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Hydrogels and scaffolds for immunomodulation

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Abstract

For over two decades, immunologists and biomaterials scientists have co-existed in parallel world with the rationale of understanding the molecular profile of immune responses to vaccination, implantation, and treating incurable diseases. Much of the field of biomaterials-based immunotherapy has relied on evaluating model antigens such as chicken egg ovalbumin in mouse models but their relevance to humans has been point of much discussion. Nevertheless, such model antigens have provided important insights about the mechanisms of immune regulation and served as a proof-of-concept for plethora of biomaterials-based vaccines. After years of extensive development of numerous biomaterials for immunomodulation, it is only recently that an experimental scaffold vaccine implanted beneath the skin has begun to use the human model to study the immune responses to cancer vaccination by co-delivering patient-derived tumor lysates and immunomodulatory proteins. If successful, this scaffold vaccine will change the way we approached untreatable cancers, but more importantly, will allow a faster and more rational translation of therapeutic regimes to other cancers, chronic infections, and autoimmune diseases. Most materials reviews have focused on immunomodulatory adjuvants and micro-nano-particles. Here we provide an insight into emerging hydrogel and scaffold based immunomodulatory approaches that continue to demonstrate efficacy against immune associated diseases.

Keywords

immunology; hydrogels; biomaterials; scaffolds; immunomodulation; polymers

1. Introduction

Immunomodulatory biologics such as antigenic proteins and peptides, nucleic acids, natural and synthetic adjuvants, drugs, and extracellular matrix components can be utilized to treat a broad spectrum of immune-related diseases. The ultimate goal of immunomodulation is (i) to either reduce immune cell activation in hyperactive immune conditions such as transplant rejection, autoimmune and inflammatory diseases; or (ii) to induce immune cell activation to

reverse hypoactive immune responses in cancer and chronic infections [1]. The immunomodulatory agents engage and modulate the intracellular or cell surface receptors on host immune cells such as toll like receptors on dendritic cells (DCs), antigen-specific T-cell receptor (TCR), and B-cell receptor (BCR). Together, modulation of these signaling cascades determines the direction, magnitude, and persistence of B and T-cell responses.

Prophylactic vaccines have become one of human medicine's most potent weapons and in the past century, research has contributed tremendously to the significant decrease in mortality and morbidity from infectious diseases [2]. Yet we are far from treating established or unexpected chronic diseases and demonstrating global success. Seasonal diseases such as influenza imposes substantial risk due to the transient nature of immune response and the adaptability of viruses. With incurable cancers like melanoma [3] and lymphoma [4], the recurring outbreaks of emerging infectious diseases such as the 2009 H1N1 influenza A [5] and 2014 Ebola hemorrhagic fever [6], and drug resistance infections like tuberculosis [7], there is a strong need for new translational strategies to achieve active immunomodulation. Similarly, either alone or by combining with existing therapies to treat autoimmune diseases [8] like rheumatoid arthritis and type 1 diabetes mellitus, immunomodulation using *ex vivo* programmed immune cells has the potential to improve our ability to control tolerogenic immune response.

A rapidly growing field of research is the design of scaffolds and hydrogels made of synthetic and natural materials, with ability to modulate the immune response. Greater understanding of the molecular basis of cellular phenomenon such as apoptosis, receptor-ligand interactions, and immune cell activation has aided the development of materials-based therapeutic strategies for disease treatment. Biomedical researchers and clinicians have been continuously shifting focus towards engineered approaches at multiple length-scales (macro-to-nanoscale) that could target organs like lymph nodes, lymphoid residing cells and their intracellular compartments, function as surrogate lymphoid-like tissues, and deliver multiple biomolecules that could modulate the extent of the humoral and cellular immune response. Pioneering work has been done in encapsulating antigens, immunomodulatory agents, and drugs inside micro-and-nanoparticles. Several of these have been extensively discussed by others and us elsewhere [9],[10],[11],[12]. Here we discuss emerging hydrogel and scaffold-based strategies for fighting against cancer, infections, and autoimmune diseases as well creating immune cell microenvironments.

2. Hydrogels and scaffolds as biological delivery systems

Polymeric scaffolds and hydrogels are three-dimensional polymeric networks swollen in water, aqueous media or biological/physiological fluids, which have been widely used for cell encapsulation and controlled release of therapeutic proteins, peptides, drugs and nucleic acids [13],[14],[15],[16],[17],[18],[19],[20],[21],[22],[23],[24]. The choice of polymer could be dictated by the end use such as the site of implantation and expected performance including release kinetics, biocompatibility, and immunogenicity. Synthetic polymers provide structural and mechanical design flexibility that is less achievable with natural occurring polymers, however the advantage of biocompatibility, cell adhesion, enzymatic and hydrolytic degradability, minimal inflammatory response, and ability to stimulate a specific cellular

response with natural polymers can also be critical in polymer choice. Functional design of macroscopic scaffolds and their release kinetics has been discussed earlier in excellent reviews by Schoichet [25] and Kearney and Mooney [26].

Hydrogels have become immensely popular in regenerative medicine, due to their biocompatibility, design flexibility, and a broad spectrum of choice of base material. The hydrophilic nature and high swelling ratio also make hydrogels permeable to oxygen, nutrients, metabolites, and waste products. A number of materials and crosslinking techniques have been explored to design hydrogels with varying properties such as pore size, mechanical strength, and degradation rate as reviewed by us and others elsewhere [13],[27],[28],[29],[30],[31],[27],[32],[33],[34],[35]. For example, photoinitiated polymerization of polymers that are end-functionalized with acrylates and vinyl sulfone groups provide a unique ability to spatially and temporally control the development of implantable materials for cell and protein delivery, as well as for the fabrication of scaffolding with complex structures. Choice of material for forming these hydrogels range from entirely synthetic polymers such as poly(ethylene glycol) (PEG) to purely natural polymers such as dextran, gelatin, and hyaluronic acid, with degradation based on the hydrolytic or enzymatic degradation of bonds in the polymer backbone or crosslinks. The mechanical properties of these hydrogels are controlled by network crosslinking density, polymer functionalization and reaction mixture concentrations.

Polymeric solutions that are capable of forming an *in situ* three dimensional crosslinked network have gained recent attention because of the mild crosslinking conditions required to make these hydrogels thus allowing for the encapsulation of biomolecules like DNA, RNA, proteins as well as cells. Although it is possible to create *in situ* crosslinked networks through UV radiation mediated chemical crosslinking of acrylate functionalized polymers [34],[35], the process requires long exposure to UV rays and could get hampered by the limited penetration depth of UV rays through skin. Of particular importance is the Michael-type conjugate addition reaction based on *in situ* crosslinkable hydrogels. Michael-type addition is a conjugate addition with a carbanion or enolate-type nucleophile to α,β -unsaturated carbonyl-containing groups in presence of a base-catalyzed or nucleophile-catalyzed condition [36],[37],[30]. These hydrogels have been extensively used in cell and protein delivery applications [38],[39],[40],[41],[42],[43]. We have used these hydrogels for combinatorial delivery of antigens and immunomodulatory agents [23],[24] as discussed later in the review.

Despite the long standing history of hydrogels and scaffolds for drug delivery and tissue engineering, it is only recently that researchers have begun to explore the potential of macroscopic gels, thin films, scaffolds, microscopic needles, or hydrogel-nanoparticles in a wide range of immunological disease applications, with significant clinical impulse observed in the past decade itself [44],[45],[46],[47],[11],[48],[23].

3. Dendritic cell trafficking and primary mechanism of immunomodulation using scaffolds

Landmark studies by Sir James Gowans and colleagues in 1964 demonstrated for the first time that lymphocytes, during homeostasis, sample the blood stream for foreign antigen as they recirculate from blood, through secondary lymphoid organ such as lymph nodes, into lymphatics and return to the blood stream. This immune cell trafficking is essential for maintaining the robust immunity in the body and the lymphocytes migrate as often as 1–2 times per day. Humans have nearly 450 lymph nodes scattered all over the body. Since their first identification almost 40 years ago [49], DCs have emerged as one of the most important professional antigen presenting cells (APCs) bridging the two indispensable arms of the immune system i.e. innate and adaptive immunity [50].

The innate immunity establishes the first line of immunological response during infection with early recognition of pathogenic signals and subsequent initiation of proinflammatory response. The adaptive immunity, on the other hand, eliminates infected cells and pathogens in the later stages of infection and constitutes immunological memory against invaded pathogens. DCs play a central role in a series of immunological events during infection, immunization, and immunotherapies that eventually lead to adaptive T and B-cell-mediated immunity.

DCs can be directly activated *in vivo* by injecting immunogenic antigens or primed *ex vivo* with antigens followed by injecting back into the body. For cancer immunotherapy, DC-based vaccines are frequently developed by deriving naïve DCs from peripheral blood of patients and successfully loaded with tumor-associated antigens, *ex vivo* [51],[52]. These *ex vivo* primed DCs, when infused back into the cancer patient at the peripheral tissue sites, are expected to function normally and provide anti-tumor T-cell response. It is expected that the infused, primed DCs will respond to the homing signals mediated by chemokine and cytokines, migrate to the draining lymph nodes and present antigens to the residing T-cells via major histocompatibility complexes (MHC). Promising studies in animal models has shown robust cluster of differentiation (CD)4 and CD8 T-cell responses, however the *ex vivo* DC vaccine strategy (direct cell delivery) has only provided marginal survival rate in patients compared to chemotherapeutic regimens despite showing an antigen-specific T-cell expansion. It is speculated that antigen-loaded DCs in draining lymph nodes and other lymphoid organs have limited ability to support the effector phase of the immune response following T-cell priming therefore failing to generate the necessary magnitude and duration of functional CD8+ Cytotoxic T lymphocyte (CTL) response to induce regression of tumors. Such partially effective tumor immunotherapy strategies are detrimental as they can amplify defective CTLs under the influence of tumor microenvironment that are rich in immunosuppressive cytokines such as interleukin (IL)-10 and transforming growth factor- β (TGF- β) [53],[54],[55],[56].

Engineering hydrogels and scaffolds to synchronize with the migration, activation, and target specificity of our immune system requires complex designs and properties that are important for cell modulation. Several recent reports provide evidence that delivery of biological therapeutics using hydrogels and scaffolds could actively regulate the kinetics of

multiple steps in the immune response (e.g. vaccines) or as materials that evade immune response and scar formation [45],[23],[24],[57],[58],[59],[60],[61],[62],[63],[64],[65],[66],[67],[66],[68],[69],[70],[71],[44], [72]. Using scaffolds, DCs can be exploited for immunotherapy through two primary mechanisms as illustrated in Figure 1, built upon the core concepts of conventional vaccines: (1) *ex vivo* priming and implantation of activated DCs, or (2) delivery of antigens and adjuvants captured by DCs *in vivo*.

4. Three-dimensional scaffolds as immunological microenvironments and delivery of *ex vivo* programmed immune cells

Creation of an artificial *ex vivo* site for culturing primary T-cells and DCs and allowing them to interact could be a powerful strategy to manipulate immune cell behavior prior to adoptive immunotherapy. However, very few studies have been done in this area. Current design rationale behind 3D scaffolds as secondary lymphoid organ is based on the mechanically soft nature of the lymphoid microenvironment, structural complexity, and the presence of key cell signaling molecules, as well as the organogenesis and plasticity of which has been discussed in an elegant review by Irvine *et al* [73]. Studies by Suematsu and Watanabe [74] used collagen sponge scaffolds carrying a thymus-derived Lymphotoxin (LT)- β receptor and vascular cell adhesion molecule-1 (VCAM-1) expressing stromal cell line transduced to express murine lymphotoxin LT- α and used as an implantable scaffold for synthetic lymph node formation. When implanted in mice, the scaffold based organoid formed an organized secondary lymphoid-like with compartmentalized zones of B-cell and T-cell clusters, high endothelial venule-like vessels (HEVs), germinal centers and networks of follicular DC (Figure 2A). Interestingly, implantation with activated DCs promoted the cluster formation for B and T-cells in the organoids in presence of LT- α expressing stromal cells. Using a composite macroporous PEG hydrogel scaffolds infused with collagen, Stachowiak *et al.* engineered an inverse opal hydrogel mimicking a lymphoid organ to study immune cell migration. Using colloidal crystal templating, ordered, interconnected arrays of porous hydrogel (Figure 2B) was formed with pores on the order of tens of microns length scale [73],[62]. These PEG-gels, infused with collagen, promoted intra-scaffold migration of encapsulated T-cells and DCs, with T-cell migration dependent on the connecting pore size. Both these systems demonstrate the promise of tissue-engineered approaches to generate lymphoid-like microenvironment.

Interesting studies by Lee and Kotov [75] also demonstrate the feasibility of re-engineering thymic microenvironment using notch signaling protein functionalized 3D inverted colloidal crystal (ICC) scaffolds for *ex vivo* T-cell development of human hematopoietic stem cells (HSC). The thymic microenvironment is a complex milieu of progenitors, supportive stromal cells, signaling interactions like Delta and Notch, cells undergoing clonal selection through MHC-TCR interactions, as well as abundant adhesion, expansion and homing molecules present as extracellular matrix. These polyacrylamide hydrogel ICC scaffolds were formed using layer-by-layer (LBL) molecular assembly technique and had 110 μm uniform pore size with $\sim 30 \mu\text{m}$ large interconnecting channels. The ICC hydrogels have LBL coating of clay and poly(diallyl dimethylammonium chloride) (PDDA) that promotes

stromal cell adhesion and immobilization of Notch ligands through electrostatic interactions. The repeated proliferation and notch induction resulted in differentiation of HSCs into T-cell lineage with expression of CD3, CD4, TCR α , and CD117. The study shows the possibility of generating a 3D system for high throughput regeneration of T-cells from stem cells, *ex vivo* and could potentially be applied to DCs as well.

In situ gelling hydrogels offer the unique ability to serve as depot or vaccination nodes to deliver either *ex vivo* primed DCs or recruit host's DCs *in situ* and program them for inducing cellular immune response. The encapsulation process being mild, does not alter the bioactivity of the molecules or functionality of the cells. Pioneering work by Hori *et al.* [46] in Irvine's group has resulted in an alginate based injectable hydrogels which can be loaded with *ex vivo* primed immune cells along with immunomodulatory proteins (Figure 3A). These self-gelling depots are composed of polysaccharide alginate mixed with calcium ion containing microspheres whereby upon injection, calcium (Ca²⁺) ions diffuses into the alginate solution and ionically cross-links the polymer chains in <60 min. Chemokines reversibly bind to these gels and encapsulated DCs exhibited survival and spreading *in vivo* (Figure 3A). Subcutaneous injection of soft alginate gels carrying immunomodulatory cytokines and/or DCs have shown promising *in vivo* responses with priming of naive CD8+ T-cells in the local draining lymph nodes followed by migration and infiltration of primed antigen-specific T-cells to the gels [46],[47]. Hori *et al* showed that the presence of CpG oligonucleotides in conjunction with injected crosslinkable alginate solution was important in generating activated MHC-II⁺ CD40⁺ DCs, which points out the need for danger signal in DC activation and secretion of inflammatory cytokines like IL-6 and IL-12p70 during immunotherapy.

5. Macroscale implantable Scaffolds to induce and program immune cells, *in situ*

Unrelated studies have begun to reveal the importance of simultaneous delivery of multiple biomolecules such as antigens and adjuvants that could modulate the extent of the humoral and cellular immune response. Prior studies also emphasize the importance of the delivery kinetics of immunomodulatory molecules in induction and persistence of a robust immune response. For *in vivo* immunomodulatory purposes, there are several design considerations for hydrogels and scaffolds, such as mild fabrication or gelation conditions, delivery kinetics, and recruitment/infiltration of immune cells. These design considerations are important both in the context of immunization and in natural responses to transient versus persistent infections. For example, it is critical that the vaccination material is responsive enough under physiological conditions for timely delivery of cargo that may require dissolution of the hydrogel and the release of the incorporated vaccine load. We and others have shown that the magnitude, functionality, and phenotype of antigen-specific CD4⁺ and CD8⁺ T-cell responses can be shaped by controlled release of antigen and immunomodulatory biologics over multi-week periods. The prolonged retention and persistent immunomodulatory signals elicit stronger immune responses against persistent pathogens which cannot be eradicated by bolus immunizations.

In an early attempt to program immature DCs *in vivo*, Takashima and colleagues recruited Langerhans cells using ethylene-vinyl-acetate (EVA) polymer rods (Figure 3B) [76]. Langerhans cells, the immature DCs residing in the epidermis, were recruited and entrapped using chemokine MIP-3 β incorporated into the 10 mm EVA rods. Implantation of MIP-3 β rods caused local accumulation of LCs without affecting their surface densities. This work demonstrated the feasibility of DC-based vaccination against tumor *in vivo* by co-implanting a xenogeneic antigen ovalbumin (OVA)-incorporated EVA rods in abdominal skin and the immunized animals developed robust CTL response against the OVA-transduced EL4 tumor line, E.G7-OVA [76].

Gubeli *et al.* [64] recently reported a strategy to reduce repetitive booster doses of hepatitis B vaccine by controlling the release of the vaccine from an oral drug-responsive hydrogel depot. PEG chains end-functionalized with protein Gyrase B (GyrB) were used to form a hydrogel with addition of coumermycin that would dimerize GyrB and physically entrap Hepatitis vaccine. Interestingly, addition of aminocoumarin antibiotic novobiocin competitively replaced coumermycin resulting in GyrB monomerization and hydrogel dissolution within 2 h and the release of the vaccine (Figure 3D). Mice immunized with hydrogels and receiving the novobiocin stimulus demonstrated significantly higher anti-HBs titers compared to control groups including the classical 2-dose injection regime, therefore clearly demonstrating that advantage of controlled, prolonged release of the HBsAg vaccine in a stimulus-inducible manner.

The breakthrough in scaffold-based cancer immunotherapy came in a seminal report where Mooney and collaborators [44],[45] engineered tablet-shaped macroporous PLGA scaffold (~85% of total volume represents pores) to deliver tumor lysates with other immune stimulatory proteins. Implantable scaffold, made of polylactide-co-glycolide (PLGA) consisted of DC attracting cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF), immunostimulatory CpG oligonucleotide, and tumor lysate as antigen. These scaffold recruited naïve CD11c⁺ DCs and programmed them to induce robust prophylactic immunity against murine B16-F10 melanoma tumor [45]. Importantly, the group investigated the ability of these scaffolds to provide therapeutic vaccination against established melanoma [44]. These studies showed that under the influence of GM-CSF, DCs expressing CD11c and CD11b accumulated the implantation site with nearly 87% CD11b⁺ DCs residing inside the implant. Scaffolds loaded with CpG oligonucleotides (complexed to cationic polymer polyethyleneimine) alone recruited CD11c⁺PDCA-1⁺ plasmacytoid DCs that increased with GM-CSF and with increasing dose of CpG oligonucleotides. Taken together these studies showed that by using polymeric scaffolds delivering multiple immunomodulatory molecules could stimulate CD4⁺ and CD8⁺ cellular response, increase the production of tumor-suppressive Interferon (IFN)- γ , IL-12, attenuated FoxP3⁺ T_{Reg} cells and immunosuppressive cytokines, and caused regression of established melanoma which can be attributed to the high CD8⁺ T-cells to FoxP3⁺ T_{Reg} cells that has been linked to therapeutic tumor immunity in murine and human systems (Figure 3C) [77]. The vaccine implant, called WDVAX, has since moved into clinical trials for treatment of melanoma [78].

6. Macroscale injectable Scaffolds to induce and program immune cells, *in situ*

Vaccine implants discussed so far require minor surgery, often subcutaneous, and therefore there is a significant need to make scaffold based strategies that are less invasive. Roy *et al.* have previously reported *in situ* injectable, biodegradable polyethylene oxide (PEO) based hydrogels for nucleic acid-mediated gene therapy against peanut allergy where the polymeric solution remained liquid outside the body and gelled once injected [79]. Specifically, tetrafunctional polyethylene oxide amine (P4Am) and tetrafunctional polyethylene oxide succinimidyl glutarate (P4SG) entrapped the DNA vaccine by varying the density of the polymer network and concentration of the PEG components, the release kinetics of entrapped DNA and gelling ability could be controlled between a branched viscous polymer and a cross-linked hydrogel. These hydrogels gelled in approximately 18 minutes and slowly degraded over a period of 28 days. These injectable hydrogels successfully delivered plasmid DNA encoding human secreted embryonic alkaline phosphatase (pSEAP) into skeletal muscles of immunocompetent animals resulting in high serum levels of the protein for a significantly longer period of time relative to that achieved with unformulated DNA injections. The technique offers a minimally invasive application of polymeric solution thus making it an ideal candidate for vaccine delivery applications.

The alginate gels developed by Hori *et al.* released encapsulated cytokines over a period of 1–2 weeks and remained intact for 21 days (Figure 3A) [46],[47]. This raises an important question on how the release rate of encapsulated cytokines/chemokines and degradation of depot could potentially affect the recruitment, infiltration, phagocytosis, and homing of immune cells in response to hydrogels and implanted scaffolds. To answer these questions, hydrogels with modular design are needed with preferable injectable characteristics. As discussed earlier, Michael type addition hydrogels offer a unique ability to encapsulate cells and deliver proteins at physiological conditions, 37 °C and pH ranging from 7.1–7.8. In particular, the conjugate addition of thiols onto unsaturated esters offers several benefits including high speed, easy preparation or availability of the unsaturated polymer, and, most importantly, it provides the least influence of competing nucleophiles (for example, at physiological pH, the rate of reaction of amines is slower than thiols). Michael type addition between thiol-modified polymers and acrylate or vinylsulfone modified polymers have been explored by several research groups to form hydrogels [80],[30],[42],[40],[81].

We have engineered dextran [82],[83] and PEG-based *in situ* crosslinkable hydrogels for immunomodulation against lymphoid malignancies (Figure 3E), specifically B-cell lymphoma. In these studies the goal was to encapsulate antigen/adjuvant carrying vaccine along with DC chemo-attractants within an *in situ* crosslinking hydrogel and deliver them as a single injectable formulation. To effectively synchronize with the timeline of DC trafficking, hydrogel design required that the polymer network to degrade in a timely fashion (i.e. within a 3–4 days) such that (a) the chemo-attractants are released slowly over a 2–3 day period thereby attracting large number of immature DCs to the site of injection and (b) at the same time vaccine is released from the gel (due to gel degradation) and is available for uptake by the attracted naïve DCs. For injectability and controlled degradability (slow

and fast-), we engineered Michael-type addition gels by combining vinyl sulfone functionalized dextran with 4-arm-thiolated PEG. Immunomodulation was achieved by simultaneous delivery of small interfering RNA (inhibiting T-cell suppressive cytokines) and tumor-gene encoding DNA to same DC, using a single particle carrier [24]. When compared with conventional PEG diacrylate based Michael-type addition hydrogels [30], dextran based gels swelled significantly less making them more suitable for injections as vaccine centers. This approach resulted in robust anti-tumor biased cellular immune response in a prophylactic, mouse tumor model of lymphoma as a function of degradation rate of the hydrogel with those degrading within 2 days inducing the maximum cellular immune response [23].

Using the same dextran vinyl sulfone immune priming centers, in a more recent study, Pradhan *et al.* [48] co-delivered three immunomodulatory molecules: Toll like receptor 9 stimulating and Th1-biasing CpG oligonucleotides, Th1 biasing IL-10 siRNA, and weakly immunogenic lymphoma idiotype DNA antigen. Hydrogels were used to chemoattract naïve DCs, *in vivo*. In mice challenged with lethal dose of lymphoma tumor cells, hydrogel based immunomodulation outperformed non-hydrogel groups by extending the median survival of mice to 39 days (Figure 3E) Using these hydrogels and co-delivering IL-10 siRNA and DNA vaccine, we have shown ~ 40% animal survival over 60 days and in studies by Pallab *et al.*, 30% mice were tumor free in CpG/IL-10siRNA/DNA hydrogel group and alive post 80 days as compared to only less than 15% surviving with naked DNA vaccine. Immunomodulatory hydrogels with the combination of CpG, IL-10 siRNA and DNA vaccine outperformed another combination of CpG with poly I:C adjuvant and DNA vaccine. It is noteworthy that the combinatorial hydrogel immunotherapy developed by Singh *et al.* [23] and Pradhan *et al.* [48] report robust induction of cellular immunity, yet only moderate 30–40% animal survival is observed against lethal lymphoma challenge. This anti-lymphoma response is still superior compared to other tested vaccine delivery platforms. Certainly, at the moment, the *in vivo* protection is below the levels needed to effectively translate these hydrogel vaccine into clinic against B-cell Lymphoma. We speculate that future direction should focus on overcoming the immunosuppressive tumor microenvironment, understanding the role of immune tolerance with very high immune response, and taking into consideration that lymphoma originates in lymph nodes, the primary site of immune reaction, which could play a key role in further suppressing the immune response.

7. Micro and Nanoengineered Hydrogel Vaccines

Numerous materials and strategies have been explored to engineer micro- and nanoscale particles for immunomodulation application. A detailed discussion on such micro or nanoscale approaches can be found in some recent reviews [10],[11],[84],[9]. Nanoengineered hydrogels represent an innovative platform for the protection and delivery of proteins and peptides to the sampling DCs leading to T-cell stimulation and B-cell mediated antibody response.

Sexton *et al.* [70] developed LBL-assembled disulfide poly(methacrylic acid) (PMASH) hydrogel capsules as immunogenic vaccines with ability to encapsulate model antigen

ovalbumin (OVA) as well as multiple OVA peptides. LBL assembly has proven to be a robust and versatile technique for synthesizing polymer capsules, largely due to the ability to fine-tune the physicochemical properties and functionality of the obtained capsules. LBL-assembled hydrogel capsules provide a fine control over size and material composition, allowing for tunable loading of the vaccine cargo that can be intracellularly processed by DCs and other APCs. Therapeutic molecules were immobilized onto the surface of a template particle and localized within polymer hydrogel capsules. Once the polymer thin film was fabricated, the core particles were removed.

It is important to note that in these hydrogels, therapeutic molecule encapsulation relied on both size exclusion and electrostatic repulsion provided by the negatively charged capsule. This protective hydrogel vaccine induced OVA-specific CD4⁺ and CD8⁺ T-cell response in transgenic mouse model of OVA tumor. The particulate nature of the capsule makes them easy target to APCs that phagocytose them. Such vaccines not only provide high payload ability but also a protective environment to degradable antigens. Interestingly, conversion of thiol moieties on PMASH make them stable under oxidative extracellular microenvironment however allows for degradation under reducing intracellular conditions, allowing for intracellular delivery of vaccine components. The kinetics of intracellular delivery and T-cell stimulation was dependent on several competing factors, such as the degree of substitution (thiolation) and number of deposited polymer layers.

Another interesting strategy is the use of fibrillized peptides, peptidomimetics, and peptide derivatives for immunomodulation purposes. Injectable self-assembling peptide nanofibers have been studied to create intramyocardial microenvironments and prevention of glial scar formation and axon elongation after spinal cord injury [85],[86]. Rudra *et al.* reported self-assembling β -sheet nanofibers fused with short peptide epitopes from Plasmodium falciparum circumsporozoite (CS) protein, NANP(3). C57BL/6 mice primed with nanofibers-NANP(3) induced T-cell- and MyD88-dependent antibody responses that lasted up to 40 weeks, making these self-assembling biomaterials a robust self-adjuncting multi-antigenic immunotherapeutic platform [87]. Park *et al.* called engineered Nanolipogel (NLG) that encapsulate TGF- β inhibitor SB505 and IL-2 for stimulating T-cells [88]. The nanoparticles consisted of a core-shell structure of PEGylated liposomal coating. Since the goal was to deliver multiple immunomodulators altogether, the bioactive molecules were encapsulated inside the core surrounded by a hydrogel that consisted of water-soluble PEG, lactide groups, and terminal acrylate groups. Nanolipogel delivering SB505 and IL-2 not only activated CD8⁺ T-cells but also the natural killer (NK) cells, causing marked reduction in tumor mass and higher animal survival. Taken together, nanoscale gels are still in preliminary development stages but hold tremendous promise to provide a unique and attractive vaccine strategy for the control of several chronic diseases ranging from cancers to infectious diseases.

8. Scaffolds and hydrogels to induce systemic tolerance and reduce inflammation

Immunomodulation by suppressing immune cell activation plays a critical role in treating autoimmune diseases and inflammation. Adoptive transfer of *ex vivo* antigen primed

tolerogenic DCs generated from myeloid precursors could suppress autoimmune response by inducing activation-induced cell death, anergy (a tolerance mechanism), and interaction with regulatory T-cells. Compelling preclinical results indicate that adoptive transfer of regulatory T-cells (T_{Reg} cells) can prevent or cure autoimmune diseases and allograft rejection (transplants), by restoring immune tolerance to self-antigens or alloantigens [89]. T_{Reg} cell immunotherapy has been employed to modulate the immune response in preventing graft rejection after hematopoietic stem-cell transplantation (HSCT) after bone marrow transplantation. T_{Reg} cells function by suppressing effector T-cells (CD4 and CD8) as well as regulate the activation of DCs by creating a microenvironment rich in immunosuppressive cytokine IL-10 and TGF-β1, and often through cell-cell interactions. Various CD4⁺ regulatory T-cell subsets have been extensively reviewed elsewhere [89] and are beyond the scope of this review. Here we specifically review a subset of CD4⁺ T helper cells phenotypically characterized as forkhead box P3 (FoxP3)⁺ CD4⁺CD25⁺ cells, commonly called T_{Reg} cells.

Studies in preclinical models indicate that polyclonal T_{Reg} cells can prevent autoimmune diseases, whereas only self-antigen-specific T_{Reg} cells can cure active autoimmunity. Similar to *ex vivo* priming of DCs, antigen-specific T_{Reg} cells can be generated *ex vivo* by exposing CD4⁺ T helper cells to TGF-β1 in the presence of DCs and target self-antigen such as insulin and GAD65 for type 1 diabetes mellitus myelin basic protein in multiple sclerosis, and type II collagen in rheumatoid arthritis [90],[91].

Type 1 diabetes, a disease in which the insulin producing β-cells are destroyed due to autoimmunity (Figure 4), is currently being treated in clinics by infusion of cadaver derived allogeneic pancreatic islet cells. Islet transplantation requires administration of immunosuppressive drugs to nonspecifically suppress hyperactive immune system in patient's body which makes them prone to infections and increased chances of neoplasia. In Type 1 diabetes, several preclinical models have shown that the transfer of *ex vivo* primed T_{Reg} cells can block disease development. Shea and colleagues have explored the possibility to establish islet antigen (BDC2.5 mimotope)-specific immune tolerance using *ex vivo* primed T_{Reg} cells in macroporous PLG scaffolds [72]. The study demonstrated that co-localized T_{Reg} cells could protect islet grafts in an extra-hepatic and extra-renal transplant site with robust insulin production and co-localized Foxp3⁺ T_{Reg} cells around islets. The functional design of macroporous scaffolds permitted efficient seeding of islets and T_{Reg} cells and shielded the transplanted islets from transplant associated inflammation and diabetogenic immunosuppression therapy. This effect, however, could also be attributed to the immunomodulatory properties of antigen-specific T_{Reg} cells along with direct cell-cell interactions. In summary, this study highlights the importance of scaffold-based immunomodulation using *ex vivo* primed T_{Reg} cells for enhanced islet survival and engraftment and addresses several shortcomings of current islet transplant-based clinical approaches to treat **type 1 diabetes mellitus (T1DM)**.

Another important area of hyperactive immune condition is rheumatoid arthritis (RA). Similar to T1DM, DCs and T-cells are central to immune tolerance in rheumatoid arthritis in their role as professional APCs and T_{Reg} cells, respectively. Immunomodulatory therapies for RA are aimed towards exploiting the tolerogenic capacity of DCs, behavior of T_{Reg} cells,

and targeted suppression of specific cytokines such as IL-1, 6, and 17 [92]. Several of these aspects are illustrated in Figure 5A and have been discussed elegantly in reviews by others. Similar to RA, inflammatory response is prominent in osteoarthritis (OA) (Figure 5A). Although the OA levels of pro-inflammatory cytokines are lower than those observed in rheumatoid arthritis, IL-1 and tumor necrosis factor- α (TNF- α) have been implicated in OA pathogenesis by promoting synovial inflammation and activating chondrocytes and synovial fibroblasts [93],[94],[95]. These cytokines stimulate their own production and induce synovial cells and chondrocytes to express IL-6, IL-8, and other inflammatory mediators as well as proteases and prostaglandins [93],[94],[95].

We recently reported self-assembling nanoparticles presenting IL-1 receptor antagonist (IL-1Ra) for enhanced delivery, retention, and bioactivity in the OA joint [96]. We demonstrated a RAFT-chemistry based self-assembly nanoparticles (300 nm) that efficiently bound IL-1Ra, targeted fibroblast-like synoviocytes (FLS) that are located inside joints in the synovium and inhibited IL-1 β mediated signaling [96]. These 300 nm nanoparticles demonstrated significantly longer retention time of IL-1Ra in the rat stifle joint compared to that of soluble IL-1Ra and no adverse effect on the cartilage structure in the joint.

We next engineered nanoparticles made of hydrogel materials PHEMA, modified with hydrophobic pyridine (Figure 5B). The unique hydrophilic/hydrophobic balance of properties of the polymer allowed us to precisely control the size of nanoparticles in the 300–900 nm range and demonstrated a size dependent retention of protein-nanoparticle in rat knee joint with 900 nm protein particles remained localized to the knee for at least 14 days compared to bolus protein that was depleted within hours of injection. These nanoparticles made from hydrogel forming biomaterials can have a wide range of immunomodulatory applications for protein, peptides, and other therapeutic delivery to treat inflammation and can be expanded to treat diseases like infections and cancer.

9. Outlook and Future Perspective

The advancement and rapid translation of recent experimental scaffold vaccines into human clinical trials have emphasized the need for engineered strategies to quantitatively manipulate, *in situ*, immune cell recruitment, activation, and homing to the draining lymph nodes. Novel engineered macro- to nanoscale hydrogels and scaffolds continue to show promise as immunomodulatory platforms against cancer, infection, and autoimmunity. Clinically, tumors like melanoma, multiple myeloma, and lymphoma are poorly immunogenic because the target-antigens are often non-mutated self-antigens, making it difficult to induce or enhance an anti-tumor immune response. Therapeutic vaccines for B-cell non-Hodgkin lymphoma using the clonal tumor immunoglobulin idiotype, have been under development for more than three decades. Results from some of these Phase III trials have recently been released and in essence most of them failed to achieve their primary endpoints. Failure to eliminate poorly immunogenic cancers is often because of inadequate priming, low cell numbers and suboptimal phenotype of effector T-cells. These barriers could be overcome by biomaterials-based immune-engineering approaches that lie at the interface between material science and immunology. We recently showed that the above barriers in lymphoma could be “partially” overcome by applying bioengineering approaches

to precisely bias the type of immune response. There is also a need to engineer systems that could manipulate diseased tissue microenvironment. Likewise, lymphoid tissue engineering is another promising arena for personalized immunotherapy however several aspects of the DC, B, and T-cell development like positive selection, antigen specificity, graft versus host rejection upon transplantation needs to be addressed. Although, initial studies in adoptive DC therapy have shown promising immune response, there exist several major limitations, including morbidity associated with patient cell isolation, high cost of *ex vivo* cell manipulation, time lag in “training” the immune cells, regulatory concerns, as well as the fact that ~90% of transplanted DCs die before they home to lymph nodes [45]. Nevertheless, with such provocative possibilities of generating secondary lymphoid tissues, thymus, and immune cells *ex vivo* from stem cells, new possibilities towards more effective personalized adoptive immunotherapy remain opened.

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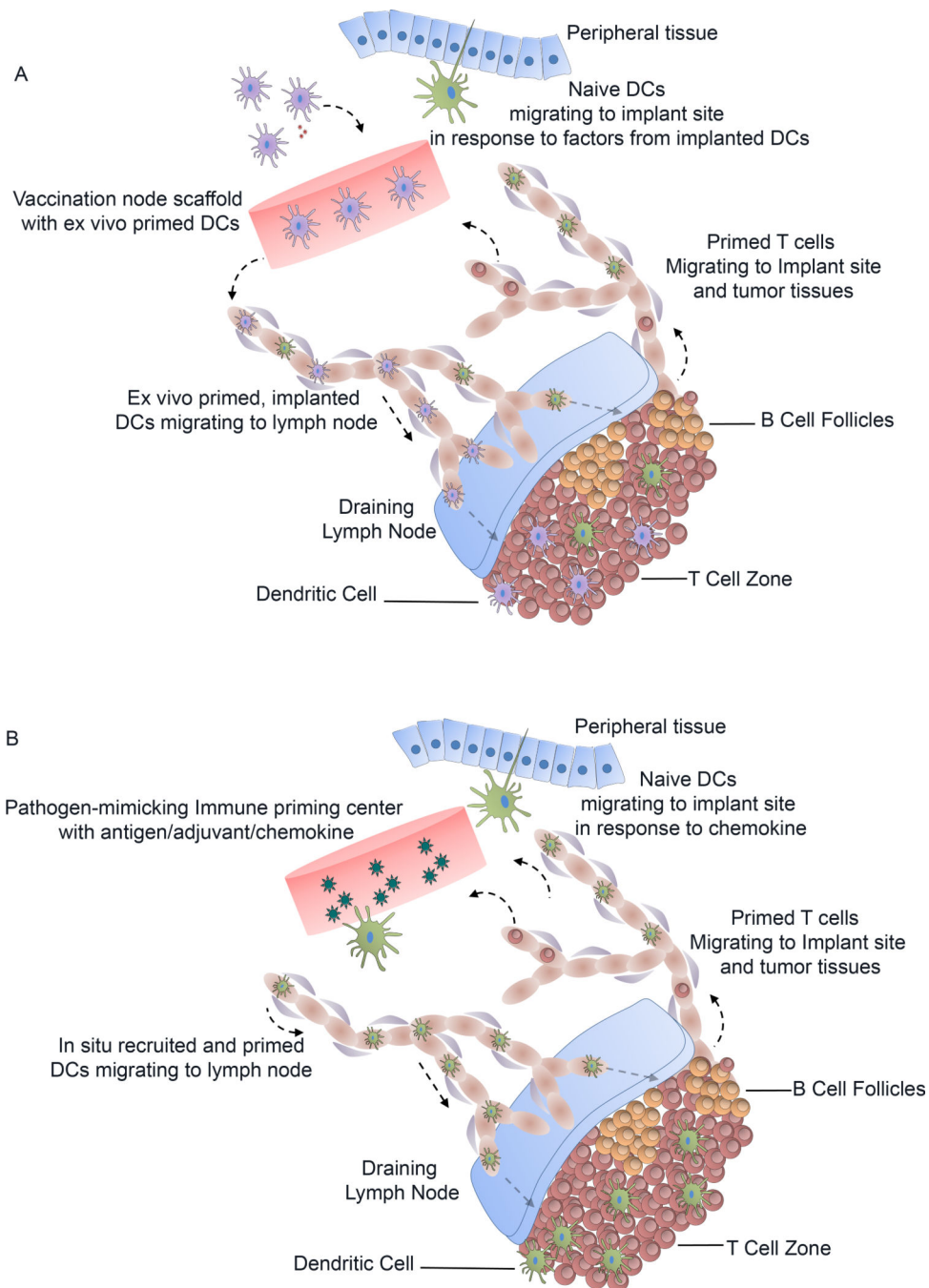


Figure 1. Dendritic cell trafficking and modulation using scaffolds

A) Cell-loaded vaccination nodes: Ex vivo primed DC (purple) are delivered through injections or implantation of pre-fabricated scaffolds, subcutaneously into mice. A few of the programmed DCs migrate to the draining lymph nodes and present antigen to the T-cells in T-cell zone. Cytokines and chemokines secreted by programmed DCs at the implantation site recruits host's own naïve DCs (green) and programmed T-cells to induce a robust immune response. B) Pathogen-mimicking immune centers with no cells: *In situ* crosslinked hydrogels or implanted scaffolds release chemokines and growth factors in the tissue to

attract naïve DCs. Recruited DCs engulf antigenic vaccines and return to draining lymph node to present antigen to naïve T helper cells (CD4+ T-cells). Programmed T-cells can then migrate to the implant site or nearby tumor regions to destroy malignant cells.

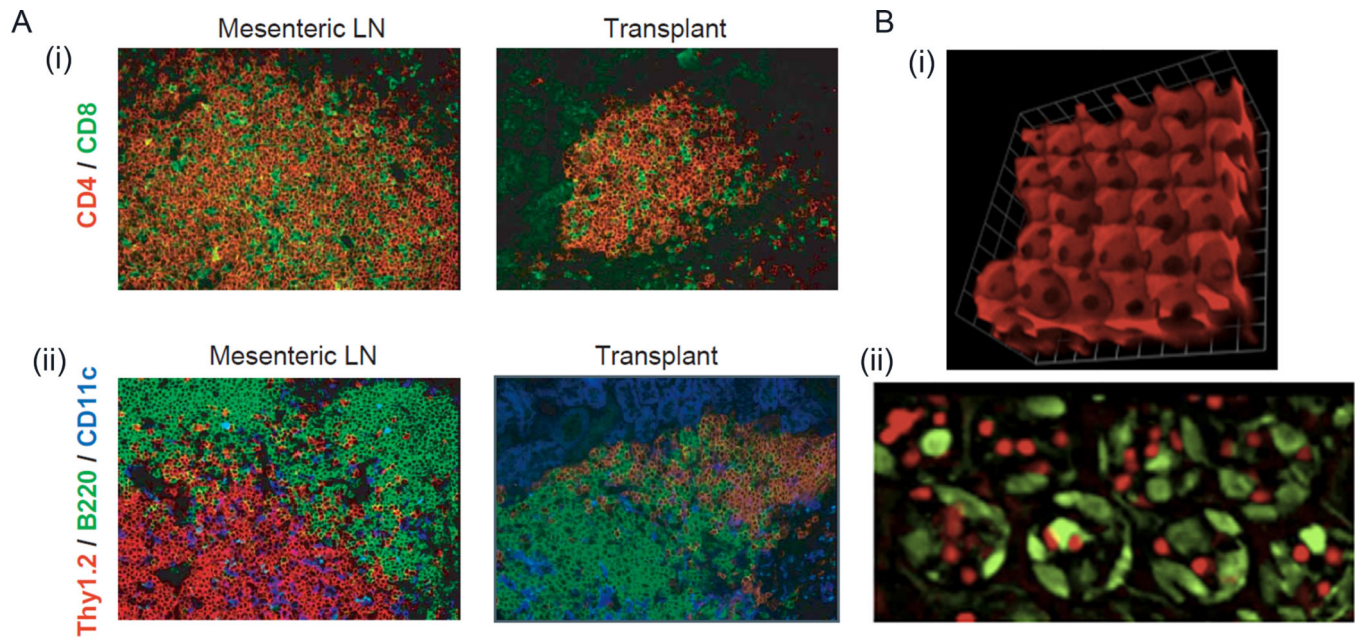


Figure 2. Lymphoid tissue engineering using scaffolds

A) Naturally occurring collagen-based lymphoid organoids. When transplanted in mouse, tissue-engineered organoids are structure similar to secondary lymphoid organs such as mesenteric lymph node (LN). (i) CD4⁺ T-cells in stained red and CD8⁺ T-cells are stained green. (ii) Mesenteric LN and transplant were stained for Thy1.2⁺ T-cells in red, B220⁺ B-cells in green and CD11c⁺ DCs in blue. B) Composite PEG-based inverse opal scaffolds: (i) Image represents an oblique view of fluorescently-labeled inverse opal PEG scaffolds with macroscale pores. The interior of these scaffolds could be decorated with cell supportive ECM proteins such as fibronectin or laminin. (ii) Plan view of inverse opal scaffolds with naive CD4⁺ T-cells (red) interacting with DCs (green) that are spread on the interior side of the ECM protein-conjugated PEG scaffold. Reproduced with permission ^{[73],[74]}.

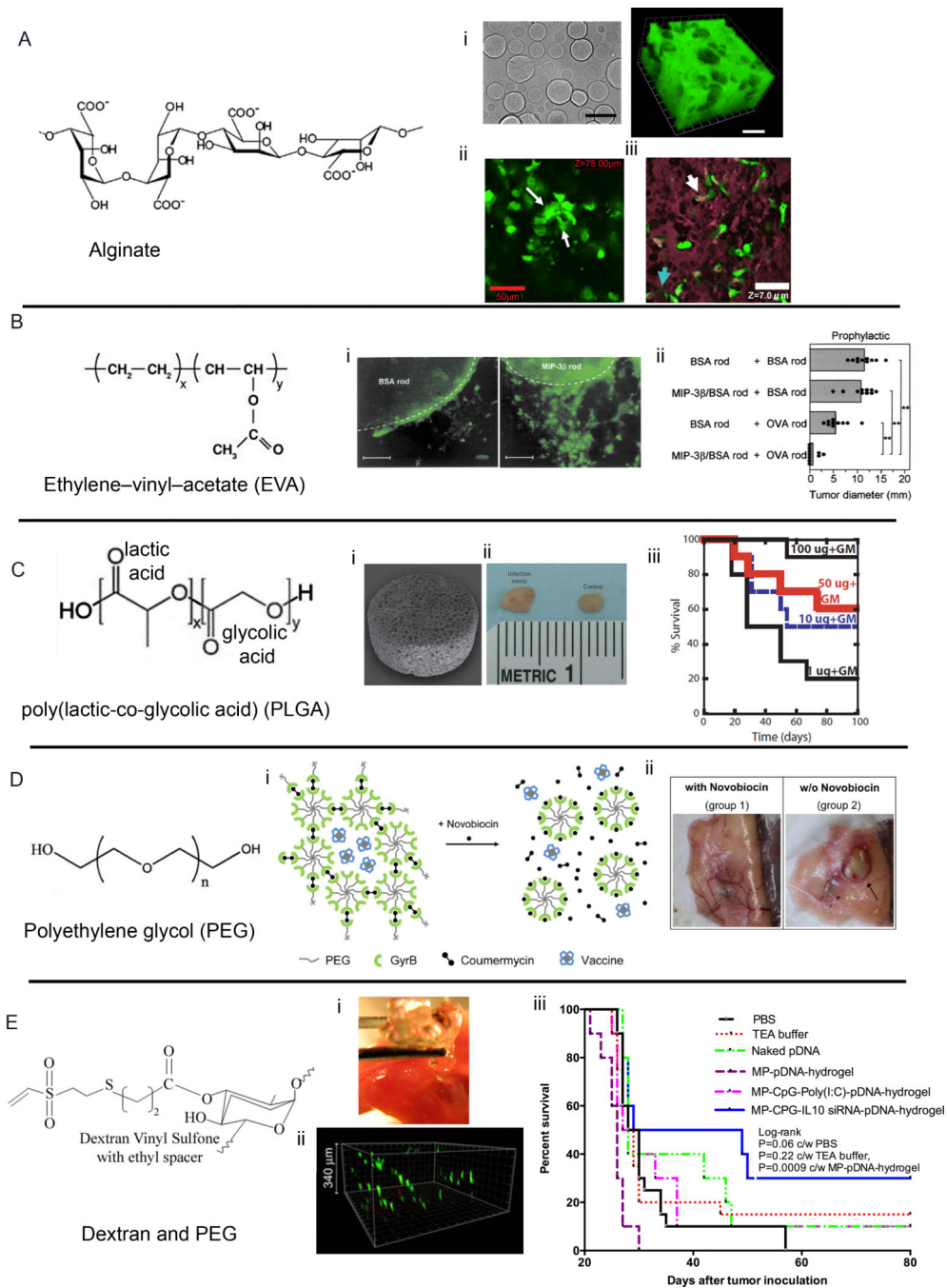


Figure 3. Injectable and implantable strategies for immunomodulation

A) *In situ* crosslinkable alginate gel co-delivering immunomodulatory factors. (i) 3D injectable alginate gel with calcium-crosslinked alginate microspheres. Alginate microspheres (non-fluorescent voids) were distributed throughout the fluorescent alginate matrix. (ii) GFP⁺ DCs in alginate hydrogels, explanted 22 h after s.c. injection in C57Bl/6 mice. White arrows indicate spread DCs. (iii) Infiltrating GFP⁺ cells infiltrate and occupy void spaces in porous alginate matrix (purple) explanted from mice 48 h after injection Scale bars: 50 μ m. **B)** Implantable EVA rods with chemokines and antigens. (i) Accumulation of

Langerhans cells around BSA rod (left) and MIP-3 β rod (right) implanted in mice and examined after 24 h. Original magnification, 200; scale bars, 100 μ m. (ii) Co-implantation of MIP-3 β rods and OVA rods initiates protective immunity in mice challenged with E.G7-OVA tumor cells in the scapular region five days after rod implantation (C) Engineered PLGA scaffold vaccine against tumor (i) SEM of PLGA scaffold. (ii) Photograph of lymph nodes from control mice and infection mimic mice after 10 days of implantation of matrices incorporating 10 μ g CpG-ODN+3,000 ng GM-CSF. (iii) Survival times of mice vaccinated with PLGA vaccines 14 days before B16-F10 melanoma tumor challenge. (D) Drug-responsive hydrogel vaccine depot. (i) Gyrase B (Gyr B) functionalized 8-arm PEG hydrogel providing a molecular switch based on the interaction of the GyrB to the aminocoumarin antibiotics coumermycin and novobiocin. (ii) Mice were sacrificed at day 98 after treatment with or without Novobiocin. Non-dissolved hydrogel of the group 2 mouse is indicated by an arrow. (E) An injectable synthetic immune-priming center made of Dextran Vinyl Sulfone and 4-arm PEG-SH mediates efficient T-cell class switching and T-helper 1 response against B-cell lymphoma (i) Formation of *in situ* crosslinkable hydrogels in mouse quad muscle, retrieved after 8 h post injection. (ii) Primary DC infiltration through 3D hydrogels (iii) Kaplan–Meier survival curve indicating protection against A20 B-lymphoma in Balb/C immunized mice with various indicated formulations. Reproduced with permission [64],[46],[47],[48],[23],[24]

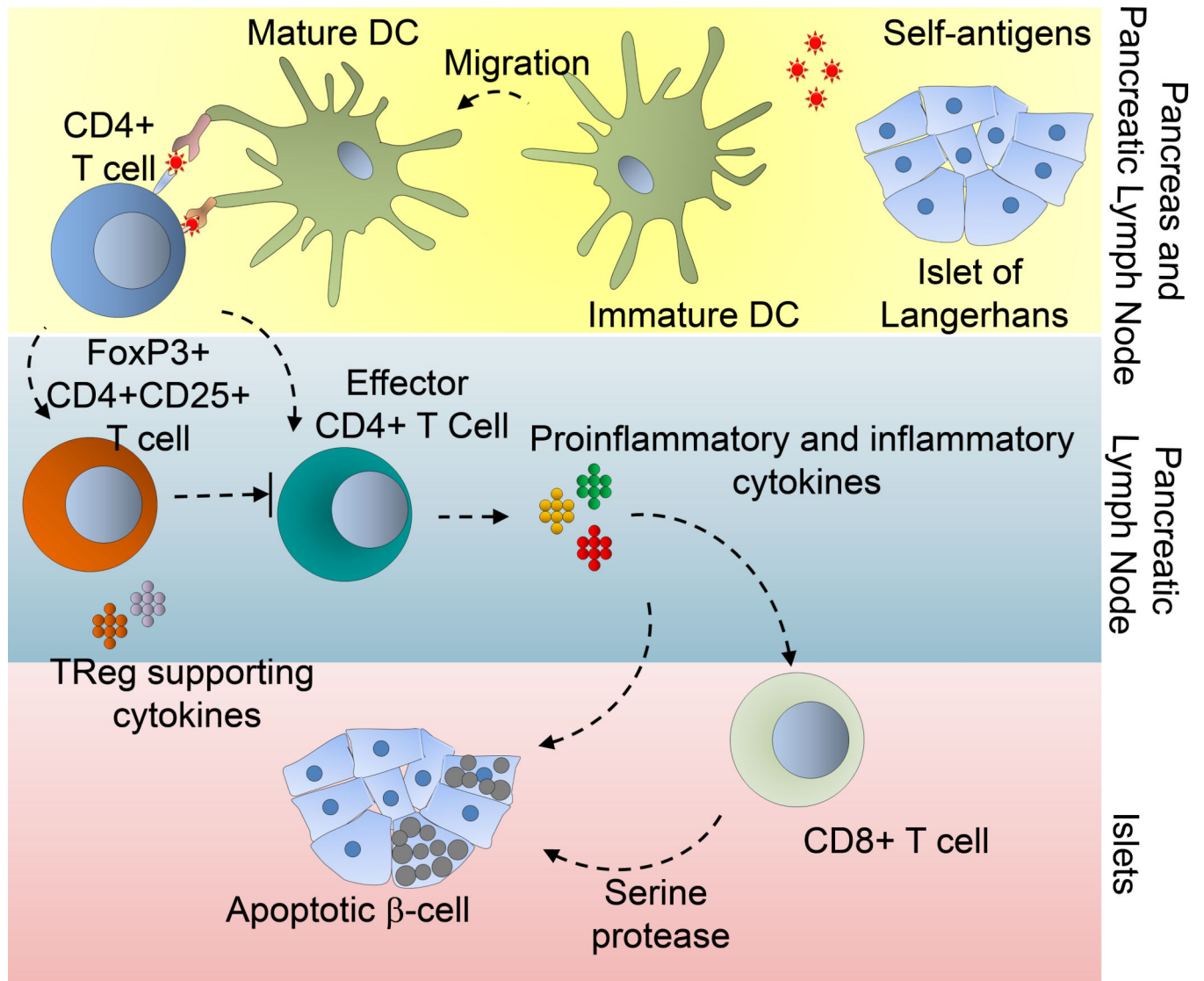


Figure 4. Scaffold based immunomodulation of T_{Reg} cells in type 1 diabetes mellitus (T1DM)
Schematic represents immunological mechanism associated with T1DM leading to apoptosis of pancreatic β -cell islets. Islets can be encapsulated in scaffolds made of polymeric materials such as PLGA. Protection of scaffold transplanted islets by T_{Reg} cells can be associated with insulin production and Foxp3+ T_{Reg} co-localization around islets.

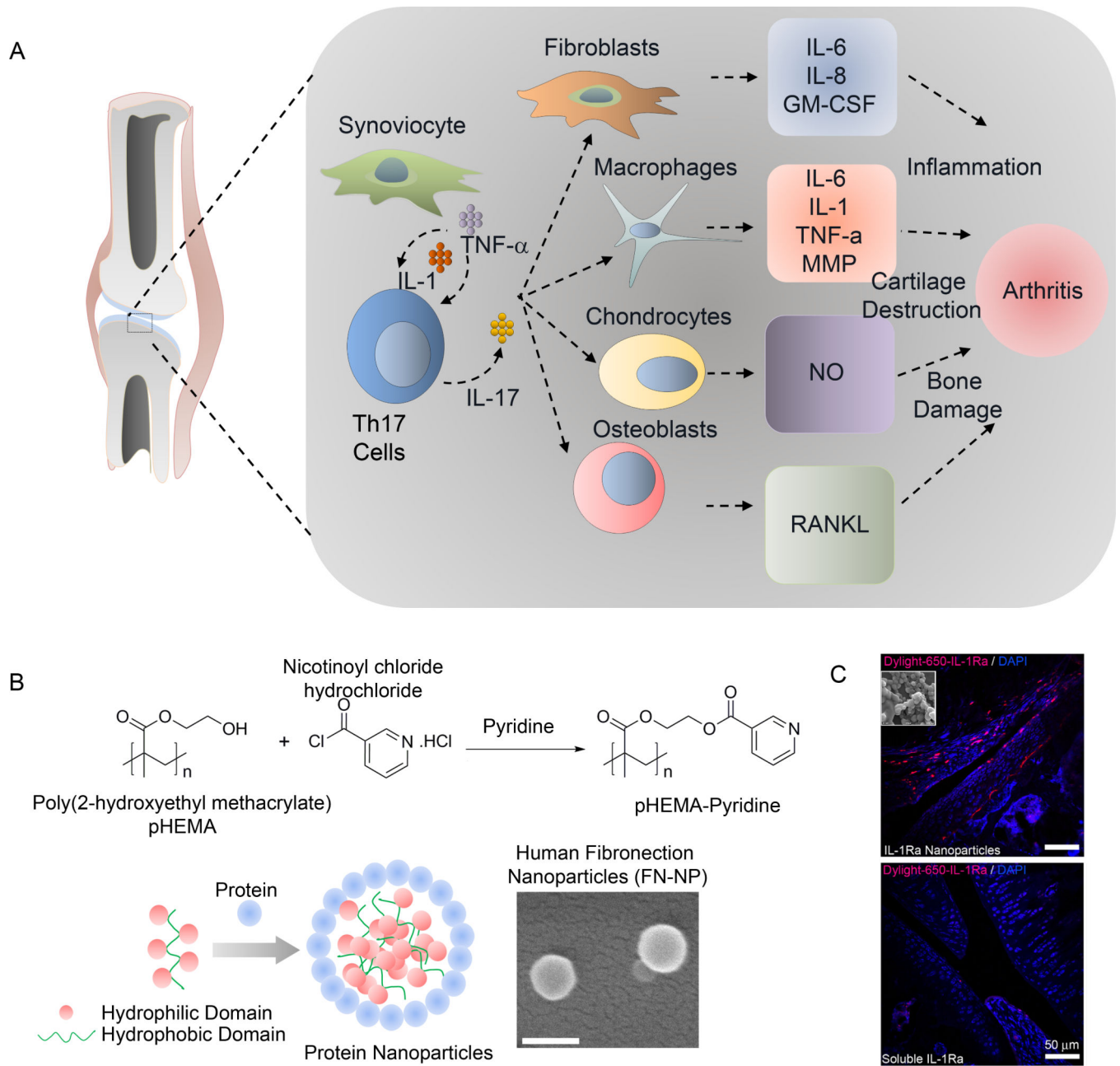


Figure 5. Immunomodulation in arthritis

A) Schematic of immune regulation in arthritis; B) hydrogel nanoparticle-based for enhanced knee joint retention to reduce inflammation in osteoarthritis. Schematic of nanoparticle self-assembly based on protein/polymer complexation. PHEMA-pyridine was synthesized by reacting PHEMA with nicotinoyl chloride hydrochloride in tetrahydrofuran and pyridine. SEM images of Fibronectin nanoparticles (FN-NP, Scale: 200 nm); C) IL-1Ra-tethered particles are distributed throughout the intra-articular joint space. IL-1Ra was tagged with a Dylight-IR-650 dye prior to tethering IL-1Ra to particles. Tagged IL-1Ra-tethered particles or soluble IL-1Ra was injected into the right stifle joint of 8–10 wk old rats while the left stifle joints received saline. Cryosectioned samples were counterstained

with DAPI to localize dye tagged protein. Scale bar = 50 μm . Reproduced with permission ^{[97],[96]}.