vessels. Once a nanoparticle leaves the interstitium and enters the lymph, it transits through the lymphatic vessel over the time scale of minutes,[162] to pass through draining LNs and eventually be returned in efferent vessels to the blood circulation at the thoracic duct. During this brief time the particle may only release a fraction of its payload,

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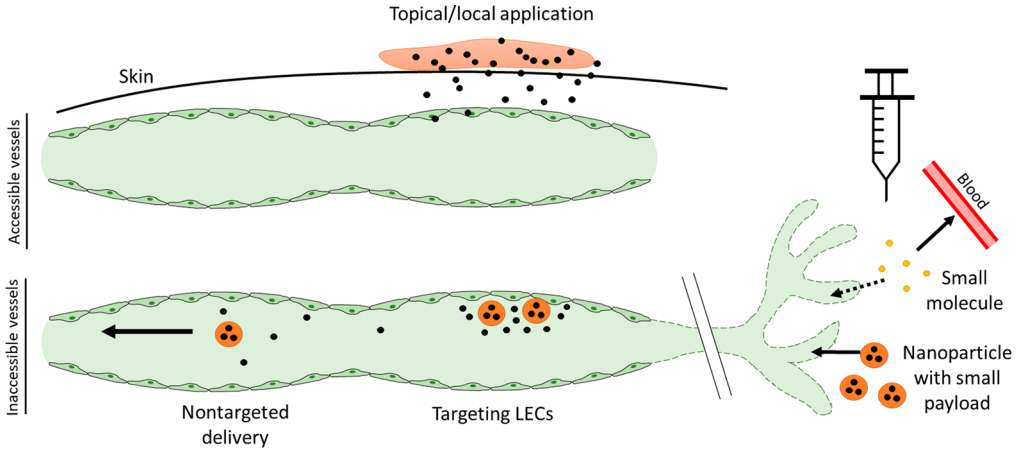

Figure 4. Limitations and future directions for modulating lymphatic collecting vessel function. While local administration of small molecule therapeutics is useful in situations where lymphatic vessels are readily accessible, injection of small molecules can result in poor lymphatic uptake and systemic distribution. Employing lymphatic-draining nanoparticles that release a small molecule payload within lymphatic vessels could overcome this limitation, and targeting the nanoparticles to LECs could help ensure local release of drug and a higher concentration within the collecting lymphatic vessel.

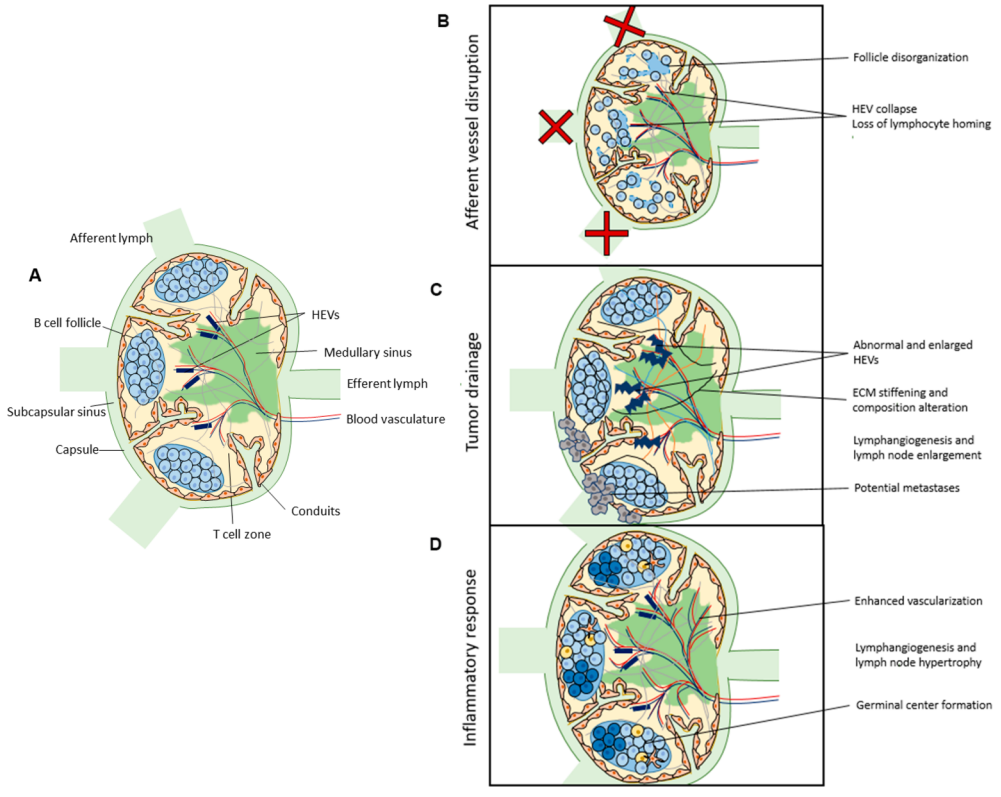

Figure 5. Lymph node remodeling in disease. (A) The biophysical organization of the LN remodels in the context of disease, including (B) afferent vessel disruption, (C) tumor drainage, and (D) inflammatory response.

and any payload retained within the drug delivery system can no longer target the collecting vessels. To enhance the retention time of drug delivery systems within collecting lymphatic vessels and create a drug depot within lymphatic vessels, drug delivery systems with LEC targeting moieties could be employed. While this approach is mostly unexplored in a therapeutic context, it has been used for lymphatic imaging. Metallic nanoparticles have been conjugated to antipodoplanin antibody for imaging of breast cancer lymphatic vessels,[163] and lectins have been used to visualize blood and lymphatic vasculature.[164] Using similar targeting approaches for more localized and controlled drug release in lymphatic collectors is a promising tool for the treatment of disorders of lymphatic pumping, and could be broadly applied in inflammation and lymphedema (Figure 4).

Lymph Node Remodeling. *Regulation and Impact on Immune Response.* LNs are complex secondary lymphoid organs that are critical in the immune response; they have particular structure defined by precise distribution of immune cells, extracellular matrix (ECM), blood and lymphatic vessels, and supportive stromal cells.[22] LN biophysical remodeling, or changes in the organization of the LN, occurs in a variety of

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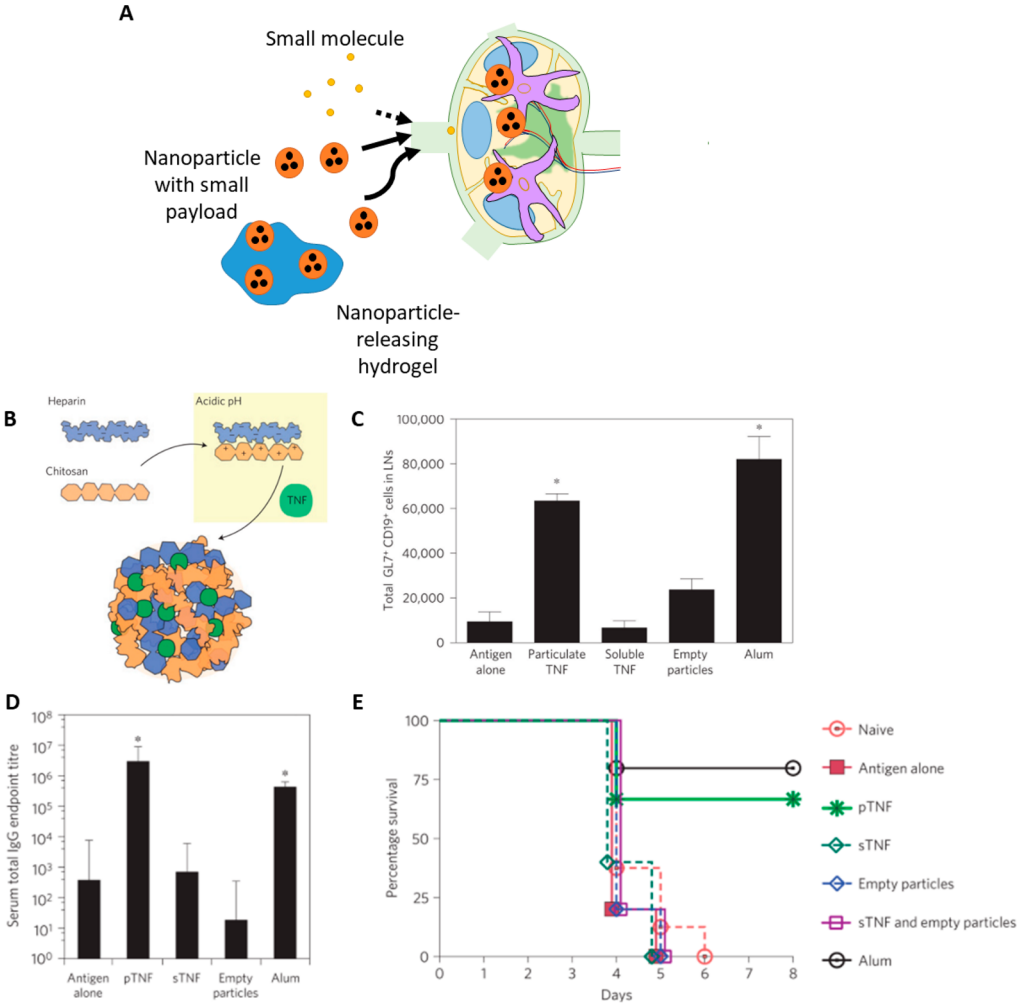

Figure 6. Biomaterials enable LN-targeted drug delivery to regulate LN remodeling and immune response. (A) While small molecules and proteins may poorly drain to lymph and yield low concentrations at target immune cells, nanoparticle formulations can enhance lymphatic drainage, enhance uptake by target immune cells, and precisely deliver combinations of payloads. (B) St. John et al. developed artificial mast cell granules composed of heparin that could be loaded with TNF-α and other drugs. (C) These synthetic granules increased germinal center formation, as measured by the total number of germinal center B cells, compared to controls. (D) After vaccination with hemeagglutinin, granule delivery resulted in improved antibody titers and (E) protected mice from lethal flu challenge. Panels B–E adapted with permission from ref 18. Copyright 2012 Springer Nature.

situations, both healthy and pathological, and includes physical changes in LN structure, such as LN size, stiffness, and matrix composition;[165,166] cellular changes as cells migrate and proliferate; and chemical changes as chemokine and cytokine distributions are altered. The organization of these components regulates lymph flow, antigen, and chemokine distribution within the LN, and adhesion, migration, and activation of immune cells. Because the structure of the LN is so tightly tied to the activation, behavior, and location of the cells within it, it follows that changes at the level of the LN can have drastic effects on the subsequent immune response.

Physical features of the LN, such as stiffness, pressure, and size, are regulated by the cells of the LN, both stromal and immune.[167] Of particular interest in this context are fibroblastic reticular cells (FRCs), supportive cells that produce extracellular matrix components such as collagen[168] and are critical in enabling LN elasticity during expansion.[169] LNs can also remodel with respect to the organization of resident cells. Antigen-presenting cells (APCs) must migrate to interact with B and T cells for the induction of adaptive and humoral responses; B cells migrate and proliferate during the formation of germinal centers;[170] FRCs remodel and migrate to enable LN expansion;[171] and lymphocytes must migrate extensively through the LN during circulation. All of these migratory processes and the maintenance of distinct LN regions are governed by complicated chemokine gradients produced by LN stromal cells, and disruption of these pathways in disease can have predictably significant consequences on immune cell organization and the immune response.

Lymph Node Remodeling in Disease. Cancer. Significant biophysical remodeling is also observed in malignancies (Figure 5); tumor-draining LNs (tdLN) that receive lymph and thus also lymph-borne soluble antigen and tumor-associated factors from the tumor interstitium are a striking example (Figure 5C). Stromal cells play an important role in tdLN remodeling.[172,173] For example, BECs, LECs, and FRCs in LNs draining melanoma tumors proliferate as tumors develop,[173] and altered chemokine production by FRCs contributes to disorganized immune cell distribution and altered immune cell profiles.[173] tdLN show enhanced expression of genes regulating ECM deposition,[174] and LNs

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draining melanomas are enlarged and stiff compared to naïve LNs with high intranodal pressure and altered ECM composition, including increased collagen and hyaluronic acid content,[165] changes that are simultaneously observed in the tumor microenvironment during tumor development. These changes in stiffness and ECM density and composition may limit immune cell mobility and recruitment, and limit the immune response to the upstream tumor.

Vascular remodeling is also observed in tdLN; high endothelial venules (HEVs) are observed to remodel in LNs draining tumors, enlarging and becoming abnormal particularly in patients with LN metastases,[175] and tdLN displayed enhanced BEC proliferation and functional vascularization, measurably enhancing blood flow within the LN.[176] LN lymphangiogenesis is also enhanced in the context of nasopharyngeal carcinoma, where an increase in the number of LYVE1+ vessels and dilation of those vessels is observed.[176] Together, these changes are postulated to prime the LN for metastasis. Tumor drainage to LNs thus significantly alters LN organization, ECM, and vascularization, potentially altering the LN's ability to mount an immune response to the growing tumor or priming the lymph node for subsequent metastasis.

Inflammation/Lymph Node Expansion. LNs must expand significantly in a normal adaptive immune response, increasing in size in a reversible fashion without permanent damage to LN structure (Figure 5D). During this expansion, FRCs, BECs, and LECs all proliferate[177] and lymphocytes are recruited to the LN,[178] resulting in notably enhanced LN size and cellularity. During infection, LEC expansion results in an increase in the number of LYVE1+ vessels within the LN,[177] and LN sinus expansion is induced by VEGF-A distributed by interstitial flow alongside FRC expansion and remodeling.[178] B cells of inflamed LNs have also been shown to induce lymphangiogenesis to enhance the influx of antigen-presenting DCs from the periphery.[179] FRC network remodeling is critical in enabling LN size change, as FRC interaction with C-type lectin receptor 2 (CLEC-2) on migratory dendritic cells relaxes the FRC cytoskeleton and enables its stretching.[169] This FRC remodeling maintains the size restriction of molecular access to conduits but enhances conduit permeability, allowing soluble molecules in the conduits improved access to antigen presenting cells and advancing the immune response.[180] LN vasculature also undergoes remodeling—BECs proliferate[177] and the LN feed arteriole expands to recruit additional lymphocytes from circulation.[181] These observed changes in physical structure and cellular properties and localization are critical to the normal immune response.

In disease and chronic inflammation, these normal processes can be interrupted. In HIV infection, for example, CD8+ T cells express high amounts of TGF-β1, inducing the deposition of collagen and subsequent LN fibrosis;[182] this fibrosis is associated with damage to the LN T cell zone and limitation of CD4+ T cell populations.[183] Enhanced collagen deposition and LN stiffening is observed in LNs draining melanoma tumors,[165] and LN fibrosis associated with poor lymph flow through nodes has been observed in lymphedema patients.[3,184,185] Fibrosis associated with such chronic inflammation and pathogen exposure can inhibit future immune responses and response to vaccination.[166]

Impaired Lymph Flow. Lymph flow through LNs from afferent lymphatic vessels is critical for the maintenance of normal LN structure (Figure 5B). Disruption of the afferent lymphatic vessels destroys the physical structure of the LN, causes the collapse of HEVs, and eliminates lymphocyte homing to LNs from the vasculature.[4] In mice lacking dermal lymphatic vessels, and thus normal drainage to dermal LNs, LNs show similarly minimized HEVs, along with disorganized FRCs and scattered B cells, perhaps due to altered distribution of CXCL13,[2] a chemokine that guides B cell organization. In lymphedema, LNs have been observed to show increased collagen and hyaluronic acid deposition, fibrosis, and lymphocyte depletion.[3] These observations together highlight the importance of fluid flow through the LN in regulating the structure, and thus subsequent immune response, of the LN.

Biomaterials for Therapeutic Modulation and Remodeling of Lymph Nodes. Biomaterials enable targeted drug delivery to LN for the induction of therapeutic or investigative remodeling (Figure 6A). By taking advantage of size-based access of molecules to lymphatic vessels, and thus the LNs, biomaterials can be used to enhance the delivery of small molecules, antigen, cytokines, and other modulatory agents to the LN to regulate remodeling and the subsequent immune response. NP carriers from 20 to 100 nm in diameter drain primarily to lymphatic vessels after injection in the interstitial space as they are too large to enter the pores of the blood vasculature, with molecules on the smaller end of the range showing improved lymphatic uptake compared to larger molecules.[186] Once in the LN, NPs can modulate the immune response by delivery of antigen, immunogenic agents, or tolerogenic agents. NPs are well-suited for delivery to the APCs that are critical in generating both immunity and tolerance, as APCs readily capture nanoscale particulates. Co-delivery of antigen and drugs by NPs thus promotes the simultaneous exposure of APCs to antigen and immunity- or tolerance-promoting agents to elicit the desired immune response.

Particle systems have thus been employed for LN-targeted delivery of adjuvants and antigen to generate immunity. For example, synthetic particles designed to mimic mast cell granules have been developed.[18] Mast cell granules are TNF-containing particles secreted by activated mast cells in peripheral inflammation. These particles easily enter lymphatic capillaries and carry their TNF payload to the draining lymph node, where they induce significant lymph node hypertrophy.[187] Synthetic mast cell granules can recapitulate the properties of native granules; these heparin-based, TNF-loaded particles (Figure 6B) drained to LNs and induced LN remodeling and germinal center formation with increased numbers of activated B cells (Figure 6C) and improved antibody titers (Figure 6D), and showed improved adjuvant activity compared to free TNF, protecting treated mice against lethal flu challenge after vaccination (Figure 6E). Not only were these particles effective adjuvants, but they could also be loaded with IL-12 to polarize the resulting immune response toward a Th1 phenotype,[18] highlighting the potential for simultaneous delivery of multiple cytokines to elicit LN remodeling and a desired immune response. Particle systems can also be used to codeliver an antigen along with stimulatory cytokines for the enhancement of the immune response. Multilamellar lipid vesicles have been used to codeliver VMP001 malaria antigen and monophosphoryl lipid A (MPLA) to LN, and induced improved LN remodeling in response to vaccination. These particles promoted germinal center formation where they accumulated in LNs, while soluble antigen delivery did not, and simultaneously increased proliferation of follicular helper T cells. They also generated a

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broader humoral response at a lower dose compared to soluble antigen.[188]

Other biomaterials can be used for the induction of tolerance. In one group, polymer microparticles that are retained in the LN upon intra-LN injection were loaded with myelin oligodendrocyte glycoprotein (MOG), a myelin peptide, and rapamycin, a common immunosuppressant, and delivered to LN in a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE).[189] While particles loaded with rapamycin alone did not improve disease symptoms, codelivering both MOG and rapamycin resulted in systemic tolerance and reduced disease severity. The delivery of the particles to LN was critical in the improved outcomes; when MOG/rapamycin-loaded particles were injected intramuscularly rather than intra-LN, no clinical changes were observed, highlighting the importance of LN localization in the generated response.[189] A polymeric NP system has also shown promise for tolerance induction when used to codeliver rapamycin and disease-specific antigen.[19] Upon subcutaneous injection, the NP drain to LN and promote tolerance that alleviates symptoms in a variety of diseases, including hypersensitivity and EAE. Importantly, tolerance was only induced in the presence of encapsulated rapamycin; when antigen-loaded NPs were delivered with free rapamycin, the tolerogenic effects were lost, highlighting the importance of context and codelivery.[19] Other biomaterials systems besides polymeric NPs have been used to deliver antigen alongside other tolerogenic drugs to suppress immune responses; for example, liposomes loaded with antigen and NF-κB inhibitors have also been delivered to LNs, where they are taken up by antigen-presenting cells and induce antigen-specific regulatory T cells to reduce symptoms of arthritis in mice.[190]

Similar approaches can be employed to deliver LN-targeted therapeutics for the activation of an anticancer immune response. One group employed a silica NP to codeliver CpG DNA and OVA antigen to LNs and observed significantly improved generation of antigen-specific T cells compared to soluble administration, in addition to improved humoral response. The NP formulation induced a protective response against an EG.7-OVA lymphoma, and reduced systemic CpG effects as evidenced by a reduction in spleen size.[191] Polymeric NPs made of Pluronic F127 and poly(propylene sulfide) have also been employed for this purpose; when loaded with adjuvanting Toll-like receptor 4 and 9 ligands and injected in the limb ipsilateral to a B16−F10 melanoma (hence resulting in payload accumulation within LNs codraining the melanoma), they promoted dendritic cell maturation and the generation of antigen-specific T cells that reduced tumor size compared to free adjuvant.[192]

Particle systems have also been developed for the delivery of chemokines to modulate immune cell migration. Because cell migration is based on chemokine gradients, bulk release of chemokine may be insufficient to induce the desired trafficking. Particle delivery can overcome this by providing sustained release and producing necessary gradients of chemokines to mediate cell attraction *in vitro*[193] and *in vivo*.[194] This approach has been used to induce neutrophil recruitment to LN.[195] Hydrogel NPs were synthesized that codelivered interleukin 8 and macrophage inflammatory protein 1α. Upon subcutaneous injection, the particles were taken up into lymph and rapidly accumulated in draining LNs where they induced remodeling, causing subcapsular sinus expansion and an influx of neutrophils to the site, while chemokines delivered without the particle carrier showed a much reduced effect,[195] highlighting the potential advantages in targeted, sustained release of chemokines that biomaterials can provide.

Peripherally implanted hydrogels can also be employed in drug delivery for LN remodeling in several contexts. Hydrogels are an excellent tool for providing sustained, local release of drug. Hydrogels can thus be employed as depots for sustained release of lymphatic-draining nanoparticles or molecules, providing spatially controlled delivery of drug-loaded nanoparticles for draining LN remodeling over longer time periods than a single injection of nanoparticles could provide. In one example, pH-sensitive polymeric nanogels containing 50 nm particles loaded with Toll-like receptor 7/8 agonist IMDQ were injected in the mouse footpad.[196] Following, 50 nm particles were slowly released from the nanogel and delivered to the draining LN, where they improved the immune response to tuberculosis antigen. Filomicelle scaffolds can also be employed for this purpose,[197] converting slowly to lymphatic-draining micellar delivery vehicles during gradual oxidation. The released micelles accumulate in LNs draining the site of injection and are taken up by LN-resident APCs. The use of technologies like these could further enhance current LN-targeted drug delivery systems by enabling the controlled release of NP drug carriers, removing the need for repeated bolus injections.

In addition to biomaterials designed to remodel existing LNs through LN-targeted drug delivery, biomaterials may also be implanted to generate new LN-like tissue. While the generation of LNs *in vitro* has not yet resulted in implantable lymphoid structures, lymphoid tissues have been generated *in vivo* using biomaterials approaches. The implantation of collagen scaffolds seeded with dendritic cells and thymic stromal cells expressing lymphotoxin alpha (LTα), a critical regulator of lymphoid tissue development, resulted in the formation of lymphoid-like organoids that showed distinct T and B cell zones, follicular DC networks, germinal centers, high endothelial venule-like structures, and were capable of mounting both humoral and cellular immune responses.[198] Further studies revealed that these artificial LNs were capable of supporting antigen-specific secondary antibody responses, and that cells from the generated organoid migrated to the spleen and proliferated at the new location.[199] Biomaterials approaches to generating lymphoid tissues *in vivo* is a young field, and there is much room for further investigation in this area.

While these examples are not exhaustive, they reveal the usefulness of a range of biomaterials including hydrogels and polymeric, silica, lipid, and protein NPs for delivering cargo to the LN, inducing LN remodeling, and regulating the subsequent immune response with improved outcomes compared to the delivery of soluble components, and highlight the potential of biomaterials for the *in vivo* generation of lymphoid tissue.

CONCLUSIONS

The lymphatic system is potently immunomodulatory, with critical roles including antigen transport, leukocyte trafficking, and direct immune cell regulation that make it a valuable target for regulating immune responses. Biomaterials are a powerful tool for lymphatic function regulation, enabling the carefully controlled delivery of cues to modulate lymphatic vessel growth and remodeling, fluid transport via vessel pumping, and LN structure, subsequently regulating the immune response for

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the treatment of a variety of pathologies. Though the myriad applications of biomaterials in lymphatic modulation and immunoengineering are beginning to be appreciated and experimentally explored, significant work remains to be done to translate these discoveries into the clinic. Applications of engineered biomaterials in immunoengineering via regulation of lymphatic structure and function will continue to expand as the role of the lymphatic system in immunomodulation is further clarified, regulatory molecular targets are identified, and novel biomaterials are developed.

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Notes

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