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Designing natural and synthetic immune tissues

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Abstract

Vaccines and immunotherapies have provided enormous improvements for public health, but there are fundamental disconnects between where most studies are performed – in cell culture and animal models – and the ultimate application in humans. Engineering immune tissues and organs, such as bone marrow, thymus, lymph nodes, and spleen, could be instrumental in overcoming these hurdles. Fundamentally, designed immune tissues could serve as *in vitro* tools to more accurately study human immune function and disease, while immune tissues engineered for implantation as next-generation vaccines or immunotherapies could enable direct, on-demand control over generation and regulation of immune function. In this review, we discuss recent interdisciplinary strategies that are merging materials science and immunology to create engineered immune tissues *in vitro* and *in vivo*. We also highlight the hurdles facing these

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approaches and the need for comparison to existing clinical options, relevant animal models, and other emerging technologies.

ENGINEERED IMMUNE TISSUES AS TOOLS AND THERAPEUTICS

Vaccines have transformed human health by providing specific, long-lasting protection, even enabling widespread eradication of diseases such as smallpox and polio. Recently, immunotherapies have also led to remarkable successes. In cancer, for example, antibody-based checkpoint inhibitors re-engage the immune system, dramatically improving outcomes for some patients with cancers of the skin, blood, lung, kidney, and others^{1, 2}. In autoimmune disease – where the immune system mistakenly attacks host tissue – antibodies that reduce inflammatory cues improve the selectivity and effectiveness of treatments for diseases such as multiple sclerosis (MS) and rheumatoid arthritis.

Despite these advances, clinical translation still relies on costly, inefficient trial-and-error methodologies. Further, existing animal models are often not predictive of human disease, leading to failure of late stage trials. In infectious disease, Novavax's vaccine for RSV – a respiratory virus impacting children and elderly – lacked efficacy during a phase III trial (NCT02608502). In cancer, Juno's engineered T cell therapy for leukemia was paused twice following patient deaths in phase II trials³. Meanwhile, in autoimmunity, Biogen's MS therapy, Opicinumab, missed efficacy endpoints in phase II⁴, while Circassia's cat allergy therapy failed in phase III (NCT01620762). Some of these trials failed because of efficacy, while others encountered safety issues that trials with smaller patient cohorts missed. Both shortcomings emphasize the way vaccines and immunotherapies are developed – first in cells and animals, then in humans – does not provide a complete picture of how these technologies will perform in humans. Engineered immune tissues could help fill in these gaps by serving both as tool to study and diagnose immune disease, and as next generation vaccines and immunotherapies with new capabilities.

In this review, we first highlight the major immune tissues, then discuss efforts to build immune organoids or organs-on-a-chip as tools, to reprogram the function of existing immune tissues, and to design immune tissues for implantation as vaccines and immunotherapies. As tools, these technologies are significant in several ways. Firstly, engineered immune tissues integrating human cells in culture could provide a more accurate research niche compared with 2D human cultures or pre-clinical mouse models that poorly recapitulate human immunity. Secondly, engineered immune scaffolds could support proliferation and analysis of rare immune populations isolated from patients. Watching these cells develop in a controlled human niche *ex vivo* might reveal new insight into pathologies difficult to study in patients, or enable renewable sources for blood cell production. Lastly, engineered immune tissues allow control over stromal components and extracellular cues – such as antigens, cytokines, and mechanical properties. This control could help isolate links to differentiation and function of specific immune cell subsets.

Beyond use as tools, engineered immune tissues could serve as next generation clinical options. A major challenge in the vaccine and immunotherapy field is targeting lymph nodes and spleen – organs that coordinate and expand immune cells. This challenge could be

bypassed by engineering implantable lymph nodes pre-primed against a particular pathogen or seeded with specific immune cell subsets. These designer tissues could be valuable in many translational contexts: generating durable responses against infectious disease, eliminating the requirement of *ex vivo* expansion before reinfusion of immune cells during adoptive cell cancer therapy, or programming immune cells to develop regulatory functions that control inflammation or autoimmunity. Engineered generative immune tissues or primary lymphoid organs – such as bone marrow (BM) or thymus – could create accurate humanized animal models. Such hosts could make clinical trials safer and improve understanding of cell behavior in patient samples that are currently only detectable *in vivo*. For example, 3D structure and defined stromal cues have recently allowed engraftment of tumors from cancer patients in humanized mice that do not engraft using simple cell infusions⁵. As summarized in Table 1, below we discuss exciting possibilities to leverage engineered immune tissues, highlight key hurdles that remain to be addressed, and provide comparisons to existing clinical options and emerging technologies.

DISTINCT ORGANS CONTROL IMMUNE CELL DEVELOPMENT AND FUNCTION

Immune cells develop in the BM and thymus – the generative or primary lymphoid organs (PLOs), then migrate to lymph nodes (LNs) and spleen – the secondary lymph organs (SLOs). In SLOs, immune cells are activated against specific pathogens or molecules, then migrate back to the blood and sites of infection, cancer, or disease (Fig. 1). These migration patterns reveal that immune cells programmed in a distinct organ – such as LNs or spleen, can migrate throughout the host to initiate responses that are both systemic and specific.

In the BM, hematopoietic stem cells (HSCs) develop into common precursors that differentiate to the immune cells populating the body. Common myeloid precursors give rise to antigen presenting cells (APCs), including macrophages and dendritic cells (DCs), that survey blood and tissue for foreign molecules⁶. A common lymphoid precursor gives rise to T and B lymphocytes in the BM that display receptors reactive against specific peptide fragments of degraded pathogens (“antigens”) (Fig. 1a). T and B cells are key players in adaptive immunity owing to immunological memory that enables these cells to mount potent, highly-specific, and fast-acting responses against pathogens. The large majority of B cells develop and exit the BM, migrating to LNs and spleen until activated (Fig. 1a). A small population of B cells that strongly bind self-antigens – which could lead to autoimmune disease, are deleted or undergo receptor editing to reduce self-reactivity (Fig 1a)^{7, 8}. T cell precursors exit the BM and migrate to the thymus for positive and negative selection. These processes ensure T cells display the molecular machinery needed to bind and process immunological signals presented by APCs, without inappropriate binding of self-antigen that could drive autoimmune disease (Fig. 1b)^{9, 10}. Mature T cells then migrate to the LN and spleen.

Generation of antigen-specific T and B cell responses requires correct presentation of an antigen matching the specificity of the receptors on the T or B cell (i.e., a “cognate” antigen). Antigen is processed and displayed by APCs in LNs and spleen after free antigen

drains from skin or peripheral tissue, or after APCs in the periphery collect antigen and migrate to LNs or spleen (Fig. 1c). Additionally, the spleen removes foreign material from blood – such as bacteria, providing systemic antigen surveillance (Fig. 1c)¹¹. Within the LN and spleen, B and T cells are trafficked by a dynamic structural network of stromal cells that control local interactions through structural proteins, such as laminins and secreted chemokines. These interactions are also influenced by mechanical tissue properties and specific microanatomic domains within the SLOs that segregate immune cells and functions¹².

In SLOs, T cells are activated upon encountering DCs presenting their cognate peptide antigens in the context of the major histocompatibility complex (MHC), along with required co-stimulatory signals (Fig. 1d). Following activation, the chemokine receptors on T cells change to support migration of T cells back to the periphery to fight infection in sites displaying the antigen to which they have been primed. Cytotoxic T lymphocytes directly destroy infected host cells to prevent viral replication, while helper T cells secrete supportive signals, such as cues that trigger destruction of phagocytosed bacteria (Fig. 1c). B cells produce low affinity antibodies when B cell receptors are directly crosslinked by antigen. High affinity antibody production requires B cells to interact with helper T cells (Fig. 1d). This step initiates a cascade by which B cells form specialized domains in LNs – germinal centers (GCs) – where they interact with follicular DCs (FDCs) and helper T cells (Fig. 1d)¹³. In GCs, B cells undergo class switching and affinity maturation, processes that result in differentiated B cells called plasma cells. These cells secrete high affinity antibodies that neutralize and tag pathogens for destruction (Fig. 1c). Some of the B cells developed in GCs become long-lived memory B cells that enable rapid response against future infections¹⁴.

IMMUNE ORGANOIDS AND ORGANS-ON-A-CHIP

2D cultures have limited ability to recapitulate the immune interactions in the body. These models over-simplify the host environment using simplified cell subsets, extracellular matrix features, fluid flows, and mechanical forces. 3D microenvironments that better mimic tissue structure can be created using organoids seeded with stem cells or heterogenous cell populations, or using microfabrication to design well-defined organs-on-a-chip^{15, 16}. Both of these approaches are being extended to immune organs – LNs, thymus, and BM – to create tools to elucidate immune cell interactions and the link to immune response and disease. This work has already identified matrix porosity, fluid flow, cytokine expression, and stromal cell interactions as important design parameters^{17, 18, 19, 20, 21}.

Recapitulating organ-level immune function with organoids

Recently, Purwada *et al.* generated immune organoids to create GC-like structures using porous hydrogels reinforced with nanoparticles to match the stiffness of natural SLOs (Fig. 2a)^{20, 21}. B cells were encapsulated in these gels, along with fibroblasts transduced to express two important B cell survival and activation signals (CD40L, BAFF) (Fig. 2b).¹⁸ This approach allowed B cells to develop phenotypic markers, such as GL7, indicative of GCs (Fig. 2c). Further, the mechanical properties of the gel impacted formation of a stromal organoid network and the expression levels of the activation signals. 3D organoids – but not

2D approaches – promoted a 100-fold increase in naïve B cell proliferation and differentiation to a GC phenotype (B220⁺ Fas⁺ GL7⁺) (Fig. 2d). Antibody class switching to IgG1 and IgE isotypes also occurred in the organoids; these antibodies promoted phagocytic destruction of pathogens and provided protection in mucosal tissues, respectively (Fig. 2e)^{22, 23}. While class switching is one important process in GCs, these organoids have not yet integrated secretion of antigen-specific antibodies or affinity maturation; the latter generates antibodies that strongly bind their cognate antigen. As such capabilities are integrated into LN organoids, these technologies will be increasingly useful for *in vitro* study or diagnosis of GC pathologies – such as B cell malignancies – through seeding organoids with patient cells. Engineered SLOs might further allow for more realistic *in vitro* assessment of vaccine performance by determining which antigen and adjuvant combinations maximize antibody affinity. There are also opportunities to generate fundamental insight; for example, LN organoids recently revealed the role of control pathways involved in unconstrained cell proliferation characteristic of GCs²⁴.

In addition to SLOs, thymus and BM organoids are being developed by seeding scaffolds with stem cells or other progenitors. As generative tissues, thymus and BM organoids could provide insight into immune cell development, and how these environments malfunction during diseases that cause deficient or self-reactive immune cells. Models of the thymus, where T cell selection occurs, have confirmed that 3D structure, ligand composition, and scaffold dimensions all impact function^{25, 26, 27}. Similarly, BM organoids – where many immune cells and precursors are generated – are influenced by 3D structure and mechanical properties such as scaffold stiffness and extracellular matrix (ECM) porosity^{28, 29, 30}. Kotov and colleagues developed a BM model from porous 3D colloidal crystals seeded with HSCs and human BM stromal cell lines²⁸. Compared with 2D cultures, 3D constructs enhanced differentiation of HSCs to B cells. These cells matured and secreted antigen-specific antibodies upon stimulation with inactivated virus. While this work did not test how strongly the antibodies bind target antigens, the studies make clear that 3D structure and a small number of defined cell populations – three in this case – can mimic some of the key processes of B cell development.

Natural BM microenvironments are enormously complex. Thus, in contrast to the approaches above using defined cell populations, other engineered BM niches are exploring scaffolds populated with heterogeneous mixtures of cell types and stromal cues. Torisawa *et al.* prepared a poly(dimethylsiloxane) PDMS device loaded with demineralized bone powder, collagen, and bone-inducing factors (BMP-2, BMP-4)³⁰. This device was implanted subcutaneously in mice and cultured for 8 weeks to populate the BM niche before excision (Fig. 3a, b). After removal, the engineered BM construct was inserted in a microfluidic device (Fig. 3c), allowing maintenance of B cells, T cells, and myeloid cells in proportions characteristic of natural mouse BM for at least 1 week (Fig. 3d). This *in vitro* niche mimicked responses of lymphoid cells to radiation when compared to responses observed in irradiated mice (Fig. 3e).

Functional tests – such as the radiation response just mentioned – are important in pushing engineered immune tissues forward. Most reports seek to confirm an immune cell population of interest develops, or replicate a particular native immune process. Much less

frequently are data reported that are relevant to the end-goal of diagnosis or therapy. Thus, translational applications of engineered immune tissues could directly accelerate clinical progress. For example, BM constructs could be used to screen for radiation toxicity and countermeasures *in vitro* as above, or to generate red blood cells for transfusions. Blood donations are limited by supply and compatibility. As one solution, synthetic blood that carries oxygen has been explored, but these carriers have limited capacity, lack cell function, and could trigger host inflammatory responses³¹. Stem cells and immortalized cell lines have demonstrated promise in animals and in one human volunteer, but these cultures do not produce cells at acceptable quantities or costs^{32, 33, 34}. Since 3D structure appears to improve performance of engineered BM niches, exploiting this idea might create viable options for large-scale blood production.

On-a-chip tools to study immune cell interactions

Beyond recreating organ- or tissue-level function in culture, new strategies are employing on-a-chip designs that harness microfabrication for precision control over the interactions of distinct immune cells or with defined extracellular signals. Considering the strong spatially-governed nature of immunity (see Fig. 1), immune cell migration is a key area of study for these technologies. For example, the migration of both APCs and lymphocytes are being probed through perturbations in adhesion molecule type and concentration³⁵, polymer scaffold composition and mechanical properties³⁶, and fluid flow or cytokine gradients^{37, 38, 39}. Additionally, real-time quantitative analysis of APC and T cell interactions – which mimic those occurring in SLOs – is helping to isolate the roles of T cell receptor binding affinities and valencies in controlling T cell activation^{40, 41}.

Hybrid devices integrating aspects from organoids and organs-on-a-chip are also being developed to study immune function. As one illustration, Ross *et al.* designed a strategy to place live LN slices in a perfusion chamber equipped with a microfluidic injector⁴². This approach allowed introduction of fluorescent probes and model therapeutics to distinct LN microdomains with spatial and temporal control. Such approaches could be useful in initiating, perturbing, or blocking specific processes – such as GC reactions – or T cell egress from LNs during activation. However, the ability to generate functional responses to immune cues, and how well those responses mimic native LN processes have not yet been addressed.

REPROGRAMMING EXISTING IMMUNE TISSUES IN ANIMALS

In this section we highlight studies aimed at reprogramming the structure and function of existing host immune tissues. One approach exploits 3D scaffolds to recruit and prime immune cells that then migrate back to SLOs to direct the function of these tissues. Another strategy uses direct injection of micron-scale depots into LNs to retain modulatory immune cues in these sites and reprogram the local microenvironment. Lastly, we discuss efforts to expand T cells using particles that mimic the function of APC without reliance on stromal cues present in SLOs.

Scaffolds to recruit immune cells and direct SLO function

Traditional vaccines are designed to drain or be carried to SLOs, where APCs and lymphocytes cooperate to generate adaptive responses to antigens in the vaccines. In contrast, biomaterial scaffolds are now being used to mimic the immunogenicity of natural infection environments by recruiting and activating APCs that subsequently return to SLOs to program the specific features of immune responses arising in these tissues⁴³. One application of particular interest for these scaffolds is cancer vaccines, which contain fragments of tumors in an effort to generate potent and selective anti-tumor immune responses. Because tumors are comprised of host cells, a challenge for cancer vaccination is the difficulty generating a large number of tumor-specific T cells. These cells must also remain functional despite the local immune-suppressing tumor environment. Toward addressing these challenges, early scaffolds were implantable structures loaded with tumor lysate as an antigen, granulocyte macrophage colony-stimulating factor (GM-CSF) as a signal to recruit APCs, and CpG as a stimulatory adjuvant.^{44, 45} This approach increased DC recruitment to scaffolds in mice, allowing these cells to encounter tumor lysate and become activated. The tumor antigen-presenting DCs migrated back to LNs and greatly expanded tumor-specific T cells. As a result, expanded CD4⁺ and CD8⁺ T cells infiltrated tumors at much higher levels (Fig. 4a), ultimately clearing established tumors in an aggressive melanoma model.

More recently, surgical implantation has been eliminated using scaffolds that gel or self-assemble upon injection. Kim *et al.* used mesoporous silica rods (MSRs) to incorporate model antigens (ovalbumin, OVA), GM-CSF, and CpG (Fig 4b)⁴⁶. The MSRs create an environment to recruit DCs by spontaneously assembling into scaffolds, enhancing both migration of tumor-primed DCs to LNs (Fig. 4c) and survival in mice (Fig. 4d). These effects required the scaffold component. Additionally, MSR scaffolds generated superior antibody responses in mice compared to the human vaccine adjuvant, alum⁴⁶.

Recruiting APCs to implanted scaffolds could be useful in several translational contexts, including infectious disease and cancer, or to promote immune tolerance by omitting stimulatory signals⁴⁷. Controlling APC activation and antigen loading to subsequently direct the function of T cells generated in LNs might also eliminate *ex vivo* manipulation during adoptive transfer cancer therapies. Adoptive therapies require isolating patient T cells, engineering the cells *ex vivo*, then a step to reinfuse the cells back to the patient. Eliminating *ex vivo* processing would dramatically decrease cost and complexity. However, realizing these possibilities using scaffolds will require more clinically-relevant models and comparisons. Further, cancer vaccines are not administered as monotherapies, but in combination with resection, checkpoint blockade, or other therapies. Thus, new materials approaches should be tested against or in combination with existing clinical regimens.

Another important direction in the biomaterials field centers on non-tissue engineering strategies for vaccines and immunotherapies, such as nanoparticles that drain to SLOs to direct responses to a foreign antigen^{48, 49, 50, 51, 52}. This goal overlaps with some of the goals of engineered immune tissues, for example, generation of a response to a foreign antigen by implanting an engineered SLO pre-primed with the antigen. Similarly, the APC-recruiting scaffolds discussed for cancer vaccines could be compared with injectable

nanodiscs that have recently generated exciting results in aggressive mouse cancer models using personalized tumor antigens⁵³, or against new peptide-antibody conjugates exploited in models of melanoma and breast cancer⁵⁴. Benchmarking tissue engineering approaches against nanoparticle technologies that target similar immunological goals could create frameworks for earlier assessment of parallel technologies, and identify where each approach provides distinct advantages.

Diffusion-limited depots to reprogram the local LN environment

Another emerging strategy to control immune tissue uses degradable polymer depots injected directly to LNs to reprogram the LN microenvironment. This approach leverages microparticles too large to drain from LNs, allowing mechanical retention of depots in LNs. Local controlled release of adjuvant from these particles increases adjuvant persistence in LNs, quickly and potently expanding antigen-specific T cells in mice⁵⁵. These responses are much higher than adjuvant-loaded nanoparticles or free adjuvant, both of which drain out of LNs more quickly. While the added complexity makes this approach less appealing for traditional prophylactic vaccination, as a tool, intra-LN depots offer opportunities to directly control the kinetics and combinations of signals in LNs to study immune cell function. For example, release of defined doses of regulatory cues in LNs during vaccination reveals the role of local concentration in polarizing antigen-specific T cells between suppressive, inflammatory, and memory functions⁵⁶.

Several clinical trials have used ultrasound-guided injection of free peptides into LNs to investigate tolerance induction for allergies (NCT01982474) and type 1 diabetes (NCT01536431)⁵⁷. Tostanoski *et al.* recently combined intra-LN injection with microparticle depots to combat autoimmunity by encapsulating a modulatory immune signal, rapamycin (Rapa), with a peptide self-antigen, myelin (MOG), that is attacked during MS⁵⁸. A single injection of these depots into LNs of mice during a model of MS (Fig. 5a) at the peak of disease permanently reversed disease-induced paralysis (Fig. 5b). This reversal was MOG-specific, as OVA-loaded depots conferred no therapeutic effect (Fig. 5c). Further, the composition and structure of treated LNs and distant, untreated LNs both exhibited reorganization and increased regulatory T cells (Fig. 5d). In the CNS – a location far from the treated node, inflammatory cell infiltration was reduced, correlating with reduced paralysis (Fig. 5e)⁵⁸. This work showed that reprogramming the local environment of distinct LNs can promote tolerance that is systemic and myelin-specific. Thus, understanding the parameters to induce desired structures and functions in LNs can also enable control over immune function in other tissues.

Despite the feasibility of intra-LN administration in clinical trials, implementing this approach involves some unique considerations which may highlight its advantages. First, treatments for autoimmune disease involve non-specific suppression of excess inflammation accompanying these diseases with small molecules drugs, and more recently, monoclonal antibodies. These treatments – such as methotrexate, fingolimod, natalizumab, and rituximab – provide patients with real gains, but cause off-target effects resulting from lack of selectivity. Monoclonal antibodies, for example, can target specific receptors but do not differentiate between expression of these receptors on normal and self-reactive cells. Thus,

intra-LN reprogramming may be beneficial if more selective tolerance is demonstrated during direct comparisons to existing therapies. Second, while there are a number of injectable nanoparticle strategies being explored to promote tolerance⁵⁰, a distinction between these approaches and direct LN reprogramming is the distribution and dose of cargo (i.e., local LN injection vs. systemic injection). Therapies integrating immunosuppressive components to polarize immune cells may be safer or more effective if concentrated in LNs. For example, control over the cells developing in a target SLO after depot injection could enable the programmed cells to subsequently migrate to sites of disease while exposing only the patient's SLO to a local immunosuppressant dose. In contrast, systemic inflammatory conditions might benefit from conventional injections that distribute immunosuppressant throughout the patient.

Using artificial APCs to control T cell expansion *in vivo*

Artificial APCs (aAPCs) aim to directly expand and control T cells. This approach involves nano- or micro-particles that present co-stimulatory molecules and antigen to locally stimulate T cells in peripheral tissues without requiring priming in LNs. Artificial APCs first gained attention in combination with adoptive transfer therapies^{59, 60}; however, *ex vivo* cell handling is complex and expensive, motivating use of aAPCs to directly expand T cells *in vivo*. Altering aAPC shape to maximize contact with T cells^{61, 62}, and combining aAPCs with modulatory signals^{63, 64}, have both been used to enhance aAPC function. The potential to directly enhance T cell expansion *in vivo* using aAPCs is becoming more intriguing as the power of T cell therapy has emerged. For example, recently-approved T cell gene therapies (Novartis, Kite) are on the order of \$500,000 per treatment because these therapies require expansion of patient cells *ex vivo* before reinfusion. Harnessing shape and molecular interactions to maximize valency and contact time could improve the efficiency of T cell expansion. aAPCs enabling this control might eventually allow T cell therapy to be carried out in patients without need for *ex vivo* expansion, greatly reducing treatment cost and preparation time. Another clinically-relevant example is Provenge, the first approved DC vaccine, which relies on infusion of modified DCs to provide a modest survival increase for patients with advanced prostate cancer (NCT00065442). Future approaches using aAPCs as artificial DCs that efficiently expand tumor specific T cells against target antigens – such as prostate cancer antigens – might provide new opportunities to improve these therapies. As with the other emerging ideas above, aAPCs should be tested in combination with checkpoint blockade and other common components of cancer treatment plans⁶⁴.

DESIGNING IMMUNE TISSUES FOR IMPLANTATION

Perhaps the ultimate goal of immune tissue engineering is the implantation of designed tissues that promote or restore desired immune functions. Implantable LNs or other SLOs primed for a specific response might effectively clear infections or tumors – or regulate distinct inflammatory cells to combat autoimmune disease, without the hurdle of vaccines or immunotherapies reaching an existing SLOs. On another front, engineering generative lymph organs – thymus and BM – might help correct immune deficiencies, improve BM transplants, relax donor-recipient matching requirements, and enable more accurate humanized animal models to study immunity and disease.

Mimicking adaptive functions with engineered SLOs

Tissue engineered LNs and spleens are being investigated in mice using scaffolds formed from natural materials – such as decellularized LNs that retain ECM components⁶⁵. Alternatively, more defined materials, including alginate and collagen⁶⁶, provide easily controlled mechanical and chemical properties⁴³. The inclusion of stromal components needed for artificial SLO function distinguishes constructs in this section from scaffolds for recruitment of immune cells in the previous section; the latter aim to prime APCs that return to existing SLOs.

In one approach decellularized mouse LNs were seeded with splenocytes from donor mice, then implanted into syngeneic (the same mouse strain) or allogeneic (a different strain) mice⁶⁵. While functionality was not tested, the cells survived for at least 14 days and the implants did not induce host responses. Suematsu *et al.* developed LN-like organoids by loading 3D collagen sponges with a mouse thymic stromal cell line and activated primary DCs⁶⁶. In mice, these constructs developed distinct B and T cell zones resembling those in natural SLOs (Fig. 6a). These scaffolds also exhibited B cell proliferation and development of high endothelial venules, a tissue microdomain that enables immune cell entry from blood (Fig. 6b). The scaffold structure was important, as neither collagen gels nor synthetic sponges recruited cells as effectively. As a functional proof of concept, DCs loaded with a model antigen were used to generate antigen-specific responses in healthy recipient mice (Fig. 6c). Subsequent retransplantation of the organoids into a second mouse lacking functional B and T cells (i.e., SCID) resulted in migration of B cells to the spleen and BM of recipients. The immunocompromised hosts were then able to generate antigen-specific antibodies, indicating artificial SLOs restored at least some immune function (Fig. 6c). Implantation also promoted B cell affinity maturation, and notably, memory T and B cells⁶⁷. However, in all of these studies, donor mice required pre-injection of the model antigen before organoid implantation. A related approach eliminated the requirement to seed scaffolds direct cell distribution and recruitment, as well as GC formation⁶⁸.

For artificial SLOs to have a direct translational impact as vaccines and immunotherapies, these strategies will ultimately need to be compared against or tested with current human options or emerging candidates. For example, as vaccines, the magnitude and duration of antibody and T cells responses induced by pre-primed implantable SLOs might be more long-lasting than conventional vaccines by establishing a local tissue niche. These comparisons could build understanding of potency and durability that lead to artificial SLOs able to protect against pathogens that are otherwise intractable using conventional vaccine technologies, such as HIV, hepatitis C, and malaria. Similarly, in cancer, tumor-primed SLOs might serve as potent new cancer vaccines, but should be tested in tandem with common combination therapies (e.g., checkpoint blockade or adoptive transfer), since most treatment plans are multi-pronged. In autoimmunity, artificial SLOs incorporating self-antigens and regulatory signals might provide a route to promote antigen-specific tolerance that selectively controls disease. However, comparison to approved immunosuppressants and monoclonal antibodies is needed since these therapies currently provide benefits to patients but are hindered by the lack of specificity. Using the best available pre-clinical animal models is also important for meaningful comparisons in pre-clinical studies.

One recent approach in a more translational cancer model assessed alginate scaffolds containing T cell binding domains and particles loaded with stimulatory T cell signals (Fig. 6d)⁶⁹. These scaffolds were seeded with engineered tumor-specific T cells, then implanted in mice. To mimic challenging clinical settings, the scaffolds were tested in an incomplete resection model of breast cancer and in an inoperable, disseminated model of ovarian cancer. Scaffolds containing particles with proliferative T cell signals greatly expanded tumor-specific T cells compared to other aggressive adoptive cell therapies using the same cells and signals, but without scaffolds (Fig. 6e). The expanded T cells migrated to tumors and caused complete regression in both cancer models (Fig. 6f). Subsequently, Smith *et al.* used a similar design to deliver tumor-targeting T cells displaying chimeric antigen receptors (CAR)⁷⁰. In solid tumor models of pancreatic cancer and melanoma that are difficult to treat, scaffold-based delivery was critical to eradicate tumors and protect against relapse.

As is becoming apparent from the examples above, an important theme is the need for comparison of emerging technologies aimed at similar problems. For example, the T cell scaffolds above require *ex vivo* preparation and seeding in the scaffold before implantation; a parallel approach by the same lab uses injectable nanoparticles that can introduce CAR T cell genes into circulating T cells to clear leukemia in mice at levels similar to adoptive transfer therapies⁷¹. Direct comparisons between emerging technologies – scaffolds versus nanoparticles in this case – could help reveal settings where each is most useful. Further, artificial SLO technology has not yet leveraged a number of ubiquitous tissue engineering tools. Some of these include rigorous mechanical characterization of stress, strain and fatigue, along with techniques to control structure and signal density with high precision, such as 3D printing and lithography. These capabilities are of particular importance since the LN, spleen, thymus, and BM all have spatial microdomains with distinct functions and mechanical properties, yet none of the examples here integrate spatial control beyond simple porosity.

Engineered thymus to enable organ transplantation

As introduced above, the thymus is critical in T cell selection, deleting cells that strongly bind self-antigens. Thymic tissue engineering offers potential to combat autoimmune disease driven by self-reactive cells, or improve transplantation by eliminating recipient- or donor-reactive T cells without broad or permanent immunosuppression.^{72, 73} Thymic epithelial cells (TECs) are important in thymic T cell recruitment and selection, as are the other stromal cell interactions, chemokines, and ECM cues needed for development of mature T cells. Decellularized thymic tissue can be seeded with thymic-derived cells (including TECs) to mimic this environment, creating a simple thymic framework⁷². When implanted in athymic nude mice lacking T cells, the resulting scaffolds attract hematopoietic progenitors from BM that can produce naive CD4⁺ and CD8⁺ T cells.

To induce tolerance in transplanted tissue, Fan *et al.* combined decellularized thymic tissue scaffolds with thymic stromal cells containing TECs, fibroblasts, and BM-derived T cell progenitors (Fig. 6g)⁷³. Implanting these organoids in athymic nude mice generated naive T cells that could migrate to SLOs and provide functional T cell support required for B cells to generate high affinity, antigen-specific antibodies. Mice with the implants also rejected skin

allografts displaying non-self antigen, indicating functional, antigen-specific T cell response. Importantly, however, when the organoids were instead designed with a mixture of TECs from donors and recipients, donor-derived skin grafts were accepted by the recipients, demonstrating immunologic tolerance (Fig. 6h). Since the studies above used athymic mice which do not generate a host response, an important question is how this approach would translate to human transplants; mature T and B cells in the periphery would still be donor reactive. Perhaps some gains could be realized through generation of tolerized cells in the thymus, or combining such approaches with immunosuppression to limit peripheral donor-reactive cells. Engineered thymus may also have utility in treating patients with immunodeficiencies – where human thymus transplants are being investigated – to restore missing immune functions, such as DiGeorge anomaly and severe combined immunodeficiency (SCID)⁷⁴.

Engineered BM implants to support hematopoiesis and immunity

Implantable BM scaffolds loaded with stem cells or other progenitors that differentiate into functional immune populations could help treat immune deficiencies, blood cancers, or self-antigen specific lymphocytes arising during autoimmunity. However, engineered BM in this therapeutic context shares many of the translational challenges discussed for thymus with respect to mature lymphocytes present in recipients with intact immune systems. While implantable BM niches may one day be used in humans, engineered constructs may be useful in the nearer term for diagnosis and therapeutics screening *in vitro* or in humanized animal models. Better understanding how material properties impact BM scaffold function could lead to constructs that support efficient engraftment with fewer patient cells for screening, or that maintain viability of cells in the niches for longer intervals. These properties would be useful for diagnostic applications where patient cells are limited or particular phenotypes are rare. Already scaffold stiffness and cell density have both been shown to impact differentiation of BM stromal cells encapsulated in collagen gels,⁷⁵ while mimicking the modulus and porosity of native BM improves HSC homing and retention in mice⁷⁶. One study exploring the idea of distinct spatial domains within engineered immune tissues has used scaffolds mineralized externally to recreate the function of bone, and non-mineralized internally to provide features of the hematopoietic niche that enable cell engraftment⁷⁷. These studies might help define parameters for constructs designed to screen responses of patient cells to therapeutics in humanized mice.

In addition to material design considerations, advancing engineered BM niches to clinical utility will require human cells. One emerging strategy to generate these humanized niches involves seeding scaffolds with human stromal and progenitor cells^{5, 78, 79, 80}. Holzapfel *et al.* generated a BM mimic using polycaprolactone scaffolds coated with calcium phosphate and bone inducing factors (rhBMP-7)⁷⁸. Scaffolds cultured with human mesenchymal progenitor cells were then implanted into mice lacking key components of the immune system (NSG mice). After 10 weeks, these constructs mimicked many mechanical, cellular, and biochemical features of human BM. During similar studies, mice were irradiated 10 weeks after scaffold implantation, then infused with human HSCs. The humanized BM environment preferentially recruited and maintained these HSCs, enhancing human leukocyte, T cell, and progenitor cells in the construct and the host BM.

Engineered BM may be particularly useful in studying or eventually treating hematopoietic diseases and malignancies. Several reports combine bone-forming scaffolds and human mesenchymal stem cells (MSCs) to engraft leukemic cells^{5, 79, 80}. In one example, NSG mice were irradiated and infused with hematopoietic progenitor cells from acute myeloid leukemia (AML) patients with distinct risks groups; alternatively, calcium phosphate scaffolds seeded with human mesenchymal stromal cells were implanted 6-8 weeks prior to injecting mice with AML cells (Fig. 6i)⁵. Without scaffolds, only 43% of AML patient samples could be engrafted; scaffolds, however, allowed engraftment and development of AML from 74% of the patient samples. Of note, scaffolds supported engraftment of some risk groups that simple infusions did not. Thus, 3D structure and tunable scaffold properties might improve study or diagnoses through faster, more robust engraftment of cancers from patients using these animal models to accurately mimic the human setting. While cancer is a key area for these tools, recent evidence reveals that patient-derived tumor grafts implanted in immunodeficient mice quickly lose resemblance to the patient tumor during selection pressures occurring in host animals⁸¹. Coupling patient grafts with engineered BM scaffolds might be useful in overcoming this divergence by recreating human immune selection pressures in humanized mice displaying human tumors.

Most of the work in this section focuses on the function of an organoid itself, but the physical connection of the organoids to the lymphatic system is another area that should be pursued. For example, lymphatic endothelial cells, fibroblasts, and adipose-derived stem cells have been incorporated in scaffolds comprised of fibrin or collagen I^{82, 83}. When combined with lymphangiogenic factors, such as VEGF-C, or fluid flow, lymphatic endothelial cells in these constructs grow into lymphatic capillary networks *in vitro*⁸⁴. Lymphatic vessels are vital for immune cell homing and surveillance, so controlling lymphangiogenesis in or around engineered immune tissues could increase antigen and immune cell recruitment to, or dissemination from, these sites.

NEW IMMUNOLOGICAL INSIGHT INFORMS IMMUNE TISSUE ENGINEERING

In this section we highlight some recent immunological advances in fields distinct from tissue engineering that have important implications for engineering immune tissues. The ways in which this knowledge translates to design criteria for immune tissues, such as LNs, are presented in Fig. 7.

Dynamic stromal cues in SLOs influence cell differentiation

The stroma provides distinct cues to attract and organize immune cells at different stages of response in LNs and spleen. These interactions are coordinated through the types of signals presented^{85, 86}, as well as the locations^{87, 88, 89} and timing⁹⁰. Stromal cells, such as fibroblastic reticular cells, blood endothelial cells, and lymphatic endothelial cells, can all present antigens to T cells^{91, 92}. For example, Tamburini *et al.* found that after a viral infection or vaccination, lymphatic endothelial cells can proliferate and capture antigens, continuing to display the antigens for weeks⁹³. In designing artificial LNs or spleen, this finding could be leveraged by incorporating lymphatic endothelial cells in constructs, along

with T cell homing molecules, to provide prolonged antigen exposure and development of memory T cells (Fig. 7a).

Immune cells actively interact with stromal cues, such as the distinctive compositions of ECM laminin fibers secreted by stromal cells. Warren *et al.* discovered that during immunity and inflammation, laminin $\alpha 4$ decreases and laminin $\alpha 5$ increases, while during tolerance, laminin $\alpha 4$ increases and laminin $\alpha 5$ decreases⁹⁴. These changes alter the interactions of DCs and T cells to help promote either pro-immune or regulatory T cell responses. Thus, tissue engineering scaffolds could integrate specific compositions of laminins, or other stromal components, to direct tolerogenic or stimulatory responses (Fig. 7b).

Matrix mechanical properties are actively regulated to support immune function

Mechanical properties play an important role in controlling immune cell migration and function. Interestingly, new studies reveal that in natural LNs, ECM properties change over the course of immune response. In particular, fibroblastic reticular cells, which form the collagen network that enables immune cell trafficking, work with DCs to contract fibers during resting states, then relax the network during immunostimulatory conditions to accelerate cell mobility (Fig. 7c)⁹⁵. This discovery implies a potential route to enable “on-demand” access of immune cells to specific signals at different stages of response by coupling collagen networks of different densities with defined concentrations of antigen and adjuvant.

Beyond SLO tissues, matrix mechanical properties are important in the tumor microenvironment, where immunosuppressive conditions severely limit immunotherapies. In addition to suppressive biochemical cues, tumors confront immune cells with rigid ECM environments that create high solid stress forces not encountered in healthy tissue⁹⁶. This feature may impact immune cell function or deter infiltration in tumors; these stresses could be mimicked to limit or direct migration of immune cells to and within immune tissue scaffolds (Fig. 7c).

Peripheral tissues alter immune cell homing and function

Immune cells function and interact differently depending on their location within the body, making immune cell migration and homing critical for immune responses. These movements are directed partly by lymphatic endothelial cells displaying homing signals for specific sites⁹⁷. For example, lymphatic vasculature is comprised of two vessel types – afferent vessels which bring antigens and leukocytes to LNs, and efferent vessels by which only lymphocytes and antigens can leave LNs. Iftakhar-E-Khuda *et al.* recently used gene expression to define molecular profiles differentially expressed on afferent and efferent lymphatic endothelial cells⁹⁸. Designed immune tissues could exploit these molecular homing signals to recruit lymphocytes or promote rapid egress of activated T cells from LN during vaccination; conversely, constructs targeting autoimmunity or transplantation could display molecules that restrict immune cell egress (Fig. 7d).

Beyond promoting or suppressing inflammatory functions, immune cell interactions with peripheral tissues influence wound healing for regenerative medicine applications. Thus, much effort in tissue engineering is directed at understanding pathways involved in

inflammatory responses to design scaffolds that instead drive wound healing^{99, 100}. One recent study revealed colony stimulating factor-1 in macrophages as a key pathway to prevent fibrosis – which often leads to failure of implants – without interfering with other macrophage functions¹⁰¹. In another example, Guc *et al.* showed the importance of inducing lymphangiogenesis – lymphatic vessel generation – to promote wound healing. Controlling the release of lymphangiogenic factors promoted lymphatic capillaries that regulated the levels of leukocytes delivered to diabetic wound sites, enhancing ECM deposition and healing (Fig. 7e)¹⁰². This work highlights another important design consideration: implantable immune tissues could include factors to promote lymphangiogenesis to enhance recruitment or dissemination of lymphocytes (Fig. 7e). Whether this approach might allow for direct connection with existing lymphatic vascular or SLOs – and how important this link is – are open questions.

Vaccine and immunotherapy design informs immune tissue engineering

In tissue engineering, recent studies reveal that the choice of scaffold material itself can impact immune cell signaling¹⁰³. This observation is in line with findings confirming many common biomaterials can directly activate the inflammasome and other innate stimulatory pathways through intrinsic material properties^{104, 105}. Thus, physicochemical material properties (e.g., charge, molecular weight, chemical functionality) may provide a new route to program pro-immune or regulatory responses into scaffold frameworks used in engineered immune tissues. Further, several new studies have taken quantitative approaches toward developing immunological design parameters that could be harnessed for scaffolds. These include the impact of antigen display density on immune response^{106, 107}, and the role that specific types and ratios of stromal cells play in directing lymph node function, such as fibroblastic reticular cells¹⁰⁸. For example, Singha *et al.* combined computational modeling, immunology, and materials fabrication to show with molecular precision that changing the dose of self-peptide presented to self-reactive T cells during autoimmunity controls T cell expansion, while the phenotype of the expanding T cells is dependent on the density of the peptide-complexes¹⁰⁷. Recent studies have also shown density plays an important role in how antigen is processed in APCs. Hess *et al.* discovered delivering the same total amount of self peptide on particles at a low density was more effective in promoting tolerance than display at high density on fewer particles¹⁰⁶. Thus, controlling the number and density of antigen on designed immune tissues – or in spatially distinct regions of these constructs – could help control the phenotypes of expanding cells (Fig. 7f).

OUTLOOK

While engineering functional, implantable immune tissues to restore or reprogram immune responses is daunting, emerging immunological discoveries are bringing this vision closer. More realistic *in vitro* models and on-a-chip designs continue to improve our ability to interrogate basic immunological interactions. Combining this knowledge with emerging tissue engineering technologies could enable designer immune tissues that accelerate screening of candidate vaccines and immunotherapies, and that improve the success or robustness of vaccines and therapies targeting cancer, transplantation, and autoimmunity.

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Figure 1 | Immune tissues play distinct roles in immune cell development.

a, In bone marrow (BM), lymphocytes develop from hematopoietic stem cells (HSCs). Most B cells then travel to secondary lymphoid organs (SLOs). A small fraction of B cells that strongly bind self-antigens are deleted or undergo receptor editing. **b**, T cell precursors develop in BM and migrate to the thymus for maturation and selection. T cells that strongly bind self-antigens or do not interact with self-molecules are deleted. Non-self-reactive precursors become mature T cells. **c**, Antigen presenting cells (APCs) search for foreign antigens in peripheral tissues. After collecting these antigens APCs travel through lymphatics to lymph nodes (LNs). Foreign antigens in the blood move to the spleen. **d**, At the SLO, antigen-specific T and B lymphocytes become activated, proliferating and differentiating into effector cells. APCs present antigen and co-stimulatory danger signals to naive, mature T cells, which undergo clonal expansion and differentiate into CD4⁺ helper T

cells or CD8⁺ cytotoxic T lymphocytes. Antigen-specific B cells become activated and expand after exposure to the antigen, which the B cell presents to an activated, antigen-specific helper T cell. This T cell then secretes cytokines to activate the B cell, enabling proliferation. With T cell help, some of the proliferated cells form germinal centers, where memory B cells and high affinity antibody-secreting plasma cells arise through processes including affinity maturation and isotype switching. The effector T and B lymphocytes arising in these tissues then perform their respective protective functions at the infection site. Upon reaching this site (c), antibodies neutralize and tag pathogens for clearance or destruction, helper T cells stimulate phagocytosis and destruction of pathogens by macrophages and other innate immune cells, and cytotoxic T lymphocytes kill host cells presenting cognate antigen – such as those infected with viral pathogens.

Figure 2 | Immune organoids allow for the development of germinal-center-like B cells that undergo complex immunological processes.

a, The gel-encased immune organoids in cell culture. **b**, A schematic of immune organoid development to create germinal center-like tissues. B cells and stromal cells are encapsulated in a gelatin suspension, and B cells interact with stromal cells causing proliferation and differentiation to a germinal center (GC) phenotype. **c**, After 4 days in culture, GC-like B cells (green) are visible in the immune organoid. **d**, Both the gelatin matrix for bioactivity and the silica nanoparticles (SiNP) for mechanical support are necessary for developing GC-like B cells. **e**, B cells cultured in 3D with mid and high seeding densities of stromal cells undergo class switching to IgG1 (left) and IgE (right) more effectively than 2D or 3D cultures at low stromal cell density. Adapted from ref. ²³, Nature Publishing Group (**a**), (**b**), (**c**); ref. ²², Elsevier (**d**), (**e**).

Figure 3 | Engineered bone marrow on-a-chip replicates function of bone marrow *in vivo*. **a**, A schematic representing the process for developing the engineered bone marrow (eBM) on-a-chip. Scaffolds are loaded with bone inducing materials and implanted subcutaneously into mice, where they are cultured *in vivo* for 8 weeks before removal and placement into a microfluidic device. **b**, The eBM after culture *in vivo* has similar structure to native mouse femur. Scale bars are 500 μm . **c**, An image of the microfluidic device loaded with the eBM for study *in vitro*. Scale bar is 2 mm. **d**, The eBM exhibited similar blood cell distribution as the mouse femur bone marrow (mBM) at the time of excision, continuing to mimic mBM over 7 days. **e**, The eBM can act as a model for radiation toxicity, typically studied in live animals, more effectively than stroma-supported culture of BM cells (Dish). Four days after exposure to radiation, a significant, dose-dependent reduction in lymphoid cells in the eBM, was found, similar to the trend observed in the BM of live, irradiated mice. Adapted from ref. ³⁰, Nature Publishing Group (**a**), (**b**), (**c**), (**d**), and (**e**).

Figure 4 | Engineered scaffolds recruit APCs to access tumor antigen and improve function after migration to LNs.

a, Earlier implantable porous poly(lactide-co-glycolide) scaffolds containing tumor lysates, along with migration and inflammatory signals, recruit APCs that subsequently travel to secondary lymphoid organs. These cells expand tumor-specific CD4⁺ and CD8⁺ T cells, improving T cell infiltration into tumors. **b**, A newer injectable scaffold that self-assembles from mesoporous silica rods (MSRs) after subcutaneous injection. The scaffold releases inflammatory signals that recruit immune cells, reprogramming these infiltrates, which then leave the scaffold environment to interact with other immune cells and promote immune response. **c**, MSR scaffolds loaded with model tumor antigen ovalbumin (OVA) and inflammatory signal granulocyte macrophage colony-stimulating factor (GM-CSF) recruit significantly more dendritic cells (DCs) than MSR scaffolds alone or MSR scaffolds loaded with OVA. **d**, The MSR scaffolds loaded with OVA, GM-CSF, and an adjuvant – CpG, provide significant protection against tumors expressing OVA compared with soluble bolus vaccine with the same components, even with lower antigen dose. These results together highlight the importance of the scaffold. Adapted from ref. ⁴⁸, Nature Publishing Group (**a**), ref. ⁴⁶, Nature Publishing Group (**b**), (**c**) and (**d**).

Figure 5 |. Local changes in lymph node structure from direct injection of tolerogenic microparticles into the lymph nodes of mice with autoimmune disease drive systemic changes in immune signaling.

a, Schematic of treatment setup for introducing tolerogenic microparticles (MPs) into LN of mice using intra-lymph node (*i.LN*) injection. Following local LN injection, local and systemic changes were assessed in LNs, spleen, and the central nervous system (CNS). **b**, Mice were induced with a model of multiple sclerosis and treated with tolerogenic MPs loaded with the autoimmune disease-relevant antigen, myelin oligodendrocyte glycoprotein (MOG), and a broadly-acting immune regulatory signal, rapamycin (Rapa). A single treatment at peak of disease, where a higher clinical score corresponds to a greater degree of paralysis, results in a permanent reduction of disease severity. **c**, The MP treatment promoted antigen-specific tolerance. MPs loaded with Rapa and a disease irrelevant peptide, ovalbumin (OVA) provides no protection from disease. Scale bars are 100 μm. **d**, Treatment with tolerogenic MPs causes LN structural changes at the treated LN, as well as distant, untreated LN. Treatment with tolerogenic MPs promotes the formation of regulatory T cells (T_{REGS}, light blue) known to modulate autoimmune disease. Scale bars are 100 μm. **e**, Tolerogenic MP treatment significantly reduces T cell (green) infiltration into the CNS. Adapted from ref. ⁵⁸, Elsevier (**a**), (**b**), (**c**), (**d**) and (**e**)

Figure 6 | Engineering immune tissues can restore immune functions *in vivo*.

a, Collagen-sponge scaffolds loaded with stromal cells and activated dendritic cells (DCs) promote T cell (red) and B cell (green) recruitment to distinct regions within the LN organoids after transplantation. **b**, Transplanted organoids promote high endothelial venule (HEV) formation (red). B cells are stained in green. **c**, Retransplantation of organoids promotes humoral responses against an antigen, ovalbumin (OVA) in both healthy (BALB/c) and severely immunocompromised (SCID) mice. **d**, Biopolymer scaffolds loaded with tumor-specific T cells and stimulants inserted into tumor resection beds can target incompletely resected tumor cells. **e**, T cells delivered by the scaffold into the resection bed of an incompletely resected tumor enhance protection against tumor relapse, whereas T cells alone do not. **f**, Scaffold delivery of tumor specific T cells significantly enhances survival compared to control treatments. **g**, Decellularizing native thymic tissue preserves 3D

extracellular matrix structures. Scale bars are 10 μm . **h**, Engineered thymus organoids include donor and recipient thymic epithelial cells (TECs). Donor mouse skin grafts are well-tolerated after 4 weeks by recipients with matched thymic organoid implants, while third party skin grafts are rejected. **i**, Schematic depicting development of leukemic humanized bone marrow (BM) model. Mice were irradiated, then infused with hematopoietic progenitor cells from leukemia patients in distinct risk groups, or implanted with calcium phosphate scaffolds containing human mesenchymal stromal cells (MSCs) 6-8 weeks before infusion with the leukemic patient samples. Scaffolds created an artificial BM niche that enabled engrafting of some risk groups that could not be engrafted using the infusion alone. Adapted from ref. ⁶⁶, Nature Publishing Group (**a**), (**b**), (**c**), ref. ⁶⁹, Nature Publishing Group (**d**), (**e**), (**f**), ref. ⁷³, Elsevier (**g**), (**h**).

Figure 7 | New immunological insight informs immune tissue engineering design.

a, Including endothelial cells that present antigen and T cell homing signals could promote T cell activation and enhance immune responses. **b**, Controlling stromal structures can direct immune response, for example, using different relative ratios of laminins on a scaffold to polarize responses toward pro-immune or tolerogenic functions. **c**, Matrix mechanical properties influence immune cell function. Stiff, densely packed regions due to fibroblastic reticular cells (FRCs) contracting the stroma or tumor microenvironments limit immune cell infiltration and activity. Activated immune sites rely on relaxation of FRCs to enhance immune infiltration. **d**, Endothelial cells in afferent and efferent lymphatic vessels express different homing cues on their surfaces. These signals could be included in a tissue engineering design to promote a specific type of lymphatic vessel or immune cell in target locations. **e**, Delivering lymphangiogenic factors using a scaffold could promote lymph vessel growth, allowing for delivery of immune cells, which could enhance wound healing abilities of the scaffold. **f**, Antigen-presenting scaffolds could be used to influence both the

degree of immune cell proliferation and the specific phenotypes that arise, properties shown to be a function of both antigen dose and antigen display density.

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Table 1 |

Advances in engineering immune tissues

Approach	Goal	Tissue Type	Key Findings or Advance	Ref.
Immune organs-on-a-chip	Mimic immune organ function on-a-chip	Lymph node	Germinal center organoids support B cell expansion and antibody class switching	21,22
		Thymus	Thymic microenvironments on-a-chip depend on biochemical and mechanical cues from matrix and other cells	24, 25, 26
		Bone marrow	Engineered bone marrow niches can be designed to ensure cellular diversity	27, 29
	Study immune cell interactions using organ on-a-chip as a tool	Lymph node	<ul style="list-style-type: none"> Immune cell directionality and velocity depend on ECM adhesion ligand presentation, matrix pore size, and immune cytokine gradients 	34, 35, 36, 37, 38
			<ul style="list-style-type: none"> Microfluidic devices can be used to study individual immune cell interactions LN on-a-chip tools can be used to spatially resolve cues to control or study immune cell function 	39, 40 41
Biomaterials to reprogram the structure and function of existing immune tissues	Recruit and reprogram immune cells using implantable scaffolds	Lymph node	<ul style="list-style-type: none"> Implantable scaffolds with inflammatory cytokines, tumor antigens, and danger signals recruit and activate DCs to leave the scaffold to prime efficacious anti-tumor T cells in LN Injectable, porous scaffolds can be used to recruit and expand DCs <i>in situ</i> and promote a regulatory environment depending on method of antigen presentation within the scaffold 	48, 49 50, 51
	Control local immune tissue function by implanting depots at the tissue site	Lymph node	Reprogramming the local LN environment using diffusion-limited particle depots loaded with immune cargo causes changes in the composition and function of both local and distal LNs, promoting inflammatory or regulatory systemic responses	57, 58, 60
	Control T cell development in vivo using artificial antigen presenting cells	Lymph node	<ul style="list-style-type: none"> Ellipsoidal artificial antigen presenting cells activate antigen-specific T cells more effectively, and have reduced non-specific uptake and clearance compared to spherical aAPCs 	63, 64
			<ul style="list-style-type: none"> Artificial APCs loaded with antigen and co-stimulatory danger signals can be combined with additional molecules to enhance their ability to activate efficacious antigen-specific T cells <i>in vivo</i> 	65, 66
Engineered immune tissues for implantation	Create adaptive immune function using implantable secondary lymphoid organs	Lymph node	<ul style="list-style-type: none"> Artificial lymph nodes with collagen sponge scaffolds loaded with stromal cells and activated DCs recruit T and B cells, develop LN organizational features, and restore complex immune functions Alginate gels with stimulatory particles and tumor-specific T cells placed near solid tumors or resection sites effectively deliver tumor-specific T cells and promote inflammatory tumor-specific T cells against tumor escape variants better than cell injections 	68, 69 71, 72
	Use implantable, engineered thymus to enable transplantation	Thymus	Engineered thymic tissue containing thymic epithelial cells and hematopoietic stem cells promotes tolerance to donor tissue when thymic epithelial cells contain major histocompatibility complexes specific to donor and the recipient	75

Approach	Goal	Tissue Type	Key Findings or Advance	Ref.
	Support immune function and hematopoiesis with engineered implantable bone marrow niches	Bone marrow	Engineered humanized bone marrow niches that include human mesenchymal stem cells can replicate the structural and biochemical features of BM to preferentially recruit injected human stromal stem cells and provide models of hematopoietic diseases	5, 80, 81, 82