Review Article

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Porous Materials for Immune Modulation

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Abstract: Biocompatible materials have a great potential to engineer immunology towards therapeutic applications. Among them, porous materials have attracted much attention for immune modulation due to their unique porous structure. The large surface area and pore space offer high loading capacity for various payloads including peptides, proteins and even cells. We first introduce recent developments in the porous particles that can deliver immunomodulatory agents to antigen presenting cells for immunomodulation. Then, we review recent developments in the porous implants that can act as a cell-attracting/delivering platform to generate artificial immunomodulatory environments in the body. Lastly, we summarize recent findings of immunogenic porous materials that can induce strong immune responses without additional adjuvants. We also discuss future direction of porous materials to enhance their immunomodulatory potential for immunotherapeutic applications.

Keywords: cell recruitment, drug delivery, immunogenicity, immunotherapy, porous materials

1 Introduction

Biocompatible porous materials with nanometer- or micrometer-sized pores have been widely used for biological applications because of their unique characteristics obtained from porous structure [1]. Owing to large surface area and pore space, porous materials have tremendous potential for encapsulating various biological payloads including small molecules, peptides, proteins, antibodies, and cells. Loading capacity and release kinetics of payloads can be controlled by adjusting porosity and pore size of the materials [2–4]. Particularly, their abilities for stable loading and controlled release of large biological payloads such as proteins and cells, give porous materials a great potential for immunological applications that need the delivery of such payloads.

During the past decades, immunotherapy has been dramatically developed for cancer and infectious diseases with a better understanding of the mechanisms. Immunotherapy is an immunomodulatory process that activates immune cells to be therapeutic or inhibits immune cells to be tolerogenic. In the immunomodulatory process, antigen presenting cells (APCs) like dendritic cells (DCs) are key players as they can induce T cell immunity for specific antigens by delivering stimulatory or inhibitory signals to T cells [5, 6]. Therefore, it is important to 'educate' DCs effectively to initiate adaptive immune responses for target antigens. For activation of adaptive immunity, DCs take up target antigens, sometimes along with immunostimulatory adjuvant or immunoregulatory molecules [7], and migrate to the specific biological environments such as lymph node and spleen for T cell activation. Several immune signals are then involved when DCs activate T cells, such as antigen presentation (signal 1), co-stimulatory molecules (signal 2), and cytokines (signal 3). Antigen-specific T cell receptor recognizes the antigen peptide in the major histocompatibility complex (MHC) of DCs. If the antigen presentation is combined with binding of co-stimulatory molecules present on the surface of DCs and T cells and paracrine delivery of cytokines from DCs to T cells, it induces antigen-specific T cell activation. Therefore, in order to facilitate such processes, porous materials can be used to deliver antigens and adjuvants simultaneously to DCs for enhanced immunogenicity [8, 9], and to recruit DCs into the micrometer-scale pores for effective education [10–12].

Recently, there has been an increase in the development of porous materials for immunotherapy [13] (Figure 1). Porous nanoparticles can deliver antigens and adjuvants simultaneously and efficiently to DCs to induce strong antigen-specific immune response [14–16]. Beyond this classical ability, it has been reported that porous materials such as mesoporous silica and porous silicon particles possessed intrinsic immunogenicity [17, 18]. Since these immunogenic particles can act as adju-

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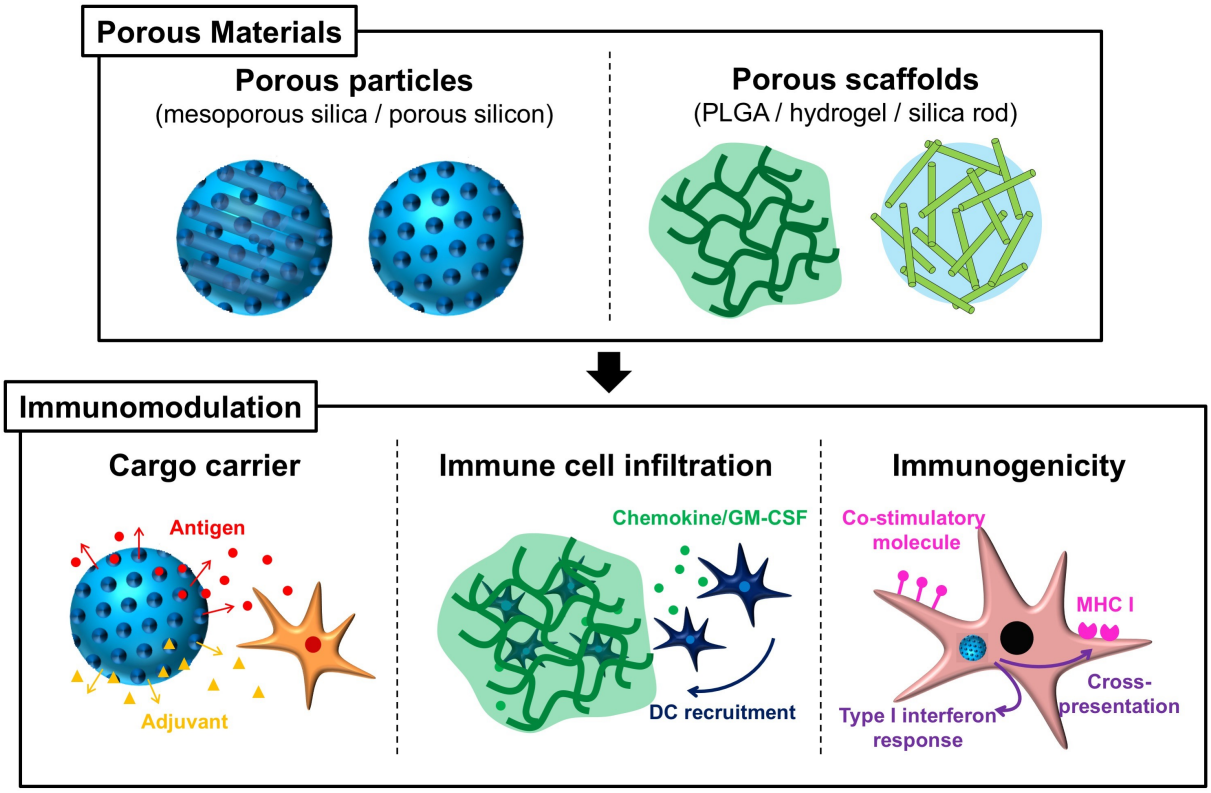

Figure 1: Schematic representation of porous materials-based immunomodulation.

vant itself, it does not need to co-deliver immune adjuvants together with antigens for immunotherapy. In addition, macroporous materials or scaffolds provide a platform for three-dimensional cellular microenvironments to recruit and educate immune cells in the pores pre-loaded with chemoattractant and antigen molecules [19, 20]. Furthermore, porous materials with cell-embracing capacity can be loaded with activated immune cells and be implanted in the body to enhance immune modulation [21–23]. Thus, this review aims to discuss current status and future prospects of porous materials for immunomodulatory and immunotherapeutic applications.

2 Porous particles as carriers

Porous particles have been used mainly as cargo carriers in the immunotherapeutic field owing to their controllable porous nanostructure. Various molecules involved in immune modulation such as peptide or protein antigens, adjuvants, cytokines and drugs can be easily adsorbed in the nanopores of particles and can sustain their bioactivity until release. In order to achieve effective immune modulation, porous particles can co-deliver antigens and adjuvants to DCs through their multiple-cargo loading capacity. In addition, APCs such as DCs and macrophages can take up the porous particles easily due to their phagocytic properties [24, 25]. Furthermore, these porous particles can be engineered to enhance uptake, activation, and antigen-specific immune response in DCs by controlling their size and surface charge and attaching targeting ligands on their surface [26–31]. In this section, we mainly discuss mesoporous silica and porous silicon particles as cargo carriers for immunomodulation. Their applications include conventional immunotherapy and chemo-immunotherapy.

2.1 Mesoporous silica particles

Mesoporous silica (SiO_2) particles are the representative porous carrier for drug delivery due to large pore volume

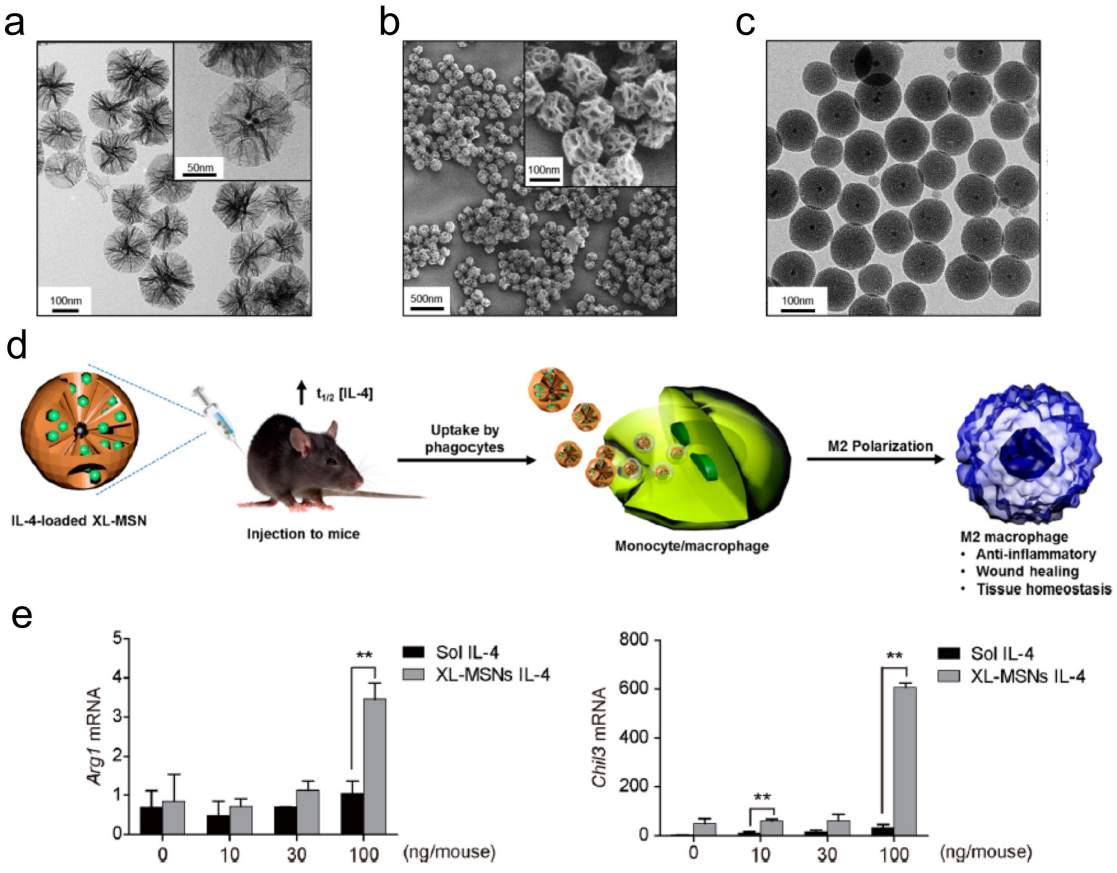

Figure 2: Immune modulation with mesoporous silica particles (a) Scanning electron microscopic (SEM) images and (b) Transmission electron microscopic (TEM) images of extra-large pore mesoporous silica nanoparticles (XL-MSN). (c) TEM image of conventional mesoporous silica nanoparticle. (d) Schematic presentation of XL-MSN-mediated IL-4 delivery for *in vivo* M2 macrophage polarization. (e) RT-qPCR results of *Arg*1 and *Chil3* mRNA levels normalized to the mRNA level of F4/80. Data are representative of three independent experiments (n=3, mean±S.D.) $^{**}p<0.01$. Reproduced with permission from ref. [30] Copyright © 2017 American Chemical Society.

and surface area that come from their porous nanostructure. Their particle and pore size can be controlled easily for various biomedical applications, in the range of 50 to 300 nm and 2 to 6 nm, respectively [2]. Mesoporous silica particles with hexagonal symmetry type are the most common forms, which include Mobil Composition of Matter (MCM)-41, Santa Barbara Amorphous (SBA)-3 and SBA-15 [32]. Hexagonal symmetric mesoporous silica particles are produced via silication and calcination through incubation of aretetraethyl orthosilicate (TEOS) and pre-stirred trimethylammonium bromide (CTAB) in ddH2O and NaOH solvent at 80°C and removal of CTAB surfactant in acidic methanol at 80°C [33, 34]. Small drugs, peptides and even proteins can be loaded easily in spherical or cylindrical pores of the particle through simple methods such as adsorption and solvent evaporation method [35]. Release kinetics of payloads is controllable with the shape and size of the pore. Since most of mesoporous silica particles are biocompatible [36] and biodegradable [37, 38], sustained release for a long period is also possible [39]. Owing to these characteristics, mesoporous silica particles have been studied as cargo carriers for effective immunotherapy.

Mesoporous silica particles can deliver various payloads to APCs for immunomodulation and immunotherapy. Kwon *et al.* produced extra-large pore mesoporous silica nanoparticles (XL-MSN) with a pore size of ~30 nm to improve the loading of Interleukin-4 (IL-4) in the pores [26]. Compared to conventional mesoporous particles, XL-MSN increased loading capacity of IL-4, and induced macrophage polarization toward M2 for modulating immune system *in vivo* (Figure 2). In addition, large-pore mesoporous silica nanoparticles can be engineered to release large payloads in response to specific biological environments. Chiu *et al.* loaded His6-tagged chromobodies into the nitrilotriacetic acid-metal ion complex-

modified pore surface of mesoporous silica particles for intracellular chromobody delivery [40]. Since this chemical conjugation broke down in acidic environments, chromobodies were released from the particles in late endosomes and lysosomes and finally functioned in cytosol. On the other hand, mesoporous silica particles with small pore size (<20 nm) can capacitate sustained release of antigens for long-term immunotherapy. Mody *et al.* developed hollow mesoporous silica nanoparticle-based vaccine which loaded E2 protein of the bovine viral diarrhea virus (BVDV) in the internal space of the particle [3]. Because the shell (thickness: 6 nm) of hollow mesoporous silica nanoparticles had pores with a diameter of 16 nm, E2 antigens were released from the nanoparticles over a prolonged period not in burst, and induced E2-specific antibody and cell-mediated response for more than 6 months with the single injection.

Mesoporous silica particles can be used to perform synergistic combination immunotherapy like chemo-immunotherapy due to multiple payloads loading capacity in the pore. Zheng *et al.* developed magnetic mesoporous silica particles which absorbed doxorubicin and CpG-oligodeoxynucleotides (ODN) adjuvant in the pore for synergistic effects of chemotherapy and immunotherapy [41]. Magnetic nanoparticles were embedded in the core of the silica particles to increase cellular uptake efficiency of the particles under external magnetic field. Tumor cell antigens produced from cell death by doxorubicin and CpG-ODN released from the particles activated DCs and then induced tumor antigen-specific immune response. Ultimo *et al.* prepared mesoporous silica nanoparticles that were loaded with doxorubicin and then capped with double-stranded RNA polyinosinic-polytidylic acid (Poly(I:C)) which is one of the toll like receptor 3 (TLR3) agonists [28]. These particles activated innate immunity with Poly(I:C) and killed cancer cells effectively via combined therapeutic effects of type I interferon and doxorubicin.

2.2 Porous silicon particles

Porous silicon (Si) particles are another useful material for immune modulation. In general, porous silicon is first fabricated by electrochemical etching of single crystalline Si wafer in hydrofluoric acid electrolyte solution [42]. Porous silicon particles are then prepared by ultrasonic dispersion of thin sections of porous silicon in aqueous or organic solvents. Pore size and shape are controllable by differing current patterns while particle size is controllable through duration of ultrasonication. In addition, high energy ball-milling process is used when pulverizing the porous silicon into particles through effective mechanical energy transfer between grinding media and substrate from collision. This method is suitable for large scale production because the operating conditions like temperature and pressure can be easily controlled [43]. Photolithographic method is also helpful to produce mono-dispersed porous silicon particles in massive amount. After electrochemical etching process, uniformly shaped porous silicon particles can be produced by patterning and shaping the porous silicon film with ultraviolet light exposure which ruptures the polymer chains [44]. Thus, porous silicon particles can be produced in various sizes with nanometer- or submicrometer-sized pores. Their porous nanostructure can be loaded with small drug molecules, peptides and proteins through covalent attachment, oxidation or spontaneous adsorption, and the loading capacity can be controlled with the porosity [42]. For immune modulation, size of porous silicon particles can be optimized to facilitate their uptake by DCs [24, 25] while the porous nanostructure can be used for simultaneous delivery of multiple immunomodulatory molecules [45].

Porous silicon particles are biocompatible [46–48], and biodegradable [42, 49, 50]. The surface of porous silicon particles is gradually oxidized and starts to degrade into orthosilicate (SiO_4^{4-}) in aqueous environments. Their by-product is also biosafe because there is the orthosilicate clearance system in our body [42]. Since porous silicon particles are degraded in the physiological conditions, release rate of payloads from the particles can be controlled with the degradation rate. The release rate can be further modulated with controlling pore size and shape. In case of bone morphogenetic protein 7 (BMP7) which has a molecular weight of ~30 kDa, most of proteins are released from the pores larger than 10 nm in 48 hours, while proteins keep being released from the pores smaller than 6 nm for more than 96 hours [51]. Owing to these characteristics, porous silicon particles have been studied as cargo carriers for effective immune modulation.

Porous silicon particles with high cargo loading capacity can induce effective immune response by delivering multiple immune-related molecules to APCs without losing their bioactivity. Gu *et al.* used luminescent porous silicon nanoparticles (LPSiNPs) for effective immune modulation with CD40 agonistic antibody (FGK45) [29]. The pore and surface of LPSiNPs were conjugated with high amount of FGK45 via biotin-avidin systems. FGK45-coated LPSiNPs showed high CD40-mediated DC targeting and uptake efficiencies because FGK45 specifically bound to CD40 and FcγRIIB receptors and induced receptor-mediated uptake. In addition, LPSiNP-mediated FGK45 delivery induced 30~40 times higher cellular responses than free FGK45

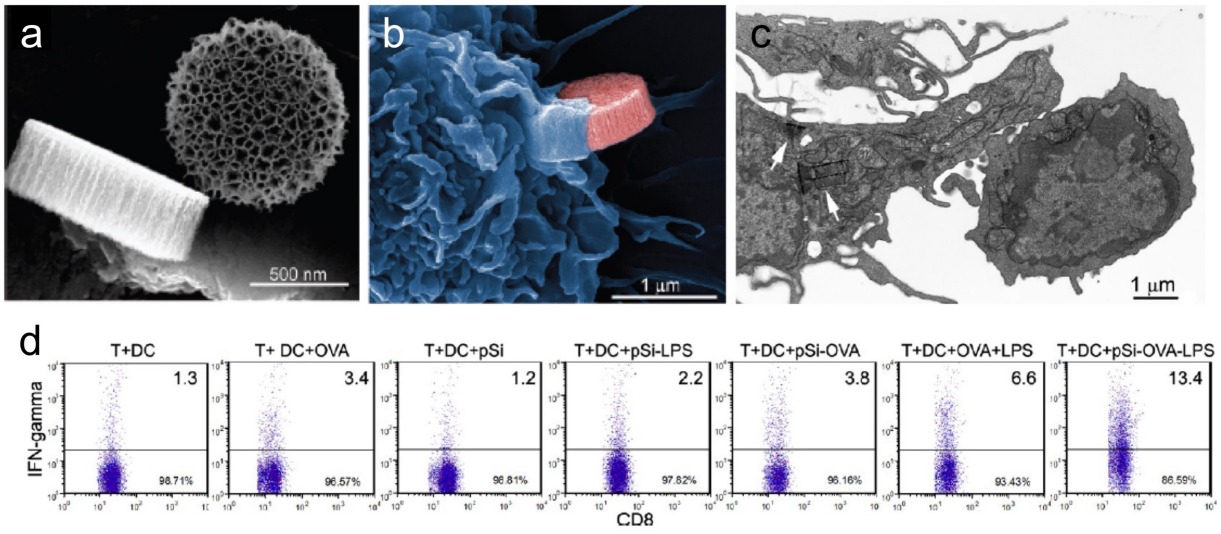

Figure 3: Immune modulation with porous silicon particles. (a) SEM image of discoidal porous silicon microparticles. (b) SEM image of granulocyte macrophage colony stimulating factor (GM-CSF) stimulated DC associated with microparticles. (c) TEM image of intracellular microparticles in the bone marrow derived dendritic cell (BMDC). (d) Flow cytometry analysis of intracellular IFN-γ levels in OT-1 $CD8^+$ T cells stimulated with C57BL/6 BMDC treated with free or microparticle-presented OVA peptide and LPS for 4 hours. Reproduced with permission from ref. [43] Copyright © 2012 American Chemical Society.

delivery. Meraz *et al.* developed immunogenic discoidal porous silicon microparticles where ovalbumin (OVA) peptides (SIINFEKL) were loaded in the pore and lipopolysaccharide (LPS) was attached on the surface [52]. This co-delivery of OVA antigen and LPS adjuvant increased MHC II and inflammasome expression in DCs compared to LPS delivery alone, inducing OVA-specific T cell immune responses and interferon-gamma (IFN-γ) production (Figure 3). Lundquist *et al.* prepared theranostic porous silicon microparticles loaded with superparamagnetic iron oxide nanoparticle (SPIONs) as well as OVA peptide and LPS [53]. These MRI-visible particles helped monitoring and understanding the particle accumulation in the biological systems.

Porous silicon particles can deliver chemotherapeutic drug molecules together with immunogenic molecules to perform chemo-immunotherapy. Shahbazi et al. fabricated undecylenic acid-modified thermally hydrocarbonized porous silicon nanoparticles (UnTHCPSiNP) in which the pores were loaded with sorafenib and the surface was conjugated with anti-CD326 antibody [31]. Antigen binding region and Fc fragment of anti-CD326 antibody on the particles bound to CD326-expressing tumor cell and immune cell membranes, respectively. The sorafenib released from the particles exerted cytotoxicity to tumor cells and tumor-specific antigens released through the death of the cells caused tumor antigen-specific immune cell activation. Meraz *et al.* prepared monophosphoryl lipid A (MPL)-loaded discoidal porous silicon microparticles together with liposomal doxorubicin for cancer chemo-immunotherapy [30]. Local inflammation induced by injection of MPL-bound microparticles promoted infiltration of innate and adaptive immune cells near tumor. Furthermore, antitumor effects were enhanced with co-treatment of MPL-loaded microparticles and liposomal doxorubicin through synergistic combination of immunotherapy and chemotherapy.

2.3 Other porous particles

In addition to mesoporous silica and porous silicon particles, other porous materials have been also studied for immunomodulation. Liu *et al.* developed alginate/chitosan porous microspheres that can highly encapsulate Interleukin-2 (IL-2) cytokine (75~98 %) and consistently release without inactivation of IL-2 due to the porous nanostructure [54]. Released bioactive IL-2 cytokine increased immune response of tumor-specific cytotoxic T lymphocyte compared to free IL-2 delivery or other non-porous carriers. Also, Jiang et al. developed *Brachyspirahyodysenteriae* membrane protein B (BmpB) vaccine in which porous PLGA microparticles were loaded with BmpB antigens and coated with M cell-targeting peptides [55]. M cell-targeting peptides helped the particles to be taken up efficiently by M cells when they were orally delivered. This M cell-targeted vaccine with the internal

porosity higher than 70% released antigens rapidly and produced BmpB-specific IgA and IgG effectively.

3 Porous scaffolds for immune cell education

Implantable porous scaffolds have been widely used as a platform for drug delivery because of its outstanding capacity for sustained delivery of drugs. Most of artificial porous networks for biological applications are composed of biodegradable polymers [*e.g.* poly(lactic-co-glycolide) (PLGA)] or hydrogels [1]. Transitionally, biodegradable porous scaffolds have been used to grow cells for tissue regeneration *in vitro* by releasing growth factor molecules in a controlled manner. Their macroporous structures and mechanical properties enable cells to reside and grow into functional tissues over time. In recent years, as the importance of immunotherapy rises, there have been remarkable advances in immunotherapeutic applications of porous scaffolds. Particularly, when loaded with chemoattractants and antigens, porous scaffolds can recruit immune cells from the body and educate them for immunotherapeutic purpose. In this section, we mainly discuss biodegradable polymeric and hydrogel porous implants as artificial cellular microenvironments for immunomodulation.

3.1 Biodegradable polymeric porous scaffolds

Polymers have been widely used for biological applications especially as a drug delivery platform because of their biocompatibility and biodegradability. Among various biocompatible polymers, PLGA scaffolds have been extensively studied for immunotherapy. Porous PLGA scaffolds are fabricated by compression molding of PLGA microspheres and particulate leaching where NaCl crystals are used as particulate progen [10, 56]. Omar *et al.* reported *in situ* regulation of DC subsets via porous PLGA scaffolds incorporating granulocyte macrophage colony stimulating factor (GM-CSF) and CpG-ODN [10, 11]. They observed that the number of infiltrating $CD11c^+$ DCs increased in the GM-CSF-loaded scaffold group. PLGA matrices encapsulating GM-CSF and CpG-ODN also increased the number of $CD11c^+CCR7^+$ and $CD11c^+$MHC II^+ activated DCs and production of anti-tumoral cytokines, interferon-alpha (IFN-α) and interferon-gamma (IFN-γ). After incorporating tumor lysates, PLGA scaffolds provided strong immune protection against B16-F10 melanoma tumor model compared to classical cell-based therapy. Importantly, they found that PLGA scaffolds lack of GM-CSF represented only a 20% survival which supports the importance of DC recruitment. From this pioneering study, DC recruitment seems to be a crucial factor for enhancing immunotherapeutic effects of porous scaffolds. In a recent study, Kim et al. further verified the effects of porosity and pore size of PLGA scaffolds on recruitment of DCs [19]. They prepared PLGA scaffolds with three different surface porosities and pore volumes by changing amounts of components. They found that higher pore volume resulted in greater numbers of infiltrated $CD11c^+CD11b^+$ DCs in *in vivo* experiment, which indicated that pore volume was an important factor for cell recruitment. However, high surface porosity showed an opposite result because it could lead to structural distortion of PLGA scaffolds due to its low compressive modulus.

Besides the cell-recruiting implant, biodegradable polymeric porous scaffolds have been also used as a cell-carrying platform for immunization. Graham *et al.* used PLGA porous scaffolds to co-transplant T regulatory cells (Tregs) for immune protection of islet grafts [57]. Co-delivery of islets and Tregs normalized blood glucose levels and extended survival of islet grafts in diabetic mice. Porous scaffold-mediated delivery of Tregs provided systemic and consistent immune protection for transplanted islets.

3.2 Hydrogel porous scaffolds

Hydrogels are three-dimensional hydrophilic networks composed of natural or synthetic polymers capable of absorbing large amounts of water [58]. Their controllable porous structures are appropriate to create cell-loading and cell-infiltrating environments. In addition, hydrogels are often used as an injectable platform combined with immune agents since they can be self-assembled by *in situ* crosslinking [13]. Singh *et al.* developed an injectable, synthetic immune priming center (sIPC) to generate effective adaptive immunity against B cell lymphoma [12]. The sIPC was *in situ* generated in an injection site by self-assembly of dextran vinyl-sulfone (DextranVS) with polyethyleneimine-poly(lactic-co-glycolic) acid (PEI-PLGA) microparticles loaded with interleukin-10 (IL-10) siRNA and DNA antigens and tetra-thiolated polyethyleneglycol (PEG-4SH) mixed with chemokine macrophage inflammatory protein 3alpha (MIP3α). As a result, the sIPC attracted a large number of immature DCs by releasing chemokine MIP3α from the hydrogel

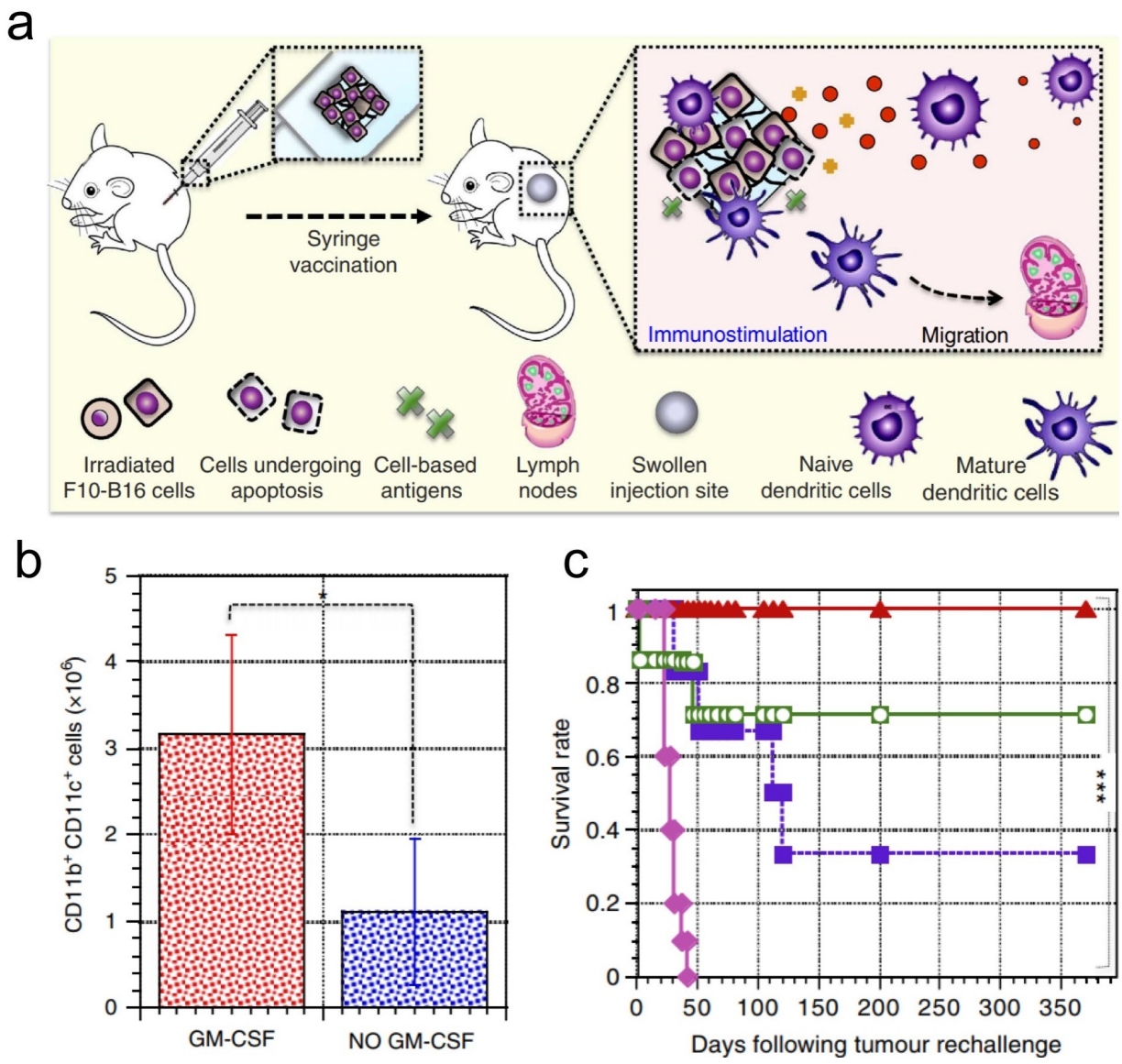

Figure 4: Immune modulation with cell-infiltrating hydrogels. (a) Schematic representation displaying the subcutaneous injection of cryogel vaccines in mice using a standard hypodermic needle, resulting in local oedema and induration at the injection site, and recruitment and activation of DCs. (b) Quantification of the number of $CD11b^+$ $CD11c^+$ cells infiltrating cryogels loaded with GM-CSF or blank (Control, NO GM-CSF) cryogels. (c) Survival rate in re-challenged mice prophylactically treated with bolus injection (purple square), cryogel vaccine (red triangle), cryogel vaccine without GM-CSF (green square) and naive mice (no immunization, pink diamond). At day 126 following immunization, C57BL/6 J mice (10 mice/group) from the first challenge study were challenged a second time with 10^5 B16-F10 tumor cells and monitored for survival. Reproduced with permission from ref. [20] Copyright © 2015 Nature Publishing Group.

and subsequently induced strong T helper type 1 (Th1) response by delivering IL-10 siRNA and DNA antigens to DCs. In this strategy, inhibition of IL-10 synthesis in DCs which phagocytosed microparticles would shift T helper cell response to Th1. Recently, the same group reported a more advanced approach with the DC-attracting hydrogels combined with microparticles encapsulating IL-10 siRNA, CpG-ODN and pDNA antigen [59]. Co-delivery of antigen, adjuvant and IL-10 siRNA successfully increased survival rate in an A20 B-lymphoma model. Bencherif *et al.* also reported macroporous cryogel-based whole-cell cancer vaccines [20]. Immunogenic macroporous cryogel was prepared with alginate sponges loaded with GM-CSF, CpG-ODN, and irradiated B16-F10 tumor cells (Figure 4a). GM-CSF-releasing cryogel significantly increased infiltration of $CD11b^+CD11c^+$ DC population compared to control group (Figure 4b). Infiltrated DCs interfaced with irradiated tumor cells in pore space and induced systemic

immune reaction for tumor cells. This cryogel-based vaccine showed remarkable immunotherapeutic effects especially against tumors re-challenged in mice, which indicated that the cryogel-based vaccine induced strong immunological memory (Figure 4c).

In addition to cancer immunotherapy, injectable hydrogels were used for tolerogenic immune modulation. Verbeke *et al.* developed a pore-forming alginate hydrogel to deliver BDC peptides to DCs in a noninflammatory context [60]. Two kinds of hydrogels were prepared by loading with PLGA microparticles carrying BDC peptides and conjugating BDC peptides directly to the alginate. Peptide-conjugated hydrogels induced greater antigen-specific Treg cells *in vivo*, leading them to accumulate in the pancreatic islets. This study demonstrated that porous hydrogels could induce tolerogenic immune responses by delivering antigens in the noninflammatory environments.

Recently, porous hydrogels were also engineered as an immunizing platform for cell therapy. Hori *et al.* used injectable alginate gels to deliver DCs for immune modulation [21]. The alginate scaffold was constructed by mixing calcium-crosslinked alginate microspheres with soluble alginate. This scaffold containing chemokine CCL21, CCL19, and activated DCs significantly attracted host T cells to the alginate matrices. Stephan *et al.* introduced an alginate scaffold implant to deliver, expand, and disperse tumor-specific T cells for cancer immunotherapy [22, 23]. The alginate scaffolds were coated with synthetic collagen-mimetic peptides (CMP) to enhance migration of lymphocytes and loaded with mesoporous silica microparticles containing IL-15 superagonists and anti-CD3/CD28/CD137 antibodies in a bioactive form to boost T cell proliferation [22] (Figure 5a, 5b). CMP modification increased release of T cell by 6.3-fold and incorporation of the stimulatory microparticles enhanced T cell proliferation by 22-fold (Figure 5c, 5d). This T cell-releasing implant granted outrageous therapeutic effects in inoperable and incompletely removed tumor models (Figure 5e, 5f). In an advanced study, stimulator of interferon genes (STING) agonist cdGMP were additionally loaded in the mesoporous silica microparticles and chimeric antigen receptor T cells (CAR T cells) were delivered using the modified implant [23]. The implant-mediated CAR T cell delivery successfully enhanced their therapeutic effects and represented strong immune protection in a mouse pancreatic tumor model.

3.3 Other porous scaffolds

Porous scaffolds can be generated *in situ* in an injection site using artificial materials. Kim *et al.* introduced mesoporous silica rods (MSRs)-based in situ self-assembling scaffolds for immune modulation [61]. As described in the previous section, mesoporous silica materials have been a great candidate for controlled drug release due to its high pore volume and biocompatibility. Authors synthesized MSRs with diameter and length of 4.5 μm and 88 μm respectively. When injected locally into the tissues, they formed interparticle spaces of tens of micrometers via random self-assembly, which were susceptible for immune cell infiltration. Encapsulation of GM-CSF successfully recruited $CD11c^{+}CD11b^{+}$ DCs to MSR scaffold. Furthermore, MSR vaccine combined with CpG-ODN and OVA displayed powerful therapeutic effects against E.G7-OVA lymphoma model. The same group further studied the effects of scaffold surface chemistry on immune cell activation and infiltration [62]. Surface of MSR was modified by poly(ethylene-glycol) (PEG), PEG-RGD or PEG-RDG. PEG modification significantly raised CD86 activation marker and interleukin-1beta (IL-1β) expressions without RGD specificity. Furthermore, highest number of total infiltrated cells was observed in the PEG-modified group. On the other hand, Choi *et al.* introduced a new hierarchical fabricating approach to prepare mechanically enhanced mesoporous silica scaffolds [63]. Mesoporous silica and salt powders were pressed to form a matrix and macroporous structures were created by leaching the salts from the matrix. The optimized porous scaffold releasing GM-CSF recruited a large number of immune cells in *in vivo* model.

4 Porous materials with intrinsic immunogenicity

For immune modulation, porous materials have been mainly used as carriers for delivery of multiple payloads or as scaffolds for carrying and recruiting immune cells. Recently, it was found that porous materials possessed intrinsic immunogenicity that could be leveraged for immunotherapy without additional adjuvants. In this section, we discuss the immunogenic potential of mesoporous silica and porous silicon particles and their immunotherapeutic applications.

In several studies, mesoporous silica particles were found to be immunogenic without any additional adjuvants. Their intrinsic immunogenicity was confirmed

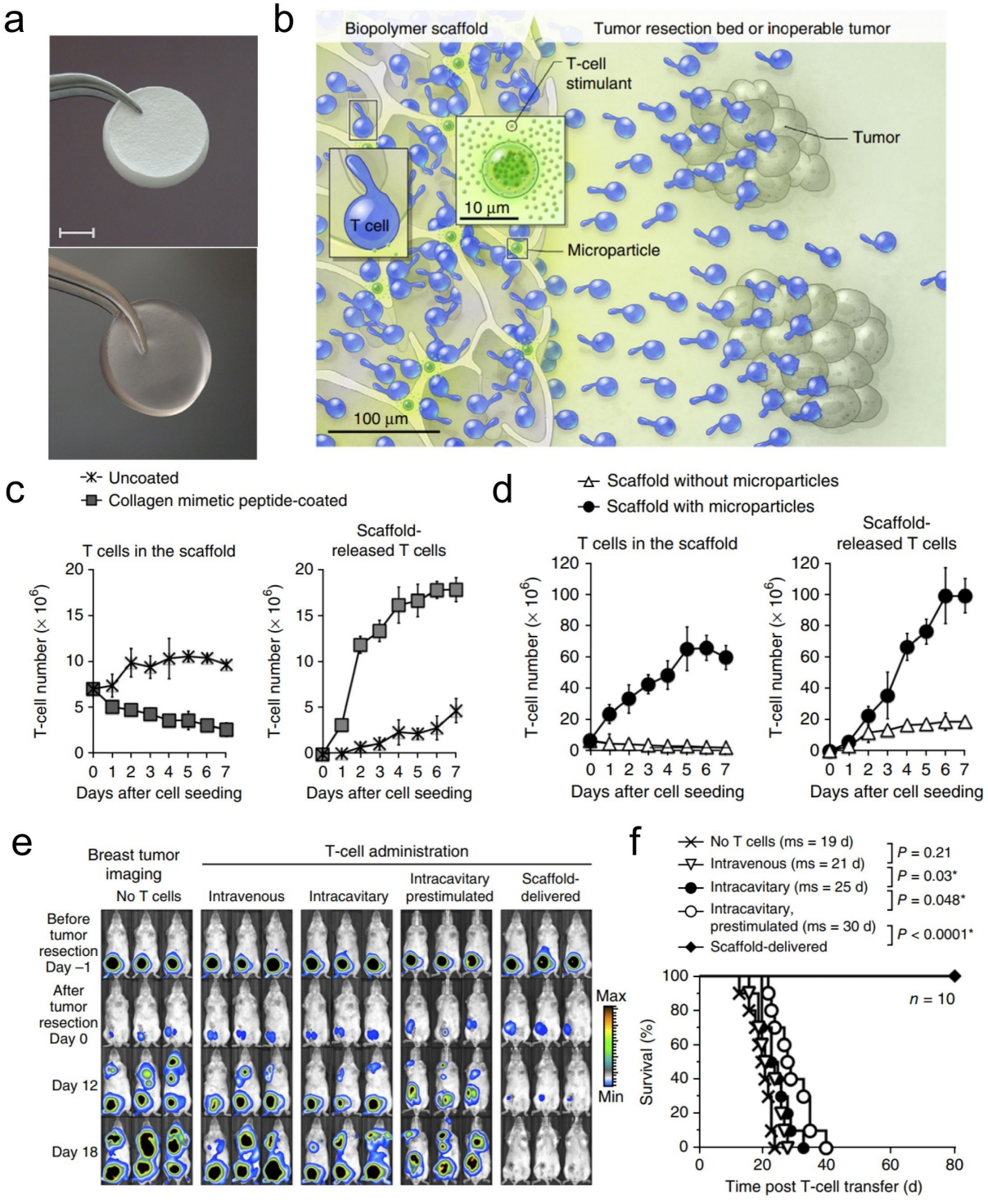

Figure 5: Immune modulation with cell-carrying hydrogels. (a) Image of biopolymer scaffold. Scale bar, 0.5 cm. (b) Schematic representation of a T cell–loaded scaffold surgically situated at a tumor site. (c) Quantification of viable (trypan blue–excluding) T cells enzymatically recovered from scaffolds versus collagen matrices at indicated time points. Error bars: mean ± S.D. (d) Absolute counts of viable T cells in scaffolds fabricated with or without stimulatory microparticles (left panel), and of cells that have transited from these implants into surrounding collagen matrix (right panel). Error bars: mean ± S.D. (e) Sequential bioluminescence imaging of the 4T1 breast tumors. (f) Kaplan-Meier survival curves for treated and control mice. Shown are ten mice per treatment group pooled from three independent experiments. (ms: median survival). Reproduced with permission from ref. [22] Copyright © 2015 Nature Publishing Group.

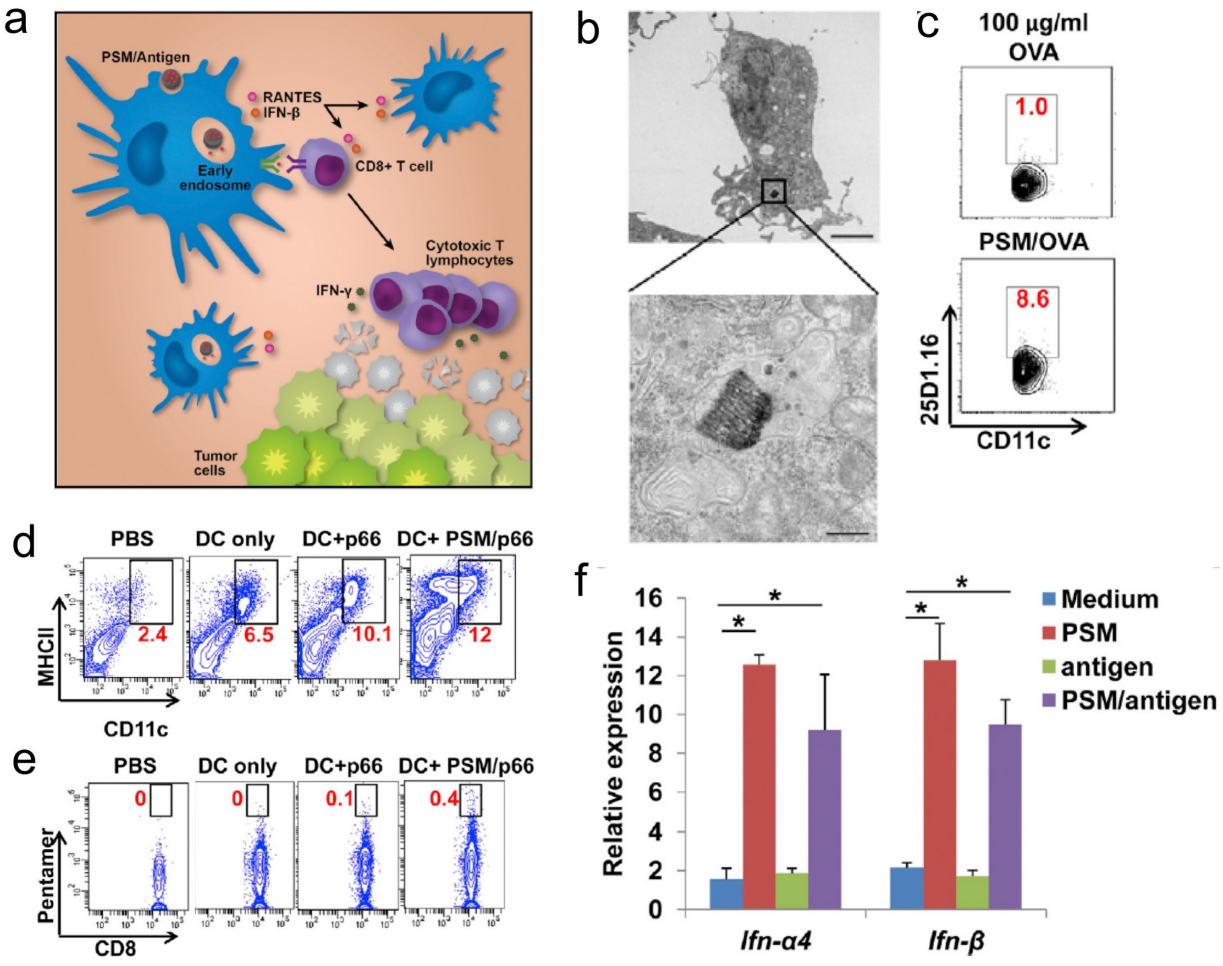

Figure 6: Immune modulation with intrinsic immunogenicity of porous materials. (a) Schematic representation of immune adjuvant function of porous silicon microparticle (PSM) by antigen cross-presentation and activating type I interferon response in DCs. (b) TEM images showing the vesicular structure around PSM/OVA particle inside the DC. Upper: scale bar, 4 μm, Lower: scale bar, 0.5 μm. (c) OVA cross-presentation by BMDCs. BMDCs were incubated with OVA or PSM/OVA for 16 hr, harvested, and labeled with anti-CD11c antibody to identify DCs and 25D1.16 antibody to identify the $OVA_{257-264}$/H-$2K^b$ complex on the DC surface. The percentages of $25D1.16^+$ staining populations in DCs are shown in red numbers. (d) Flow-cytometry analysis of $CD11c^+$MHC II^+ cells in isolated from posttreatment TUMO tumor tissues. The percentage of $CD11c^+$MHC II^+ cells in each sample is labeled in red. (e) Flow-cytometry analysis of CD8 and p66-$pentamer^+$ tumor-infiltrating lymphocytes in posttreatment tumor tissues. (f) qPCR analysis of mRNA levels of the Ifn-α4 and Ifn-β genes in BMDCs 5hr after co-incubation with PSM, free antigen, or PSM/OVA antigen. Error bars: mean ± S.D. *p<0.05, **p<0.01. Reproduced with permission from ref. [64] Copyright © 2015 Cell Press.

with DC activation *in vitro*, antigen specific antibody formation, and therapeutic effects *in vivo* [17, 18, 64–66]. Plain hollow mesoporous silica nanosphere loaded with OVA showed greater immunotherapeutic effects in OVA-expressing tumor model and increased adaptive immune cell response and antibody-related humoral immunity compared to OVA-loaded conventional alum adjuvant [17, 18]. SBA-15 type mesoporous silica particles with elongated rod-like shape induced OVA-specific splenocyte proliferation and higher OVA-specific IgG level compared to alum microparticle [65, 67]. AM-41 type mesoporous silica particles with amino-functionalized surface also activated OVA-specific splenocytes (IFN-γ secretion) [64, 66]. The proposed mechanisms for intrinsic immunogenicity of mesoporous silica particles is to activate NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasomes or to induce osmotic swelling and damage in lysosome by phagocytosed silica crystals [64, 68]. Their intrinsic immunogenicity was also thought to result from reactive oxygen species (ROS) production by NLRP3 activation [69]. However, further studies should aim to elucidate the exact mechanism for immunogenicity of the porous silica particles.

Porous silicon particles also showed the potential of intrinsic immune adjuvants. When porous silicon microparticles were treated to monocyte-derived dendritic cells (MDDCs), they increased CD80 and MHC I expression of MDDCs and effectively educated T cells to produce significant amount of IFN-γ [70]. Furthermore, Xia *et al.* showed that porous silicon microparticle-mediated antigen delivery enhanced cross-presentation of antigens and type I interferon response in DCs [71] (Figure 6). The antigens delivered by porous silicon microparticles were retained long in the endosomes and induced cross-presentation through both proteasome/lysosome-dependent pathways. The mechanism for DC activation was not mediated with TLR, but somehow with TRIF and MAVF signaling. Porous silicon microparticles loaded with HER2 antigens produced strong $CD8^+$ T cell-dependent antitumor immunity against $HER2^+$ breast tumors. In case of porous silicon nanoparticles, they increased CD86 and MHC II expression of DCs without additional adjuvant [29]. In addition, thermally oxidized porous silicon nanoparticles (TOPSi) coated with cancer cell membranes incorporating cancer specific antigen or loaded with biological antigens (Trp2) increased CD80 and CD86 expression of DCs [72]. Thus, porous silicon particles can be used as biomaterial vaccine with intrinsic immunogenicity without help of adjuvant in immunotherapy.

5 Conclusion and future work

Porous materials have been studied as immunogenic drug carriers, immune cell-infiltrating/carrying scaffolds, and self-adjuvants for effective immunotherapy. First, mesoporous silica and porous silicon particles were mainly used as porous carriers of immunogenic or immunomodulatory molecules. These particles can load immune payloads in their nanometer-sized pores with high loading efficiency, maintain the payloads in an active form, and finally release it in a controllable manner. For payload delivery, porous particles can be engineered to deliver immune payloads specifically to APCs such as DCs and macrophages by attaching targeting ligands on the surface although they can be naturally taken up by them through phagocytosis. Furthermore, porous particles can deliver multiple functional molecules with synergistic therapeutic effects to immune cells at the same time to enhance the efficacies of immunotherapy and combination therapy. Next, porous scaffolds with macroporous structure can be used as cell-carrying/infiltrating implants for immune modulation. Porous implants releasing chemoattractants can induce efficient recruitment of immune cells into the macroporous space of the implants. Such cell recruitment into the artificial cellular microenvironment can lead to effective education of immune cells for immunotherapeutic applications. Furthermore, porous scaffolds pre-loaded with immune cells and immunostimulatory molecules can be implanted into the body to induce effective immune responses. Lastly, porous particles have the potential to stimulate immune systems without additional adjuvants. These intrinsically immunogenic materials have attracted much attention in the immunoengineering field because most of immune adjuvants are considered toxic in the body [73–77]. These porous particles can act as immunogenic carriers, thus expecting to make immunotherapy simpler and more effective. However, more in-depth studies must be done to elucidate the immunogenicity mechanism of these particles before using them in the human body. Furthermore, it would be interesting to find porous materials that can induce immunosuppressive responses so that they can be used as antigen-specific immunosuppressive agents for autoimmune diseases. Collectively, porous materials have a great potential to modulate immune systems for effective treatments of immunosuppressive diseases such as cancer.

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