Emerging Strategies to Bypass Transplant Rejection via Biomaterial-Assisted Immunoengineering

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Emerging strategies to bypass transplant rejection via biomaterial-assisted immunoengineering: Insights from islets and beyond

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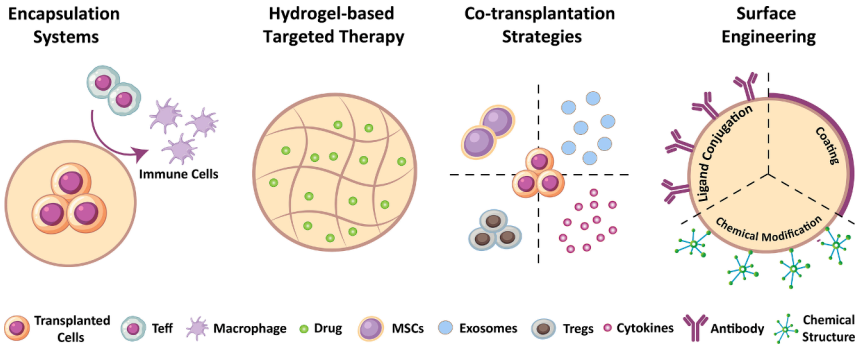
GRAPHICAL ABSTRACT



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ABSTRACT

Novel transplantation techniques are currently under development to preserve the function of impaired tissues or organs. While current technologies can enhance the survival of recipients, they have remained elusive to date due to graft rejection by undesired *in vivo* immune responses despite systemic prescription of immunosuppressants. The need for life-long immunomodulation and serious adverse effects of current medicines, the development of novel biomaterial-based immunoengineering strategies has attracted much attention lately. Immunomodulatory 3D platforms can alter immune responses locally and/or prevent transplant rejection through the protection of the graft from the attack of immune system. These new approaches aim to overcome the complexity of the long-term administration of systemic immunosuppressants, including the risks of infection, cancer incidence, and systemic toxicity. In addition, they can decrease the effective dose of the delivered drugs via direct delivery at the transplantation site. In this review, we comprehensively address the immune rejection mechanisms, followed by recent developments in biomaterial-based immunoengineering strategies to prolong transplant survival. We also

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compare the efficacy and safety of these new platforms with conventional agents. Finally, challenges and barriers for the clinical translation of the biomaterial-based immunoengineering transplants and prospects are discussed.

1. Introduction

Transplantation is a life-saving therapeutic approach to restore the function of impaired organs, granting survival and a better quality of life to individuals with end-stage organ failure [1]. However, allografts suffer heavily from inescapable host-to-graft rejection, which severely reduces the survival of transplanted organs and reduces the life quality of recipients [2–4]. The most pressing issue in the field of transplantation is how to maintain the long-term functions of the grafted cells or tissues. Another shortcoming of transplantation is the need for lifelong usage of systemic immunosuppressants by patients to halt graft rejection and prolong its survival. Currently, the immunosuppressive drugs in clinical application mainly include monoclonal-/polyclonal-antibodies, calcineurin inhibitors, corticosteroids, anti-proliferation drugs, mammalian target of rapamycin (mTOR) inhibitors, and janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway (Table 1). Unfortunately, these drugs are not specific or selective in their actions and they can affect untargeted cells throughout the body, leading to ineffectiveness and even failure in mitigating acute rejection. The long-term administration of immunosuppressive drugs also leads to severe side effects and health risks, such as the increased risk of cancer, infections, chronic kidney damage, and osteoporosis [5–9]. Although adjusting the dosage of suppressive agents in each transplant recipient can prevent graft rejection to a certain extent, low dosages of immunosuppressants fails to prevent organ rejection and high dosages of immunosuppressants often cause opportunistic infections, malignancy, severe bone marrow suppression, disruption of immune homeostasis, damage to vital organs (*e.g.*, liver and kidney), and metabolic changes [10–15]. These challenges highlight the urgent necessity for novel immunomodulatory strategies to prevent graft rejection with high efficiency and minimal side effects while skipping the need for regular administration of immunosuppressive agents.

Immunoengineering is a new approach that combines immunology principles with engineering tools, techniques, and concepts to to develop innovative solutions to combat diseases and improve human health. For example, engineering principles and techniques can be acquired to prolong immune tolerance and diminish the risk of acute and chronic graft rejection following organ transplantation. This novel strategy involves manipulating and developing biological and synthetic materials, cellular therapies, drug delivery systems, and bioengineering techniques to modulate the immune response and improve the immune acceptance of transplanted tissues or organs [14,16]. A promising approach, known as biomaterial-based immunoengineering, utilizes biomaterials with customized surface and mechanical properties to achieve the prolonged function of transplants. [17]. By combining this technology with localized immunomodulation strategies, it is possible to to bypass concerns associated with the complications of systemic immunosuppressants. Therefore, the synergy of biomaterial-assisted transplantation and immunomodulation strategies provides the opportunity for effective and graft-specific immune responses, leading to enhanced transplantation

Table 1
Traditional immunosuppressive drugs, their mechanism of action, and side effects.

Immunosuppressive category			Action mechanism	Side effects	Refs
Antibodies	Monoclonal	Rabbit anti-thymocyte globulin (rATG) and horse anti-thymocyte globulin (hATG)	By binding to and inactivating human T cells.	Thrombocytopenia, anaphylaxis, flulike symptoms, infection fever, cytokine-release syndrome, hypotension, and pulmonary edema.	[30]
	Polyclonal	Alemtuzumab	Binding to CD52 protein leads to the destruction of T cells and a reduction in the immune system's attack on the body's own cells.	Cytokine-releasing syndrome (milder compared to ATG), lymphopenia, and autoimmune phenomena (thyroid disease, hemolytic anemia, and thrombocytopenia in patients with multiple sclerosis).	[31–33]
		Basiliximab	By binding to a protein called interleukin-2 receptor alpha chain (IL-2Rα), blocking the activation of T cells.	Hypersensitivity reaction.	[34]
Corticosteroids (Prednisolone and Prednisone)			By entering the cell and binding to specific proteins called glucocorticoid receptors. Change in gene expression, affecting the production and function of immune cells and cytokines involved in the immune response.	Hyperglycemia, hypertension, fat distribution changes, protein loss, adrenal suppression, adrenal atrophy, psychosis, mood changes, cataracts, glaucoma, peptic ulceration, osteoporosis, and impaired wound healing.	[35,36]
antiproliferative/ antimetabolites		Mycophenolate mofetil	Inhibiting the activity of inosine monophosphate dehydrogenase.	Gastrointestinal distress, neutropenia, and opportunistic infections.	[35,36]
		Azathioprine	Inhibiting DNA synthesis in immune cells.	Leukopenia, thrombocytopenia, anemia, dose-related bone marrow suppression, liver impairment, cholestatic jaundice, hepatotoxicity, and hypersensitivity reactions (rash).	[35–37]
mTOR Inhibitors (Sirolimus and Everolimus)			Inhibiting the activity of mTOR protein.	Delayed wound healing, increased risk of infections, hyperlipidemia, leukopenia, thrombocytopenia, anaphylaxis, hypersensitivity reactions, life-threatening pneumonitis, mucositis, edema, and proteinuria.	[8,35,38]
CaNIs (Cyclosporine A, Tacrolimus)			Inhibiting the calcineurin pathway.	Nephrotoxicity, hypertension, diabetogenesis, hyperkalemia, hyperuricemia, hyperlipidemia, gingival hyperplasia, and hypertrichosis.	[39–41]
JAK-STAT inhibitors (Tofacitinib, Ruxolitinib)			Blocking specific proteins in the JAK-STAT signaling pathway	Opportunistic infections, such as pneumonia, nasopharyngitis, urinary tract infections, cellulitis, herpes zoster, cytopenia, hypercholesterolemia, liver enzyme abnormalities, and risk of malignancies, including lymphoproliferative disorders, cutaneous T-cell lymphoma, non-melanoma skin cancers	[42–44]

outcomes and reduced adverse effects. The first widely utilized immunomodulation approach is macro, micro, and nano-encapsulation devices that can isolate transplanted cells from immune recognition as a physical barrier while providing flexibility in choosing implantation site, functional mass transport, and enhanced oxygen and nutrient diffusion to grafts [18,19]. In addition, biodegradable and biocompatible biomaterials have been used to fabricate devices and hydrogels for co-transplantation of cells/tissues with drugs/biomolecules to circumvent the disadvantages of systemic immunosuppressants and minimize the risk of adverse effects in a controlled manner [20–24]. The co-transplantation of graft cells with mesenchymal stem cells, extracellular vesicles (EVs), and immunomodulatory cells have also been achieved using biomaterials to improve vascularization [25], increase immunomodulatory cytokines [26], and reduce pericapsular growth due to foreign body responses (FBR) [27]. Besides the above-mentioned strategies, manipulating the surface of transplants by materials has offered additional advantages toward transplantation through the apoptosis of specific immune cells or eliciting immunomodulatory responses by enhancing the population of regulatory T cells (Tregs) [28,29].

In this review, we first discuss the mechanisms that contribute to the immune rejection of transplants and current clinical challenges before introducing immune-engineering biomaterials that can bypass transplant rejection by providing a tolerogenic immune microenvironment through encapsulation systems, hydrogels, different co-transplantation strategies, and surface-engineered biomaterials. In the last part of the review, we discuss barriers to the clinical translation of biomaterials-based immune-engineering strategies.

2. Mechanisms of immunological rejection and challenges of transplantation

Similar to different biomaterial-based systems employed to elicit anti-inflammatory responses for various diseases [45,46], the prevention of inflammatory immune responses against particular antigens in transplanted cells or organs is needed to enhance the survival rate of the grafts and decreases the risk of acute or chronic rejection. In fact, the achievement of successful transplantation relies on the establishement of immune tolerance, which in the context of transplantation, it refers to a state in which the immune system of recipient accepts a transplanted organ or tissue without recognizing it as foreign to mount an immune response against it. This is an ongoing area of research that enables to reduce the essential for long-term immunosuppressive medicines, which can have significant side effects.

Despite the numerous advancement in transplant tolerance, the complex and elusive interplay between immune system and

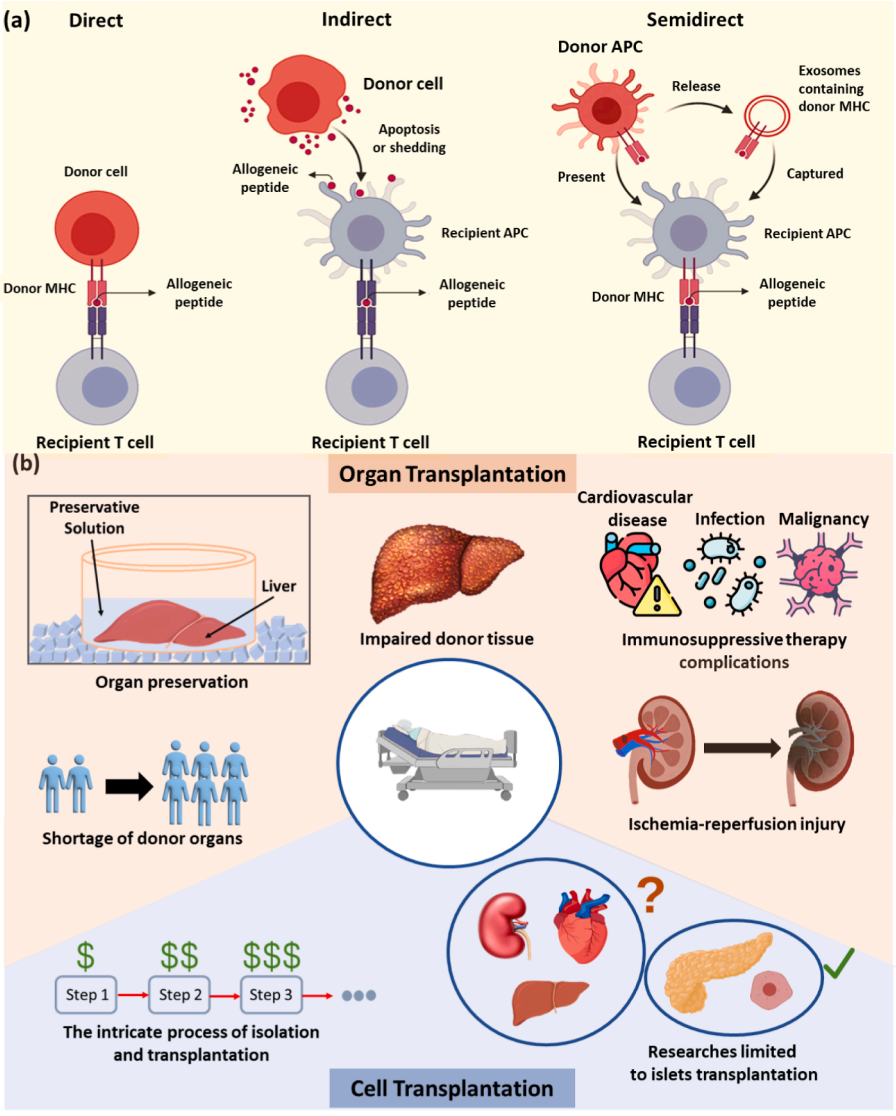

Fig. 1. a) Schematic illustration of three allorecognition mechanisms, including direct, indirect, and semidirect pathways. Direct allorecognition is the activation of recipient T cells by interaction of the TCR with intact allogeneic MHC-peptide complexes presented by donor APCs. Indirect allorecognition occurs when peptides are degraded by antigen processing pathways and presented by autologous MHC molecules on recipient APCs. Semidirect allorecognition is referred to the capture of donor MHC-peptide complexes by recipient APCs and the presentation of these complexes to prime T cell activation. Reprinted with permission from ref. [57]; Copyright© 2021, Wiley. b) Schematic illustration of current challenges in organ and cell transplantation.

immunological rejection have not been fully elucidated. Two principal arms of the immune system involved in the immunological rejection of transplants include innate and adaptive immune responses. The non-specific innate response, as an early phase of immune response after transplantation, can commence acute rejection through the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) as a result of ischemic injury developed during the transplantation process [47,48]. The immune cells can then detect DAMPs and PAMPs by pattern recognition receptors, resulting in the production of pro-inflammatory cytokines, as well as activation of effector T helper cells and natural killer (NK) cells that can induce graft rejection [48,49]. Ultimately, these factors presents the transplanted organ as a site of injury and inflammation, which evokes the inflammatory leokocytes to the graft site. Nevertheless, the adaptive immune response is the main reason for cell and organ rejection, which activates through the presentation of alloantigens to T cells by different antigen-presenting cells (APCs) mediated by direct, indirect, and semi-direct pathways (Fig. 1a). In the direct pathway, donor APCs represent allogenic MHC molecules to the T-cell receptors (TCR) on T cells, which is responsible for acute graft rejection [49]. In the indirect pathway, the recipient's APCs capture and process allogenic proteins, and alloantigens as peptides, and present them by their autologous MHC molecules to prime T cells. In semidirect reaction, alloantigens are transferred between donor and recipient APCs by fusion with EVs or cell–cell interactions followed by re-presenting as conformationally intact proteins by allogenic MHC to activate T cells [50,51]. Collectively, activation of T cells with antigens initiates the adaptive immune responses. The migration of activated $CD4^+$ Helper cells and $CD8^+$ cytotoxic T cells to graft site causes the destruction of transplanted cells by inducing apoptosis through releasing cytotoxic molecules such as perforin and granzyme B [49].

In addition to the need for proper solutions to overcome the above-described rejection pathways, there are also other pre- and post-transplantation challenges that should be addressed. The shortage of organ donors is one of those that the increasing rate of preexisting diseases in donors and the aging of the population makes it more complicated [52,53]. Organ or tissue preservation is another challenge, which is currently managed by static cold storage in the UW® solution [54].

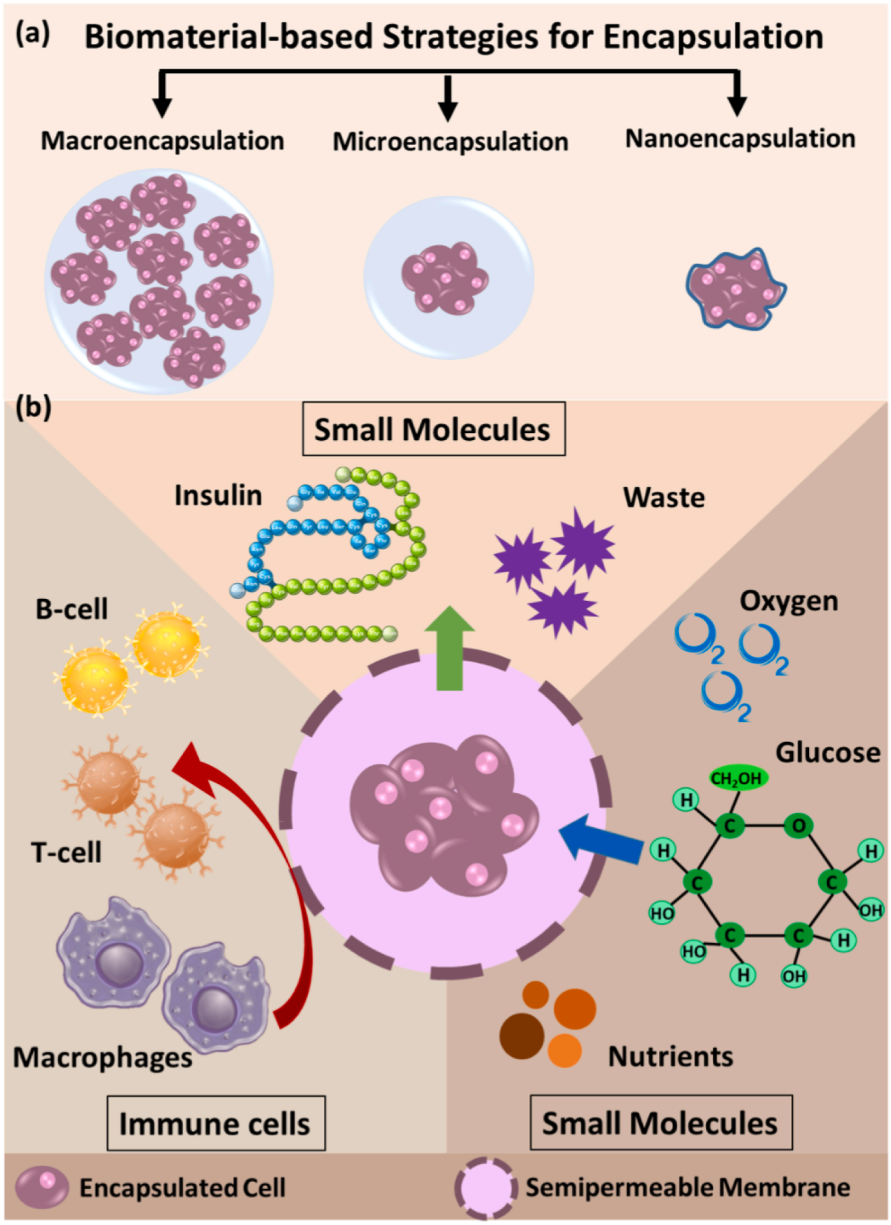

Fig. 2. (a) Illustration of current biomaterial-based strategies for cell encapsulation at different scales (macroencapsulation, microencapsulation, nanoencapsulation). (b) Schematic illustration of islet encapsulation strategy, which permits the exchange of oxygen, glucose, insulin, etc., while simultaneously acting as a physical barrier to prevent the transfer of immune cells and subsequent immune rejection. Biomaterials can be used to develop a semipermeable membrane for islet encapsulation.

However, machine perfusion techniques like hypothermic machine perfusion and hypothermic oxygenated machine perfusion have attracted attention to extend the *ex-vivo* preservation time of transplants. Nevertheless, there is still a lack of evidence on whether this approach prolongs the preservation time or not [55]. Impaired donor tissue, side effects of lifetime systemic immunosuppressive therapy, and ischemia–reperfusion injury, i.e., the paradoxical exacerbation of cellular dysfunction and death as a result of blood flow restoration to the transplanted organ, are the other challenges of organ transplantation that need more attention of researchers to be addressed using biomaterials. Cell transplantation has also several challenges, such as multistep of costly cell isolation and its current limited focus to certain diseases like type 1 diabetes (T1D) [56]. Fig. 1b summarizes the challenges of organ and cell transplantation, which should be addressed in future studies using novel biomaterials to boost cell/organ transplantation technologies.

3. Biomaterials-based encapsulation systems for immunoengineering of transplants

Biomaterial-based approaches for cell encapsulation can be classified into three primary categories. The first is macroencapsulation, which involves encapsulating a substantial mass of cells within a device. The second is microencapsulation, where one or a few cells are enclosed within semipermeable microcapsules. Lastly, nanoencapsulation focuses on encapsulating individual cells in a shell with a thickness at nano range (Fig. 2a) [58]. Encapsulation supplies a physical biocompatible barrier using biomaterials to protect the transplant from immune responses while allowing the permeability of oxygen and essential nutrients for its survival (Fig. 2b) [58,59]. Cell encapsulation has shown potential for various applications related to rejection. For example, a three dimensional (3D) printed cell encapsulated device made of polylactic acid (PLA), was developed for the transplantation of testosterone-secreting Leydig cells and demonstrated enhanced cell viability, vascularization, and testosterone secretion in the mice [60]. Moriarty *et al.* encapsulated dopaminergic neurons in a glial-derived neurotrophic factor (GDNF)-loaded collagen hydrogel in a Parkinson's disease rat model to protect transplanted cells from the immune response and to increase the graft survival and re-innervation capacity [61]. In another work, an in situ gelling collagen hydrogel was used as an encapsulation matrix for brain delivery of genetically modified MSCs expressing glial cell line-derived neurotrophic factor (GDNF). The hydrogel reduced the host brain's immune response by decreasing microglial and astrocyte recruitment at the graft site, leading to the enhancement of cell support and graft integration [62]. Despite the potential of immunoengineered biomaterials for neural transplantation, as well as the treatment of endocrine and other diseases, many of current researches focus on islet transplantation, and additional research is required to explore and exploit the diverse possibilities offered by biomaterials in this context. Islet encapsulation is a promising approach that has been studied for the treatment of T1D, demonstrating the desirable endocrine function of islets due to the imperfect attack of the host immune cells [63,64]. The different classifications of encapsulation, including macroencapsulation, microencapsulation, and nanoencapsulation are discussed in this section to provide an overview of the advancement in this field and present challenges that should be addressed for further improvements to move towards biomaterial-based clinically translated products.

3.1. Macroencapsulation to suppress transplant rejection

Macroencapsulation is a rapidly growing area for islet immunoisolation by appropriate polymers. This technology can provide some benefits compared with whole-organ transplantation, including non-invasiveness and more availability [65]. Also, as compared to microscale and nanoscale systems, more islets can be transplanted by macroencapsulation technology [58]. The most common transplantation sites for macroencapsulated devices are intraperitoneal/omentum and subcutaneous spaces [66–68]. Up to now, different polymers, such as alginate, polysulfones, agarose, and the copolymer of acrylonitrile and vinyl chloride are investigated to form membranes for microencapsulation [58]. These membranes can be incorporated with factors involved in regenerative processes and immunoprotection of transplanted cells, including vascular endothelial growth factor (VEGF) [69,70] and transforming growth factor-beta 1 (TGF-β1), respectively [71]. A variety of different macroencapsulation designs have been studied over the past years. Skrzypek *et al.* presented a novel concept for a macroencapsulation device in which islets were enclosed between two porous membranes of poly(ethersulfone) (PES)/polyvinyl pyrrolidone (PVP) polymers [63]. PES was blended with PVP through the phase separation micro-molding method to prepare porous micro-structured membranes with high stability, good mechanical properties, excellent biocompatibility, and low cell adhesion properties, which are necessary for the increment of islet survival after transplantation. The prepared membrane was microporous (1–3 μm), allowing glucose diffusion to the islet and corresponding insulin release in response to blood glucose levels while blocking the immune cells (size of ~ 10 μm) to supply adequate immunoisolation for encapsulated islets. In another study, a silicon nanopore membrane (SNM) with pores of about 7 nm in width, 300 nm in depth, and 2 μm in length was prepared to supply middle molecule selectivity by restricting the passage of the host's immune components and pro-inflammatory cytokines while providing an adequate exchange of glucose and insulin (Fig. 3a) [72]. The sieving coefficient of solutions consisting of mouse cytokines tumor necrosis factor alpha (TNF-α) (1000 U/ml), interferon-gamma (IFN-γ) (1000 U/ml), IL-1β (50 U/ml), glucose (400 mg/dL), and insulin (150 mU/L) in a 3% bovine serum albumin (BSA) solution across SNM were analyzed, which represents the ratio of the concentration of the filtrate (the substance that has passed through a membrane) over the concentration of the feed (the initial amount of the substances). The calculated sieving coefficient of TNF-α, IFN-γ, and IL-1β were 0.16, 0.27, and 0.27, respectively, following the 6 h experiment. However, the sieving coefficient of glucose and insulin quickly reached 1, which revealed the potential of SNM for immunoisolation as well as permitting the entire transport of small molecules (Fig. 3b). SNM membrane could protect encapsulated islets from pro-inflammatory cytokines, while control static culture with cytokine exposure (Control, +Ck) exhibited a significant cell death, confirming the ability of SNM membrane to protect islets from pro-inflammatory cytokine attack to maintain islets viable (Fig. 3c,d). In another attempt, Chang et al. fabricated a bilaminar nanoporous thin film macroencapsulation device incorporating human embryonic stem cell-differentiated beta cell clusters to take the advantage of the thin membrane for the optimal diffusion of nutrients for cell viability and immune protection [73]. For this purpose, zinc oxide nanorods were expanded hydrothermally onto silicon wafers to provide pores of 20 nm in diameter and 500 nm in height. Then, a polycaprolactone solution was spin-casted onto zinc oxide nano-templated silicon substrates, followed by sulfuric acid etching of zinc oxide. The final polymer membrane had a thickness of 10 μm, backed by a supporting porous layer. The results demonstrated that the designed device could notably protect cells from the host immune system while allowing ideal glucose and insulin exchange. In another interesting study, electrospun nanofibers were used as a barrier to inflammatory cell infiltration and for the local release of FTY720 to provide immunomodulatory and pro-angiogenic effects for islet transplantation [74]. FTY720 (Fingolimod) belongs to the class of medications known as sphingosine-1-phosphate receptor modulators, which was used as an immunomodulatory drug and alternative to traditional immunosuppressive drugs due to its ability to inhibit TCR-mediated T cell activation in primary human T cells [75].

In addition to membranes, some studies have also shown the potential of hydrogels as a successful islet encapsulation system against the

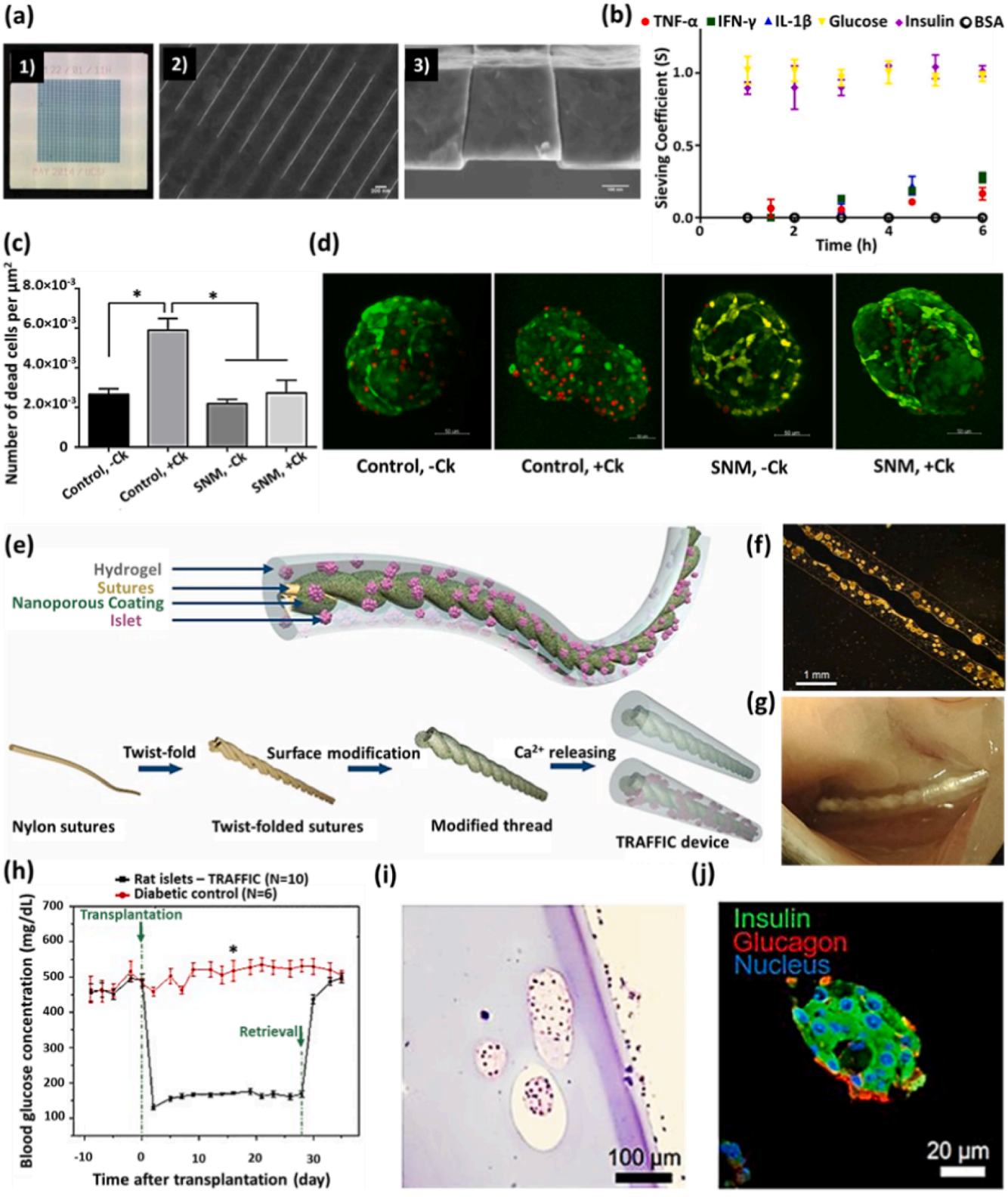

Fig. 3. (a) 1) An optical image of the SNM chip, 2) The scanning electron microscopy (SEM) image of the membrane's surface, and 3) The SEM image of the cross-section of the membrane. (b) Transportation of different molecules via slit-pore of SNM evaluated under a pressure difference of ~ 2psi. BSA was used as a negative control. Data is presented as Mean ± Standard error of the mean (SEM). (c,d) In vitro viability of control culture and SNM-encapsulated mouse islets with and without cytokine exposure. Stained viable (green) and dead (red) cells are presented. Reprinted with permission from ref. [72]; Copyright© 2016, Nature Portfolio. (e) Schematic illustration of the design and fabrication process of TRAFFIC. (f) Microscopic image of the prepared TRAFFIC device encapsulating human islets. (g) Photograph of the device in the intraperitoneal space of mice during the retrieval. (h) The blood glucose concentration of diabetic mice after transplantation of encapsulated rat islets, (N = 6–10), Data is presented as mean ± SEM, *P < 0.05. (i) H&E-stained retrieved islets in TRAFFIC treated group. (j) Immunohistochemical staining of retrieved islets in TRAFFIC treated group. Reprinted with permission from ref. [81]; Copyright© 2018, National Academy of Sciences.

host immunity environment [76–78]. Alginates are typically used for designing such hydrogels due to their biocompatibility and easy hydrogel formation [79,80]. For example, a cell-encapsulated device, named TRAFFIC (thread-reinforced alginate fiber for islets encapsulation), was prepared by crosslinking alginate around a nanoporous, wettable, Ca^{2+}-releasing poly(methyl methacrylate) derived thread. A uniform in situ crosslinking was achieved with strong adhesion of the thin layer of alginate hydrogel around the thread (Fig. 3e) [81]. The main characteristics of this system were its high mechanical strength, easy handling, and facile implantation. To evaluate the biocompatibility of TRAFFIC, the islet-loaded device was implanted in the intraperitoneal space of mice and at the end of the experiment, it was free of fibrosis with most of the islets remaining viable (Fig. 3f,g). The results of *in vivo* study demonstrated that blood glucose declined to the normal glycemic range (<200 mg/dl) in the device-implanted group two days after the transplantation and remained normal for four weeks. After retrieval, the blood glucose level returned to a diabetic state, which revealed the efficacy of the device in controlling blood glucose (Fig. 3h). The results of hematoxylin and eosin (H&E) staining of islets retrieved from cured mice demonstrated normal morphology, and additionally, the positive immunohistochemical staining of insulin and glucagon approved the islet function in the device (Fig. 3i,j) due to the suppressed immunoattack of the host body. These results confirmed that the fabricated device provides immunoprotection of rat islets, which may remarkably contribute to cell encapsulation therapy of T1D and other diseases.

One of the main considerations when designing an encapsulation device is the presence of dense vascularization around the device, which can facilitate compound exchange between the device and its surrounding physiological environment. Insufficient vascularization can hinder oxygen and nutrient supply to the islet core, which leads to the death of islet cells and transplantation failure [82,83]. Many approaches have been proposed to overcome this problem, including the usage of vascularization-promoting agents around implanted devices [84], enhanced oxygen delivery by enhanced pre-vascularization or reduced diffusional distances within capsules and devices [85–88], increased oxygen permeability of the encapsulating materials [89,90], delivery of exogenous oxygen to increase pO_2 within capsules and devices by inhalation therapy or oxygen-generating biomaterials [91–94], oxygen gas injection into a chamber within an immunoisolation device [95,96], and the usage of electrochemical oxygen generators [97]. To enhance the vasculature surrounding the encapsulation device, Weaver *et al.* developed an immunoisolating synthetic protease-degradable poly (ethylene glycol) (PEG)-based hydrogel macrodevice system functionalized with VEGF and Arg–Gly–Asp (RGD) for islet encapsulation and transplantation. This approach resulted in enhanced local vascularization at the device surface, improved oxygen and nutrient delivery to the transplanted islets, desirable insulin responsiveness, and islet immunoisolation [70].

Fabrication of encapsulation devices is also reported using selective laser sintering 3D printing of biocompatible polyamide PA 2200. The device was called NICHE (Neovascularized Implantable Cell Homing and Encapsulation) and demonstrated simultaneous in situ vascularization and local delivery of an immunosuppressant [98]. The NICHE was designed as a rectangular shape consisting of a U-shape drug reservoir that surrounded a central cell reservoir, which was enclosed by a two-layer mesh: an inner 300 μm × 300 μm mesh to provide structural support and an outer 100 μm × 100 μm mesh to facilitate vascular tissue penetration and cell retention. At each longitudinal side of the drug reservoir, 1 × 1 mm openings interconnect the drug and cell reservoirs, and each of these openings has nylon nanoporous membranes affixed over them to permit concentration-driven diffusion of immunosuppressants into the cell reservoir for localized immunosuppression. Finally, biocompatible and self-sealing silicone plugs at the end of reservoirs provide transcutaneous access for immunosuppressant replacement and islet transplantation (Fig. 4a). To achieve in situ vascularization, the cell reservoir was loaded with syngeneic mesenchymal stem cells (MSCs), and the histopathological analysis demonstrated that NICHE provided sufficient and dense vascularization in both rats and non-human primates (NHP) to provide successful engraftment and efficient therapeutic function (Fig. 4b–e).

Concerning enhanced oxygen supply to encapsulated islets, Evron *et al.* represented a retrievable macroencapsulation device incorporating islets in an alginate slab provided with exogenous oxygen from a replenishable gas chamber to prevent the damage of transplanted islets by the consumption of pO_2 in the gas chamber [99]. To provide an oxygen supply for a longer time, Liang *et al.* fabricated an implantable system containing oxygen-generating micro-beads [100]. They encapsulated calcium peroxide as a common source of oxygen generation within polydimethylsiloxane, termed OxySite in a diabetic Lewis rat syngeneic transplantation model to generate sufficient local oxygen as well as enhanced vasculature formation (Fig. 4f).

In addition to the broad investigations on transdermal drug delivery and vaccination by microneedle (MN) patches [101,102], studies have evaluated them for the delivery of insulin-producing cells to treat T1D (Fig. 5a) [103]. Hyaluronic acid was utilized to develop the MN array patch, which was further crosslinked by methacrylate groups and treated under ultraviolet light. The cells were encapsulated in alginate microgels to improve the immunoprotection and survival of β-cells and applied to the back of the MN array patch (Fig. 5b). Each needle of the prepared MN device had a side length of 400 μm at the base, a side length of 5 μm at the tip, and a height of 800 μm (Fig. 5c). A fluorescence image of pancreatic β-cells capsules-integrated MN patch is shown in Fig. 5d. The matrix of MN had a synthetic glucose-signal amplifier (GSA) to enhance the diffusion of glucose from the interstitial fluid to the alginate microgel. These MNs were inserted into the dermal tissue to adjust insulin release in response to blood glucose changes. The treated T1D mice exhibited the maintenance of blood glucose levels in the normal range for about 6 h (Fig. 5e). In addition, the repeated treatments of two MN array patches did not show the risk of hypoglycemia (Fig. 5f). These results displayed that concentrated cells could do a homogenous release of insulin surrounding the capsules while showing desirable viability and functionality of the encapsulated β-cells (Fig. 5g, h), which confirms the ability of the proposed strategy to protect β-cells from immune rejection reaction.

Taken together, macroencapsulation is a favorable strategy for cell transplantation with immunoisolation while providing mechanical and physiochemical support for therapeutic function.

3.2. Microencapsulation strategies for transplant survival

The microencapsulation strategy provides immunoprotective microsized capsules with <1 mm diameter to protect encapsulated cells using different biomaterials [104,105]. It can play an important role in the immunoisolation of pancreatic islets, which overcomes the need for immunosuppressants and the lack of donor islet cells [106]. The biocompatibility of biomaterials is an important criterion to develop these devices. In addition, the formation of fibrosis in the microenvironment of microcapsules is a critical issue to be addressed since it will induce transplant failure via the disruption of oxygen and nutrient supply to the transplanted cells [107,108]. Various biocompatible polymers, including chitosan, alginate, PEG, agarose, collagen, dextran, and hyaluronic acid, have been previously used to fabricate microencapsulation devices, in which alginates are mostly used [58,109–113]. Many studies highlight the developments in alginate-based hydrogels for islet microencapsulation [109,114–119]. However, alginate suffers from poor long-term post-transplantation stability, which should be addressed in future studies [120–122]. In addition, in some cases, immune reactions can lead to the formation of fibrotic cell layers and collagen deposition on the surface of transplanted alginate-microcapsules due to the indirect activation of the antigen recognition pathway, which subsequently can impair islet function [123–126]. In this context, researchers have co-encapsulated transplanted cells with

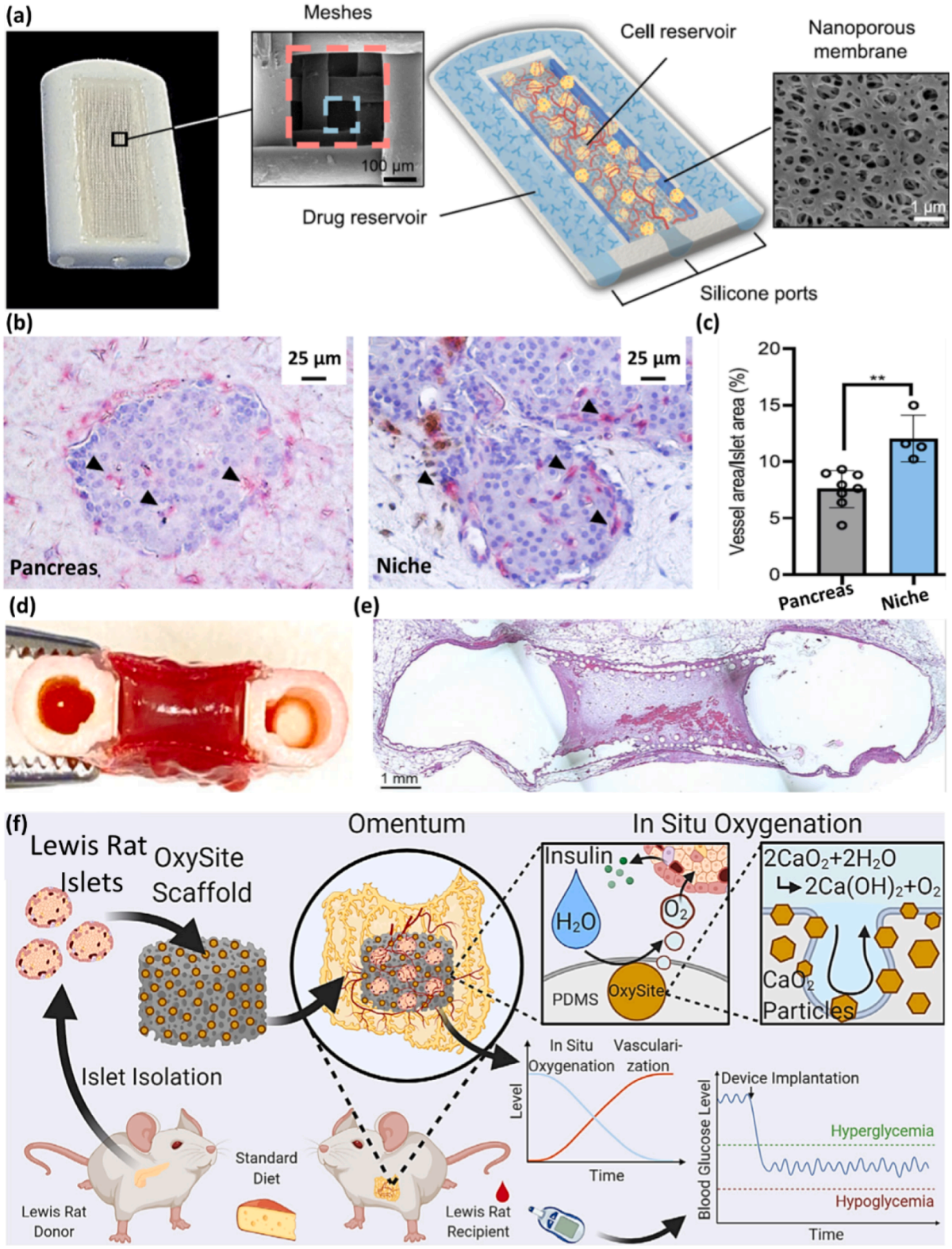

Fig. 4. (a) Optical image and schematic illustration of NICHE and representative SEM image of the two-layer mesh and nanoporous membrane. (b) Histological analysis of the pancreas of healthy rats and NICHE-transplanted group stained for blood vessels (red). Black arrows show capillaries. (c) Percent of islet area comprised of vessels in the pancreas of native rat (N = 8) and NICHE-transplanted group (N = 4; **$p < 0.01$). Data is presented as Mean ± SEM. (d) Gross and (e) H&E-stained micrographs representing a cross-section of NICHE after 4 weeks of subcutaneous implantation in NHP. Reprinted with permission from ref. [98]; Copyright© 2022, Nature Publishing Group. (f) Schematic illustration of islet transplantation in the rat using an OxySite scaffold to enhance oxygen delivery and vascularization, resulting in excellent transplantation efficacy. Reprinted with permission from ref. [100]; Copyright© 2021, Social Science Electronic Pub.

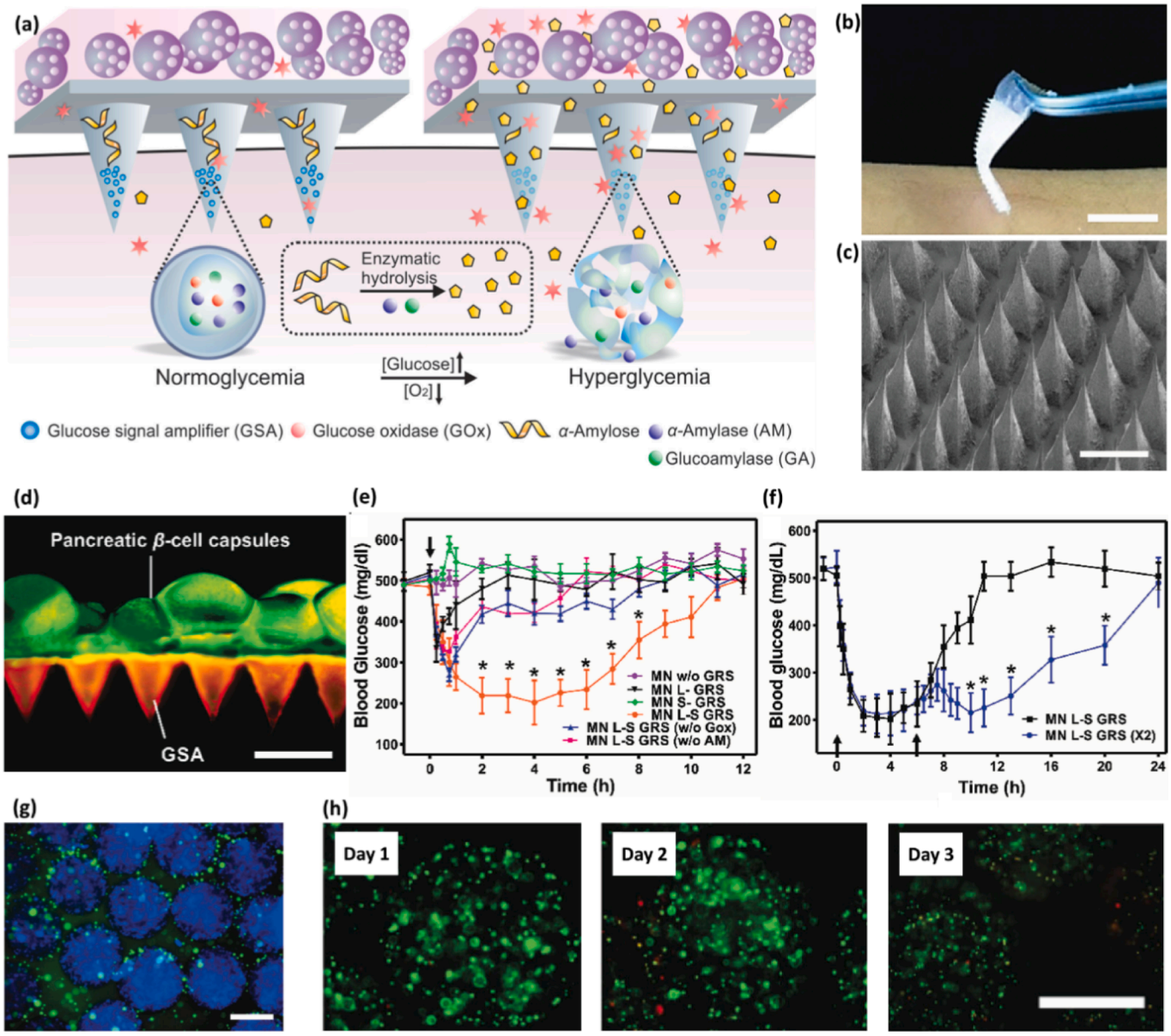

Fig. 5. (a) Schematic illustration of the glucose-responsive mechanism of a microneedle-array patch integrated with pancreatic β-cells and GSA. [Glucose] and [O_2] demonstrate the concentrations of glucose and O_2, respectively. (b) Photographs of the GSA-loaded MN patch, which can be inserted into the dermal tissue (Scale bar:1 cm). (c) SEM image of the MN patch. (Scale bar: 500 μm). (d) Fluorescence image of pancreatic cell-integrated MN patch in which GSAs and β-cell capsules were labeled with rhodamine and calcium-a-AM, respectively. (Scale bar: 500 μm). (e) The blood glucose level of type 1 diabetic mice treated with different MN patches. MN w/o glucose-responsive systems (GRS): empty MN; MN L-GRS: MN integrated with cell-encapsulated gel; MN S-GRS: MN with the amplifier but without cells; MN L-S GRS: MN was loaded with gel and was composed of amplifier system; MN L-S GRS (w/o GOx): MN L-S GRS without the usage of GOx; MN L-S GRS (w/o AM): MN L-S GRS without the usage of amylose (N = 5; *P < 0.05 for treatment with MN integrated with L-S GRS in comparison to the control group. (f) Blood glucose level of type 1 diabetic mice with the repeated treatments of two MN patches (N = 5; *P < 0.05 for X2 treatment with MN patch in comparison to no additional treatment). The black arrows demonstrate the administration points. (g) Immunofluorescence image of the β-cells stained with insulin (green) and nucleus (blue) (Scale bar:500 μm). (h) Fluorescence images of the β-cells during 3 days post-encapsulation, which shows the live (green) and dead (red) cells (Scale bar:500 μm). Reprinted with permission from ref. [103]; Copyright© 2016, Wiley.

immunosuppressants or modified the surface of the alginate to improve its properties [109,122,127–130]. The systemic side effects of immunomodulating agents could be reduced by localized delivery and reduced fibrotic growth was reported [131,132]. Bunger *et al.* examined tissue responses to alginate-poly L-lysine capsules with or without dexamethasone incorporation and reported that the temporary release of encapsulated dexamethasone significantly reduced the adherence of fibroblasts and macrophages to the immuno-isolating capsules [133]. In another study, Acarregui *et al.* developed a hydrogel-based platform composed of cell-loaded alginate-poly L-lysine-alginate (APA) microcapsules and dexamethasone-loaded poly(lactic-co-glycolic) acid (PLGA) microspheres embedded in alginate hydrogel (Fig. 6a) [134]. The results indicated that the encapsulated islets remained viable during the study period. Moreover, the cellular infiltration and collagen deposition were lower compared to the control group, which confirmed the less inflammatory reactions surrounding the cell-based grafts. However, there was no significant difference between the low and high doses of dexamethasone-treated groups (Fig. 6b). Overall, it was concluded that the prepared multifunctional hydrogel-based scaffold can extend the lifetime of the implant by localized continuous release of

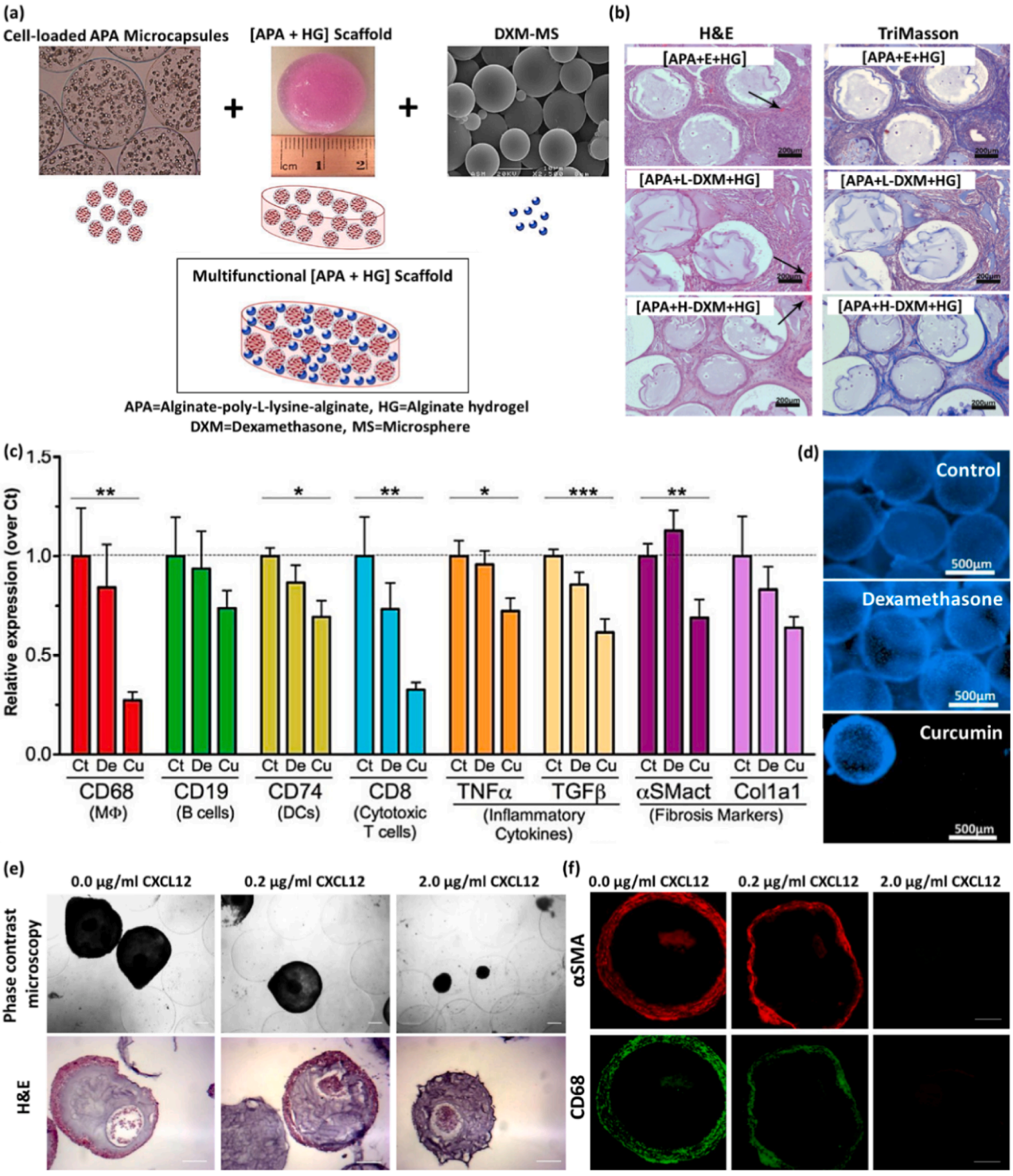

Fig. 6. (a) Schematic illustration of the prepared cell-loaded microcapsules and dexamethasone microspheres embedded in alginate hydrogel. (b) H&E and Tri-Masson stained explanted grafts. E: empty (without dexamethasone), L: low dose, H: high dose (Scale bar: 200 μm). Reprinted with permission from ref. [134]; Copyright© 2014, Elsevier B.V. (c) The expression of host immune and fibrosis markers on alginate microcapsules and surrounding fat pad tissue retrieved one month post-transplantation. The markers were macrophage (Mϕ) marker CD68, B cell marker CD19, dendritic cell marker CD74, cytotoxic T cell marker CD8, inflammatory cytokines TNF-α and TGF-β, and fibrosis-associated activated-fibroblast marker α-smooth muscle actin (α SMact) and collagen 1A1 (Col1a1). Data is presented as Mean ± SEM, (N = 7). *, **, and *** denote $p < 0.05$, 0.01, and 0.001, respectively. Ct: Control, De: Microcapsules with dexamethasone, Cu: Microcapsules with curcumin. (d) Fluorescent photographs of the explanted microcapsules stained with Hoechst 33,342 dye retrieved two months post-transplantation. Blue fluorescence exhibited the binding of Hoeschst 33,342 dye to the DNA of the fibrotic cell layers on the microcapsule surface. Reprinted with permission from ref. [135]; Copyright© 2013, Elsevier B.V. (e) Representative images of phase contrast microscopy and H&E staining of microcapsules retrieved from mice 154 days post-implantation (Scale bar: 200 μm). (f) Immunofluorescence imaging of microcapsules retrieved from mice 154 days after implantation for markers of the fibrotic response including CD68 and αSMA (Scale bar:200 μm). Reprinted with permission from ref. [116]; Copyright© 2019, BLACKWELL PUBLISHING.

dexamethasone. Co-encapsulation of drugs has also been evaluated by loading dexamethasone and curcumin inside alginate microcapsules. The results showed that co-loading significantly decreases the levels of several immune cell markers and reduced the growth of fibrotic cell layers on the capsule surface compared to dexamethasone-loaded and control microcapsules, resulting in the improved long-term release of insulin by the microencapsulated islets (Fig. 6c,d) [135]. Another suggested approach to evade the inflammatory FBR and pericapsular fibrotic overgrowth in alginate-microencapsulated cells was the application of an immunomodulatory chemokine, CXCL12, which can cause immunosuppression due to the Treg recruitment without any need for systemic immunosuppression [115–117]. Alagpulinsa *et al.* demonstrated that this strategy can lead to enhanced insulin secretion for more than 150 days without immunosuppression by remarkable reduction of the pericapsular cellular overgrowth on microcapsules and diminishing the markers of the fibrotic response, including macrophages (CD68) and

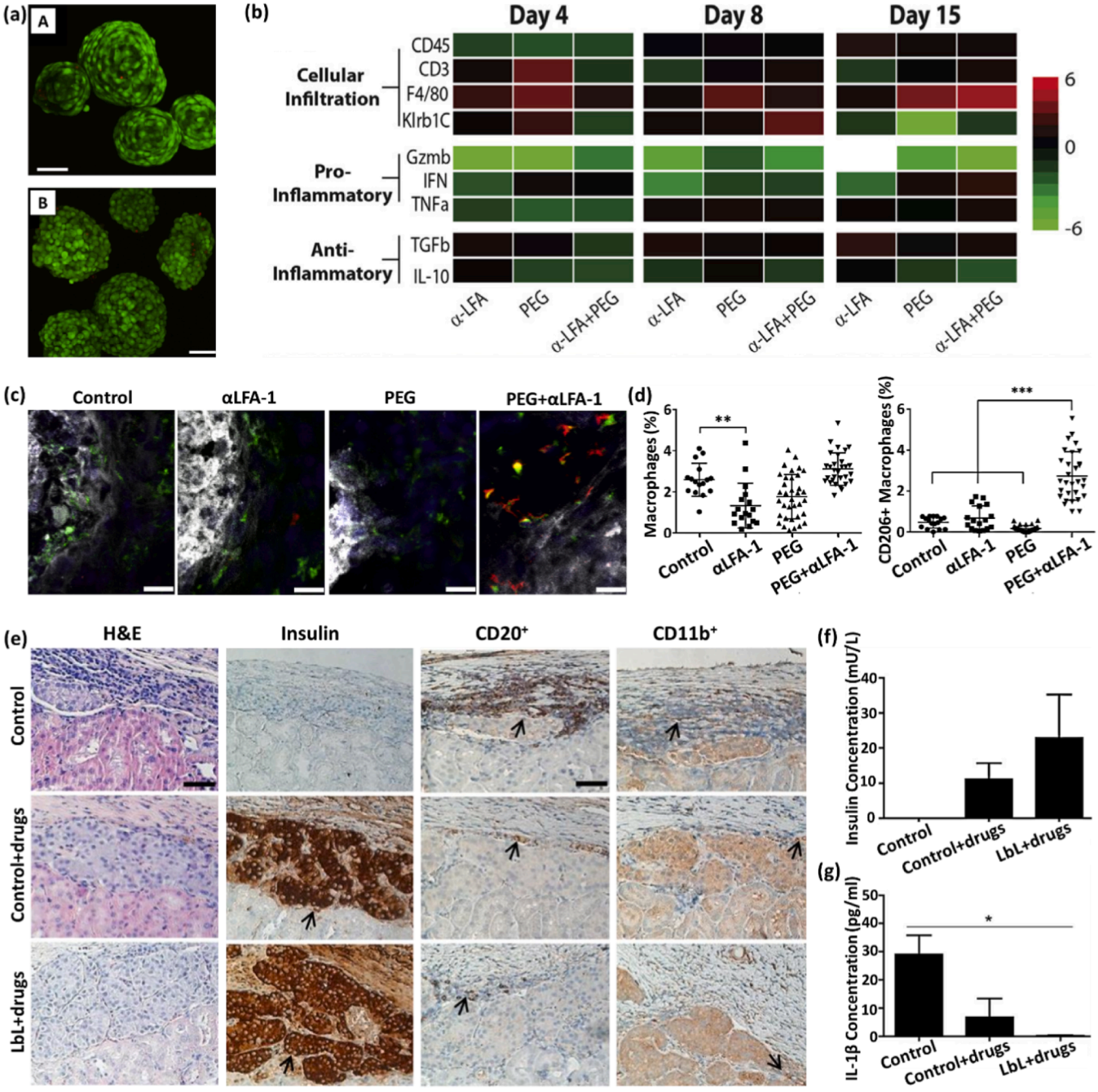

Fig. 7. (a) Representative images captured by confocal microscopy from (A) control and (B) PEGylated islets stained with live/dead (green = viable; red = dead). (b) Gene expression of cellular infiltration markers and pro- or anti-inflammatory cytokines after different treatments. Gene is expressed as fold control (N = 4). (c) Immunohistochemical analysis of grafts from different treated groups for general macrophage marker CD68 (green), macrophage M2 marker CD206 (red), and insulin (white); counterstained with DAPI nuclei stain (blue). (Scale bar: 20 μm). (d) Immunohistochemical quantification of macrophages ($CD68^+$) and M2 phenotype macrophages ($CD206 + CD68^+$); N greater than 15, $^{**}P < 0.01$; $^{***}P < 0.001$. Reprinted with permission from ref. [149]; Copyright© 2017, Elsevier B.V. (e) H&E and immunohistochemical analysis of insulin, $CD20^+$, and $CD11b^+$. Black arrows point at the corresponding staining (Scale bar: 50 μm). (f,g) The concentration of insulin and pro-inflammatory cytokine IL-1β in the serum of all three recipient groups ($^*P < 0.05$). Reprinted with permission from ref. [150]; Copyright© 2017, Elsevier B.V.

myofibroblasts (αSMA) (Fig. 6e,f) [116]. The same results were also obtained in a study by Sremac *et al.* who reported that the co-encapsulation of CXCL12 and pancreatic islets in an alginate capsule can recruit Treg cells and reduce FBR against alginate [115]. Nevertheless, despite promising results in the laboratory, the clinical translation of microencapsulated technologies is not still successful due to the inability to retrieve microencapsulated islets for a long term and more efforts and innovations are required to establish devices with the potential to enter the clinic [82].

3.3. Nanoencapsulation for immuno-engineering of the transplant environment

The nanoencapsulation technique, known as layer-by-layer coating, provides a nanoscale immuno-isolation layer to encapsulate islet cells. Nanoencapsulation possesses many advantages compared to macro/microencapsulation technologies, including improved responsiveness to biological changes, enhanced release of biomolecules from the cells, and facilitated diffusion of oxygen, nutrients, and waste due to reduced diffusional distance between the encapsulated cells and the host environment [18,136–138]. The most widespread strategy for nanoencapsulation includes surface "PEGylation" or multilayer nanocoating [139,140]. PEGylation is the attachment of PEG molecules to an islet surface to prolong islet survival and function and prevent rejection by the immune system [141–143], which is synergized when used in combination with immunosuppressive drugs [144]. Park *et al.* developed PEG with heparin (heparin nano-shielded islets) using a layer-by-layer method to protect the NHP model islet cells against the attack of immune cells [145]. Also, a hyperbranched PEG/heparin nanoencapsulation system was constructed to enhance the survival of transplanted cells by restricting humoral and cell-mediated immune activation and reducing proinflammatory cytokine generation [146]. Lee *et al.* evaluated the functionality of PEGylated islets and immune responses of the host alone or in combination with cyclosporine A [147]. They reported that PEGylation of islets can efficiently inhibit the direct cellular immune reaction, improve the functionality of islets, and inactivate the indirectly stimulated immune cells when combined with a low dose of cyclosporine A. In line with this study, the cytoprotective and anti-inflammatory effect of the PEGylation approach in combination with a low dose of cyclosporine A and heme oxygenase-1 induction was reported, which could immunologically protect the islets from rejection [148]. Giraldo *et al.* used anti-lymphocyte function associated antigen 1 (LFA-1) antibody as a complementary to the immunoprotective effect of PEGylation in an allogeneic murine model [149]. PEGylation did not have any destructive effect on the viability of cells (Fig. 7a) and systemic LFA-1 blockade prevented the expression of pro-inflammatory cytokines and reduced the infiltration of macrophages to the graft (Fig. 7b-d). They realized that the combination of PEG with short-course immunotherapy by LFA-1 antibody can efficiently reduce rejection in comparison to immunotherapy alone, confirming the capacity of the PEGylation approach as an effective immunoprotective strategy in transplantation. To improve the function of transplanted islets, Haque *et al.* designed uniform nano-shielding on NHP islets using a layer-by-layer encapsulation approach [150]. Along with encapsulation, they also used a combination of tacrolimus, sirolimus, and anti-LFA-1 monoclonal antibody in a xenorecipient. The results showed that the layer-by-layer encapsulated islets had low immunogenicity and the combination of encapsulation and drugs led to the highest amount of insulin release and a lower rate of immune cell infiltration (Fig. 7e–g).

Considering the significant advantages of alginate in nanoencapsulation, Zhi *et al.* used alternate layers of phosphorylcholine-derived polysaccharides (chitosan or chondroitin-4-sulfate) and alginate as nano-coating materials to encapsulate islets in a mouse model of diabetes [137]. In addition, Syed *et al.* coated isolated human islets using electrostatic bonding technology to deposit positively charged chitosan and negatively charged polystyrene sulfonate sodium salts for the multi-layer-by-layer nanoencapsulation, which led to enhanced function of islets and simultaneously protected the cells against the inflammatory cytokine damage [151]. All these studies demonstrate that the nanoencapsulation systems can offer localized immune protection to the graft and enhance the survival of cells post-transplantation, which shows great potential to be extended to clinical settings.

4. Hydrogels to prevent graft rejection

Hydrogels are three-dimensional and hydrophilic polymer networks, which can absorb and retain a large amount of water or biological fluid [152–157]. Since hydrogels have many advantageous features (*e.g.*, good biocompatibility, tunable biodegradability, and ability to mimic the physicochemical properties of natural extracellular matrix), they have been used in diverse biomedical fields [158]. For example, hydrogels are used as drug delivery systems that constantly release therapeutic agents over time; they can also be used as scaffolds to support tissue growth and regeneration, or as wound dressings to promote healing [159–161].

To promote the life and functionality of transplants, several investigations have been undertaken on the hydrogel-based delivery of immunomodulatory agents to the graft site [162]. For clinical transplantation of the islets, these local immunomodulatory hydrogel-based strategies may offer an alternative to prolonged systemic immunosuppression. Nevertheless, organ transplantation necessitates blood vessel adhesion and anastomosis. Anastomosis is often performed with thick sutures and takes a lengthy period. However, vessels may get blocked after vascular surgery, eventually resulting in organ damage and patient death [163,164]. Liu and co-workers developed tough, adhesive, and bioabsorbable hydrogels, which could sustain tissue tension and pressurized flow. They exposed the endothelial surface of the vessel to a spacer, used magnetic rings to push both endothelial surfaces against the hydrogel, and promptly re-opened the blood flow. Interestingly, it was found that the time required for adhesion anastomosis was much less than that required for suture anastomosis. Furthermore, the hydrogel displayed negligible detected cytotoxicity and systematic immunological responses. In pig liver transplantation experiments, the scientists accomplished hydrogel anastomosis of big veins. After the transplantation, it was observed that the hydrogel was absorbed, the veins were repaired, and the pig lived for more than a month following the procedure [165]. Preclinical data suggest that revascularization after islet transplantation takes days to weeks, leading to ischemic conditions during revascularization that may damage the transplanted islets [166–168]. This insufficient revascularization of the transplanted islets is one of the main causes of reduced viability, function, and implantation of the islets [169–171]. Weaver *et al.* constructed a synthetic hydrogel based on four-arm poly(ethylene glycol)-maleimide monomer (PEG4-MAL) functionalized with Arginyl-glycyl-aspartic acid (RGD) and VEGF as angiogenic factors. The hydrogel was cross-linked by VPM-peptide for local delivery of the islet grafts to extrahepatic graft sites under physiological conditions (Fig. 8a). The *in vivo* study results demonstrated that the hydrogel, when functionalized with RGD and VEGF and placed in the epididymal fat pad (EFP), effectively normalized blood glucose levels (Fig. 8b–d). Furthermore, the bioluminescence assay revealed satisfactory viability and functionality of the transplanted islets (Fig. 8e,f) [172].

In recent years, Matrigel^{TM} hydrogels extracted by Engelbreth-Holm-Swarm mouse tumor cells have been used as a drug delivery system to prevent graft rejection in the treatment of T1D [173–177]. For instance, Haque and colleagues maintained the viabilities and functions of locally administered islets by simultaneously embedding a macrophage-degrading agent, liposomal clodronate, in an injectable hydrogel. Further mouse experiments revealed that embedding islets in a liposome-containing matrix significantly improved the survival of mice with T1D [175]. Pancreatic islets with tacrolimus (FK506)-loaded poly (lactide-*co*-glycolide)-based microspheres (FK506M) within Matrigel^{TM}

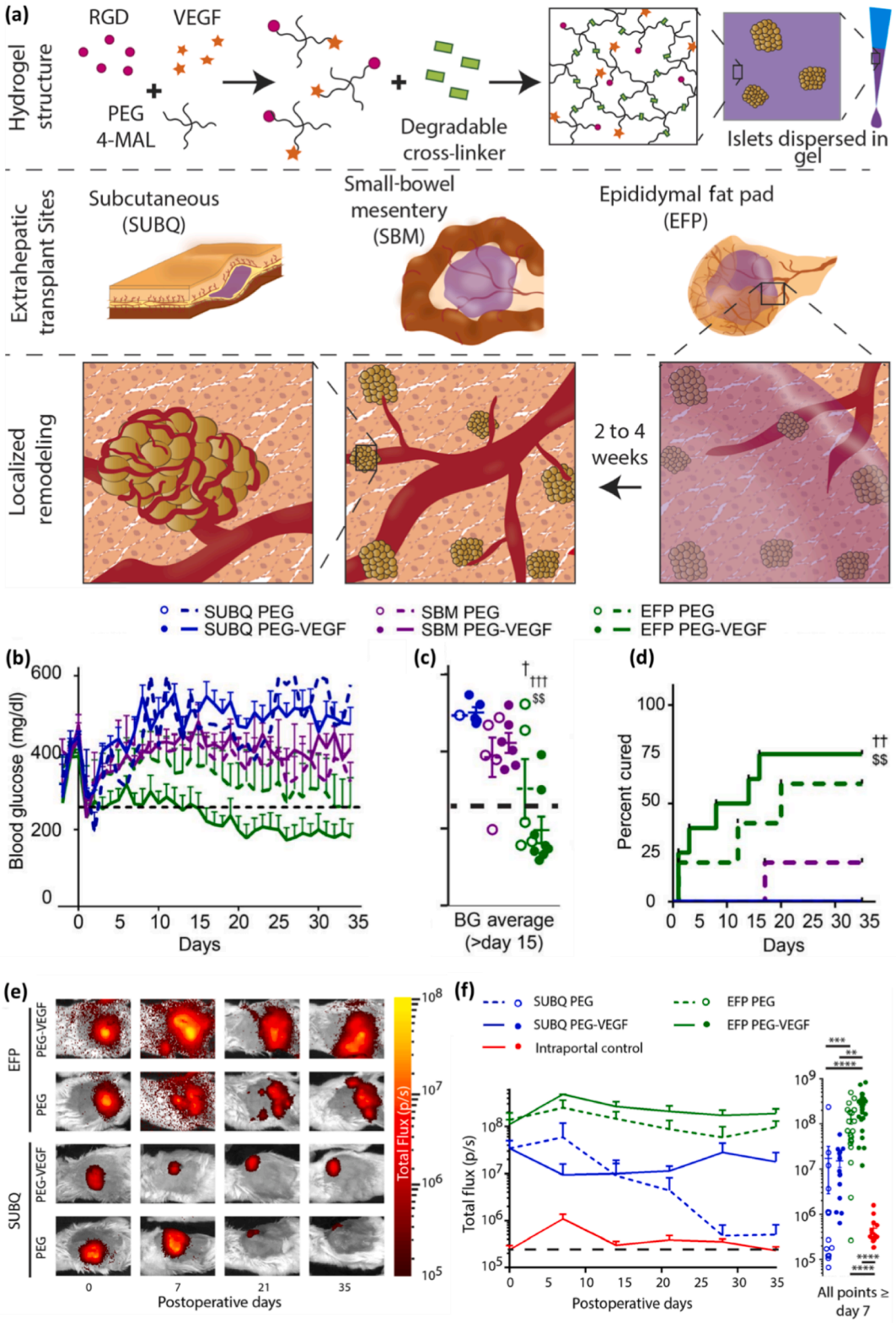

Fig. 8. (a) Schematic illustration of the angiogenic, degradable synthetic hydrogel structure, islet delivery strategies, and local gel remodeling at extrahepatic graft sites. RGD: Arginyl-glycyl-aspartic acid, VEGF: vascular endothelial growth factor, PEG 4-MAL: four-armed poly(ethylene glycol) macromer that has maleimide groups at each terminus, SUBQ: subcutaneous, SBM: small-bowel mesentery, EFP: epididymal fat pad. (b) Non-fasting blood glucose level of type 1 diabetic mice treated with PEG and PEG-VEGF hydrogels in different extrahepatic sites. (c) Survival curve of diabetes reversal. (d) Graft function evaluation by intraperitoneal glucose tolerance test on day 35. Data is presented as Mean ± SEM, (N = 5 to 8 per group). † versus SUBQ (†††$P < 0.001$, ††$P < 0.01$, and †$P < 0.05$); $ versus SBM within the same group (control or VEGF) ($$P < 0.01$). (e) *In vivo* bioluminescent images of islets. (f) Bioluminescent signal (left) by intraperitoneal luciferin injection and cumulative bioluminescent data (right) after day 7 of transplantation. Data is presented as Mean ± SEM, (N = 3 to 4 per group; ****$P < 0.0001$, ***$P < 0.005$, and **$P < 0.01$). Reprinted with permission from ref. [172]; Copyright© 2017, American Association for the Advancement of Science.

have also been utilized by Pathak *et al.* to restore the viabilities and functions of the islets to induce normoglycemia in diabetic mice. They also found that topical administration of FK506M obtained a subtherapeutic concentration of drugs in the blood, which could effectively inhibit T-cell proliferation and subsequently block the immune defense cascade mediated by macrophage activation [176]. Very recently, a robust immunosuppressive protocol proposed by Pathak *et al.* has shown that a single-dose administration of immunosuppressive agents consisting of FK506 microspheres and clodronate liposomes induced tolerance to islet xenograft. Notably, the excellent protocol achieved a graft survival rate of 100% within 120 days and 60% within 520 days. Also, the percentages of $CD4^+$ and $CD8^+$ T cells in the spleen, draining lymph nodes, and graft decreased within 14 days and even 520 days after transplantation [177].

Triglycerol monostearate (TGMS), an amphiphilic self-assembled small molecule, is a generally recognized as safe (GRAS) compound, which can be used for hydrogel formation to encapsulate diverse therapeutic agents for controlled drug release by disassembling the 3D structure in response to inflammatory enzymatic activities [178]. Gajanayake *et al.* constructed an injectable and self-assembled TGMS-based hydrogel, which could specifically release the immunosuppressant tacrolimus in response to proteolytic enzymes and further extend vascularized composite allotransplantation survival to more than 100 days [179]. By comparing systemic administration of tacrolimus (TAC) or subcutaneous injection of TGMS-TAC into the graft, Dzhuliya *et al.* found that injection of TGMS-TAC into the graft prolonged long-term graft survival and provided better toxicological and immunological outcomes [180]. In another report, Fries *et al.* investigated the preventive effect of a tacrolimus-loaded TGMS hydrogel on the rejection of the orthotopic transtibial grafts in a large animal model. They demonstrated that the efficacy and tolerability of this novel enzyme-responsive graft could result in long-term graft survival [181].

Hyaluronic acid is a biocompatible and biodegradable polymer that is used to fabricate hydrogels capable of effectively retaining and releasing drugs over an extended period [182,183]. This property makes hyaluronic acid an attractive delivery platform for immunosuppressive drugs, which often require a sustained release to maintain their therapeutic efficacy. Alvarado-Velez *et al.* designed a functional hybrid gelatin and hyaluronic acid (F-G/H) hydrogel to modulate the immunological microenvironment of the spinal cord tissues from the patients receiving the transplantation of adult spinal cord tissues (aSCTs). They discovered that the resultant F-G/H hydrogel suppressed the recruitment and activation of immune cells *via* the Toll-like receptors (TLRs) and ST-2 signaling pathways, thus enhancing the survival and function of aSCTs. ST-2, known as "interleukin-1 receptor-like 1", is a transmembrane receptor on the surface of Th2 cells, which its inhibition leads to immune tolerance [184]. Lastly, Gao *et al.* developed a hybrid gelatin and hyaluronic acid hydrogel modified with cationic polymers (generation 3 poly(amidoamine) dendrimer, PAMAM-G 3) and anti-inflammatory cytokine for allogeneic aSCTs transplantation. They showed that the F-G/H hydrogel could scavenge damage-associated molecular patterns, produce persistent anti-inflammatory cytokines, and decrease lymphocyte accumulation, thereby modulating the immune response and improving the survival and function of aSCTs [184].

Supramolecular hydrogels are a type of hydrogel formed by the self-assembly of small, non-covalent building blocks (*e.g.*, peptides, small molecules, and polymers) into complex structures, resulting in the formation of a hydrogel network. These hydrogels have unique properties, such as responsive capacity (*e.g.*, temperature, pH, and ions) and self-healing ability [185–187]. Due to their inherent biocompatibility and biodegradability, supramolecular hydrogels offer a wide range of applications for immunosuppressive drug delivery. Wu *et al.* synthesized two types of hydrogelators, namely Nap-Phe-Phe-Glu-Tyr-OH and Nap-d-Phe-dPhe-Glu-Tyr-OH, and prepared tacrolimus-assembled supramolecular hydrogel (Gel 1 and Gel 2), which could specifically release tacrolimus under the phosphorylation of protein tyrosine kinase (PTK) enzyme from activated T cells (Fig. 9a). The amount of tacrolimus released from Gel 1 or Gel 2 into the culture medium after incubation with different numbers of activated T cells at 37 °C for 6 h is presented in Fig. 9b,c, confirming the immune responsive release of tacrolimus, which in turn, suppresses the activity of T cells. Further cellular investigation indicated that Gel 1 and Gel 2 inhibited activated T cells more effectively than the free tacrolimus (Fig. 9d). Moreover, as shown in Fig. 9e, high blood drug concentration (up to 16 ng mL^{-1}) was noticed in the tacrolimus-treated group, during the first 10 h after liver transplantation, which can lead to severe side effects. However, in both Gel 1 and Gel 2 groups, a constant release of tacrolimus into the blood of the recipients was observed (the highest blood drug concentration was 9.2 ng mL^{-1}). After 7 days of treatment, the mean survival time of rats treated with Gel 2 increased dramatically to 22 days, compared with 13 days for rats treated with standard tacrolimus (Fig. 9f) [188]. In another study, Majumder *et al.* developed a noninteracting multiphase molecular assembly approach to crystallize tofacitinib in a self-assembling fibrillar peptide hydrogel network, which could improve cardiac functionality and transplantation efficiency. The microcrystalline tofacitinib hydrogel (MTH) was liable to be injected directly into the transplant sites during surgery to realize the localized release of the loaded small molecules. Systemic combination of MTH with CTLA4-Ig resulted in significantly longer survival time of grafts in mice receiving heterotopic heart transplantation, in comparison with the single treatment modality [189].

Gelatin methacrylate (GelMA), which is formed by a covalent bond between the native polymer (gelatin) and the methacrylate group, has also been used to hinder graft rejection [190]. Due to its good biocompatibility and potential for tissue regeneration [191], Uehara *et al.* investigated its effect at the interface between the wound beds and the skin grafts. They found that the encapsulation of anti-IL-6 antibodies in the GelMA hydrogel remarkably improved the median survival time of 23 days compared with the systemic injection of anti-IL-6 antibodies, and the hydrogel alone exerted no impact on the survival rate. In addition to providing a slow-release platform due to its biomaterial components, GelMA can support burn wound closure and skin allograft. It was also found that the localized release of anti-IL-6 antibodies reduced the inflammatory reactions and the infiltration of T cells and monocytes into the transplanted skin and draining lymph nodes [192].

5. Co-transplantation strategies to stop immuno-rejection

Using biomaterials to co-transplant allogeneic cells with immunomodulatory MSCs, Tregs, exosomes, and cytokines is another new strategy that is found promising for effective cell therapy to bypass transplant rejection and improve tissue regeneration. Different approaches used for co-transplantation are discussed in this section with the hope to provide an overview on its challenges and benefits for future translation.

5.1. MSC co-delivery for transplant immunomodulation

MSCs are multipotent stem/stromal cells that have attracted a lot of attention in tissue regeneration applications and immune disorders primarily due to their immunomodulatory effects. This feature of MSCs is through T cell suppression mediated by regulatory cytokines and biomolecules.

such as TGF-β, IL-10, tumor necrosis factor-inducible gene 6 protein, indoleamine 2, 3-dioxygenase, prostaglandin E2, and nitric oxide (NO). Moreover, MSCs can inhibit the proliferation of NK cells, hinder dendritic cells (DCs) maturation, promote the generation of Tregs, and transform macrophages into anti-inflammatory M2 phenotype [57,194–196]. Studies also showed the potential of MSCs on revascularization through the paracrine secretion of angiogenic factors.

including VEGF, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and matrix metalloproteases [194]. Therefore,

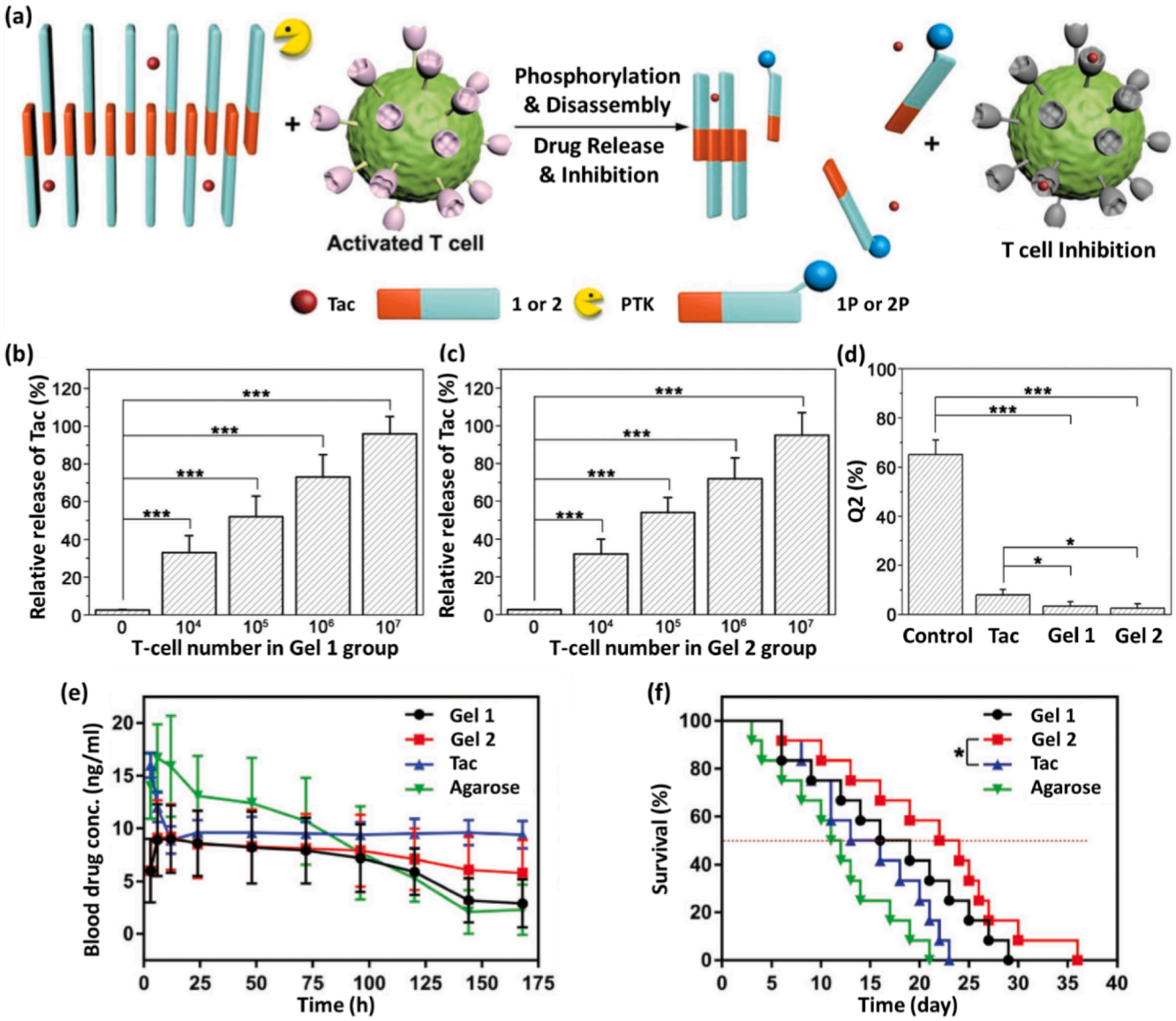

Fig. 9. (a) Schematic illustration of the disassembly of supramolecular peptide hydrogel under PTK in the activated T cell, which induces the release of tacrolimus and the inhibition of T cells. Drug release profiles of tacrolimus from Gel 1 (b) and Gel 2 (c) to the culture media after incubation with different numbers of activated T cells at 37 °C for 6 h. (d) Statistical analyses of the populations of the activated T cells in different groups. (e) Blood concentration of tacrolimus (f) survival rate of rats after different treatments. Reprinted with permission from ref. [188]; Copyright© 2018, Wiley.

many studies have co-transplanted MSCs with allogeneic graft cells using biocompatible materials and demonstrated immune tolerance induction and prolonged graft survival [195–200]. For example, Kogawa *et al.* reported the co-transplantation of MSCs combined with a recombinant protein, called MSC-CellSaic, and alginate microencapsulated pancreatic islets using a nylon macro pocket in the peritoneal cavity of Balb/c diabetic mice (Fig. 10a) [193]. The combination of macro and microcapsules could provide nutrient penetration, the ability to renew pancreatic islets, and immunoisolation to a large extent, by mimicking the native environment. As shown in Fig. 10b, MSC-CellSaic could significantly induce vascularization through the secretion of VEGF and HGF even more than MSC-Spheroid (MSC non-CellSaic). Besides, as reported in previous *in vitro* studies [201], the MSC-CellSaic platform induced anti-inflammatory properties of MSC up to 3.1 fold higher than MSC non-CellSaic through the secretion of TSG-6 as an anti-inflammatory cytokine. *In vivo* results showed a weakened inflammatory response within 14 days (Fig. 10c). Consequently, the co-transplanted MSC-CellSaic with islets induced a significant decrease in blood glucose in comparison to other groups within four weeks of monitoring (Fig. 10d). Taken together, results suggested MSC-CellSaic co-transplanted islets can promote survival and efficacy of islets through effective vascularization around microencapsulated islets and reduction of inflammation. Despite the functional outcomes of alginate microencapsulation of islets with MSC, the precapsular fibrotic overgrowth (PFO) can still cause difficulties as a result of FBR. Thus, more innovative techniques are needed to achieve optimal islets viability and graft efficacy. For this purpose, the effect of altering the immunosuppressive activity of MSCs on inhibiting PFO and improving islet survival was assessed [26]. The cytokine protein array data showed that the treatment of MSCs with a cocktail of IFN-γ and TNF-α (500 + 5000 U/ml) significantly altered the secretion of certain cytokines as shown in Fig. 10e. Also, the stimulated MSCs enhanced the gene expression of inducible nitric oxide synthase (iNOS) and consequently increased NO production 2.4 fold in the stimulated MSCs compared to the un-

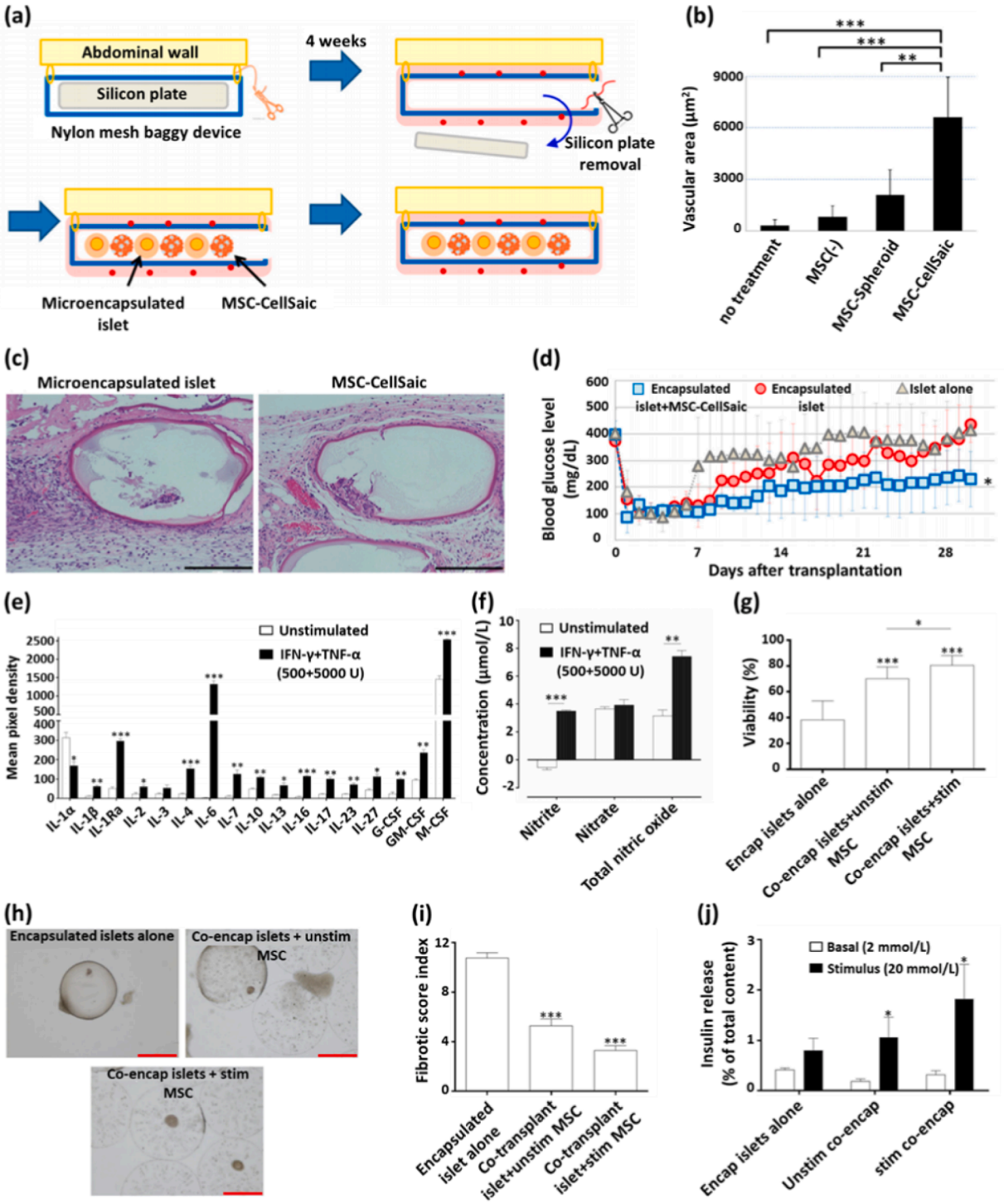

Fig. 10. (a) Schematic illustration of microencapsulated islets and MSC-CellSaic in nylon mesh macropocket transplanted in the peritoneal of a Balb/c mouse. (b) The vascular area of MSC-CellSaic or MSC-Spheroid two weeks post-transplantation. (c) H&E staining of the microencapsulated islets and MSC-CellSaic 14 days post-transplantation. Scale bar:200 μm. (d) Blood glucose changes under non-fasting conditions of three different treated groups (N = 5). Reprinted with permission from ref. [193]; Copyright© 2020, MDPI AG. Effect of MSC stimulated by IFN-γ + TNF-α (500 + 5000 U/ml) on (e) cytokine secretion (N = 2) and (f) iNOS induction and NO production (N = 3). Data is presented as Mean ± SEM, ***p < 0.0001, **p < 0.001, and *p < 0.05 when compared between unstimulated and stimulated MSC. (g) Viability of encapsulated islets retrieved from different treatment groups 50 days after transplantation. Data is presented as Mean ± SEM (N = 100 islets for each treatment group), ***p < 0.0001 and *p < 0.05. (h) Image of PFO formed on different retrieved grafts 50 days after transplantation. Scale bar:500 μm (i) Quantitation of PFO extent as fibrotic score index. Data is presented as Mean ± SEM (N = 6–9), ***p < 0.0001. (j) Ex-vivo stimulation assessment of insulin release of different treated groups. Data is presented as Mean ± SEM (N = 5–7), *p < 0.05. Reprinted with permission from ref. [26]; Copyright© 2017, Nature Publishing Group.

stimulated MSCs (Fig. 10f). The produced NO could potentially suppress the inflammation by regulating the T cell immune response. In addition, the stimulated MSCs could result in higher viability of islets 50 days post-transplantation as compared to islets + un-stimulated MSCs as well as islets alone (Fig. 10g). Moreover, less PFO was observed for the stimulated MSC-treated and non-stimulated MSCs groups than the islets alone-treated group with the fibrotic score index of 1.7 ± 0.5, 4.2 ± 1.03, and 10.7 ± 0.4, respectively (Fig. 10*h* and 10i) as well as high glucose-stimulated insulin secretion (Fig. 10j). Higher levels of immunomodulatory cytokines including IL-6 and granulocyte-colony stimulating factor for the stimulated encapsulated MSCs and islets with alginate.

confirmed the effectiveness of MSCs stimulation prior to transplantation and similar results were observed for the co-transplantation of stimulated MSCs and islets. Overall, the data suggested that co-transplantation or co-encapsulation of MSCs and islets effectively promoted the function of islets via reducing PFO, and the stimulation of MSCs could increase this effect, In another approach, Razavi *et al.* studied the effect of pulsed focused ultrasound (pFUS) stimulation on the function and survival of islets coated by adipose tissue-derived mesenchymal stem cells (AD-MSCs) [202]. The co-cultured islets with AD-MSCs were conformally encapsulated in a thin semi-permeable alginate capsule (50 ± 10 μm) to ensure the spatial localization of AD-MSCs on islets. The conformal coating could overcome the limitations of thick microencapsulations such as hypoxia and hindered/delayed release of insulin while protecting the islets from immune cells to ensure desirable engraftment. The encapsulated islets coated with AD-MSCs were non-invasively stimulated by the pFUS technique to increase the islets' function and survival either directly or indirectly via AD-MSC stimulation (Fig. 11a). The *in vitro* glucose-stimulated insulin secretion assay in normal conditions showed a significantly higher response of encapsulated islets coated with AD-MSCs plus pFUS (emf-islets) compared to islets, islets coated with AD-MSCs (m-Islets), and encapsulated islets coated with AD-MSCs (em-Islets). Also, emf-islets preserved their function and showed a higher amount of insulin secretion even after exposure to IL-1β, IFN-γ, and TNF-α, which is attributed to the trophic (i.e., angiogenic, anti-inflammatory, anti-apoptotic, immunomodulatory, and anti-fibrotic factors) and growth factor secretion potential of AD-MSC. Also, the sensitivity of islets to insulin was enhanced due to the continuous adjacent of AD-MSCs to the islets (Fig. 11b). Moreover, the function of islets could be promoted via pFUS-induced simultaneous stimulation of AD-MSCs and islets. The *in vivo* results in a diabetic mouse model demonstrated higher amounts of insulin in blood serum and kidney (Fig. 11c,d) in the emf-islet-treated group in comparison to other groups, confirming the *in vitro* results. Moreover, emf-islets-treated mice showed reduced infiltration of inflammatory cells and the least TNF-α expression in the histological analysis (Fig. 11e) as well as down-regulation of pro-inflammatory cytokines, including IL-1β, IL-23, IL-27, and IL-6 in comparison to other groups due to the immunomodulatory effect of AD-MSC. Overall, the results confirmed that combining different approaches, including transplanting alginate encapsulated islets coated with AD-MSCs and biomechanical stimulation of both islet and MSCs through wave sound prevented autoimmune mediated graft rejection and improved islet function.

Despite the promising function of bioscaffolds in preventing inflammatory reactions, providing immune isolation of islets, and co-transplantation of MSCs, there are also some limitations, including FBR and fibrotic layer growth at the interface between biomaterials and soft tissue, which impair the function of islets. Therefore, functionalization of the scaffold with anti-inflammatory drugs in addition to other approaches could help local immunomodulation of the milieu to overcome the above-mentioned limitations. For this purpose, the localized drug delivery of dexamethasone (Dex) within graphene bioscaffolds combined with co-transplantation of islets and AD-MSCs for improving islet engraftment was proposed [203]. The islet-laden graphene bioscaffold was functionalized with Dex via a polydopamine nanolayer to overcome the host-FBR without bearing the systemic side effects of the immunosuppressive drugs. The interconnected macroporous structure of graphene provided uniform distribution of the islets, diffusion of nutrients, efficient blood vessel formation similar to native islets, and localization of AD-MSCs in the vicinity of islets throughout its lattice network, which could prevent the cell clumping (Fig. 11f). It is worth mentioning that although the high doses of Dex (1 w/v%) showed the greatest islets' survival, a significantly higher functionality was observed in the islet:AD-MSC units in graphene–0.5 w/v% Dex bioscaffolds in comparison to other groups in both low-glucose and high-glucose stimulation. Therefore, the incorporation of 0.5 w/v% Dex was chosen as the optimum dose (Fig. 11g). The *in vivo* results indicated that only mice treated with islet:AD-MSC units in graphene–0.5 w/v% Dex bioscaffolds could immediately restore and maintain glycemic control (181 ± 32 mg/dl) in the blood for 30 days as compared to mice treated with islets alone (380 ± 50 mg/dl), validating *in vitro* results. Notably, the diabetic mice treated with islet alone in 0.5%w/v Dex bioscaffolds could not reestablish the glycemic control until day 14, confirming the supportive activity of AD-MSCs via enhancing angiogenesis and localized immunosuppression. Moreover, mice treated with islet:AD-MSC units in graphene–0.5 w/v% Dex bioscaffolds exhibited the least change in fasting blood glucose at 30 min in comparison to other groups, which came back to the normal range after 120 min of glucose injection (Fig. 11*h*). Furthermore, the significantly higher number of survived islets with normal size and morphology was observed in islet:AD-MSC units in graphene–0.5 w/v% Dex bioscaffolds in histological analysis in comparison to other groups. Also, insulin immunohistochemical and von Willebrand factor (vWF) staining exhibited higher insulin level and vascularization, respectively, in transplanted islet:ADMSCs units in graphene–0.5 w/v % Dex bioscaffolds compared with other groups (Fig. 11i). The combination activity of AD-MSCs and Dex-functionalized scaffold demonstrated the enhanced amount of insulin level in blood serum (Fig. 11j), reduced TNF-α expression (Fig. 11*k*), and also down-regulation of proinflammatory cytokines such as IL-1β, IL-5, TNF-α, and IL-10, indicating a promising strategy to promote the survival and function of the islets. Despite the promising immunomodulatory effect of MSCs for co-transplantation strategies in pre-clinical tests, this approach suffers from some limitations, such as the inadequate survival of MSCs and the complexity of their isolation and expansion. Therefore, scientists are investigating other novel strategies to overcome these shortcomings.

5.2. Exosome co-delivery for immunoengineering

Small EVs, namely exosomes, are small membrane-bound vesicles that release from various cells with a remarkable role in intercellular communication by transferring nucleic acids, proteins, and other molecules between cells. These nanosize particles have garnered the attention of many scientists as as potential therapeutic and diagnostic tools [204–207]. In addition, in the context of transplantation, exosomes have been used to stop graft rejection via immune response modulation and potentially promoting immune tolerance in transplant recipients. Different strategies are reported for the maintenance of transplantation by exosomes. For example, exosomes derived from Tregs have been able to suppress immune responses and increase immune tolerance in experimental models of transplantation [208]. Induction of tolerogenic DCs by MSCs-derived exosomes could also promote immune tolerance to potentially prevent graft rejection [209,210]. At present, a wide range of immune cell- and MSC-derived exosomes have been studied in cell grafts to mitigate transplantation complications, due to their ability to promote immune tolerance [211], improve neo-angiogenesis [212], and inhibit peripheral blood mononuclear cell (PBMC) proliferation [213]. In addition to the above strategies, antigen-specific tolerance could also be achieved using exosomes. For example, engineered exosomes that carry specific antigens derived from the transplanted organ or tissue would induce antigen-specific immune tolerance. In fact, the delivery of

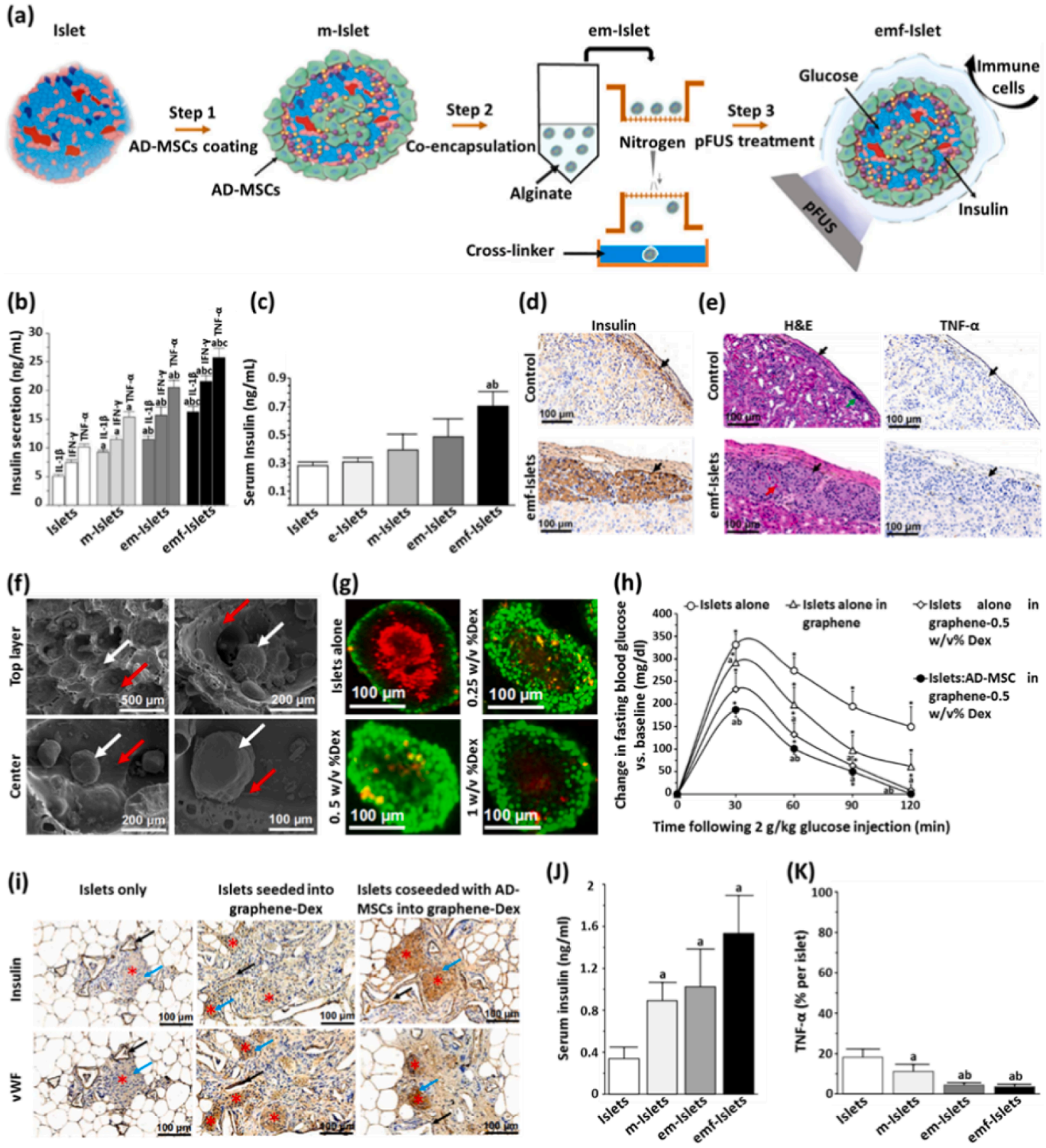

Fig. 11. (a) Schematic illustration of AD-MSCs coated islet encapsulation. step 1: coating islets with AD-MSCs; step 2: conformal encapsulation of coated islets with alginate; Step 3: pFUS treatment. (b) High-glucose-stimulated insulin secretion analysis of different treated groups in exposure to pro-inflammatory cytokines. Data is presented as Mean ± SEM. $^{a}P < 0.05$: Islets vs. m-Islets or em-Islets or emf-Islets; $^{b}P < 0.05$: m-Islets vs. em-Islets or emf-Islets; $^{c}P < 0.05$: em-Islets vs. emf-Islets. (c) The level of insulin in the blood serum of diabetic mice treated with different groups was measured by ELISA. (d) Insulin immunohistochemical staining, (e) H&E staining, and TNF-α immunohistochemical staining of the emf-islets-transplanted group and control islets. Black arrows represent islets. Reprinted with permission from ref. [202]; Copyright© 2020, BioMed Central. (f) SEM images of the top layer and center of graphene-Dex bioscaffolds seeded with islets and AD-MSC at different magnifications. (g) Confocal images of islets cultured in different culture plates on day 7. Green indicates live cells and red indicates dead cells. (h) The intraperitoneal glucose tolerance test of various treated groups after 2 g/kg glucose injection. (i) Representative immunohistochemical images of insulin and vWF staining of different treated groups. (j) Insulin and (k) TNF-α levels of transplanted islets with various treatments. Data is presented as Mean ± SEM (N = 8). Reprinted with permission from ref. [203]; Copyright© 2021, American Association for the Advancement of Science.

donor-derived antigens to the immune cells of transplant recipient can promote tolerance toward the transplanted tissue [214]. The next tested approach was the direct loading of immunosuppressive drugs into exosomes to increase the acceptance of transplant by the host [215]. All these approaches are emerged as pivotal players in immunoengineering to revolutionize the landscape of transplantation strategies [216]. In fact, exosomes can be a promising candidate to alleviate some major limitations of transplantation, which are hypoxia, secretion of pro-inflammatory cytokines, and expression of immune-activating miRNAs and immune rejection [204]. For example, considering the hypoxia-induced dysfunction as one of the key challenges in transplantation, Nie *et al.* revealed that co-transplantation of neonatal porcine islet cell clusters (NICCs) with exosomes derived from human umbilical cord-derived MSC-conditioned medium (hu-MSC-CM) protected the islets from hypoxia-induced dysfunction *in vitro* (Fig. 12a) [217]. Extracellular flux analysis revealed that the viability and function of NICCs enhanced due to increased potential in mitochondrial breathing capacity in hu-MSC-CM with exosomes-treated NICCs group (Fig. 12b). Also, the concentration-dependent protective effect of exosomes on islets was observed, while the accurate mechanism is yet to be identified. The role of exosomes in the reduction of FBR was also investigated by the fabrication of a controlled-release hybrid platform of alginate microcapsules (AlgXO) that was loaded with human umbilical cord-derived MSC exosomes (XO) and rat islets [27]. Analyzing $CD11b^{+}$ cells and MHCII biomarkers revealed 9.4%±3.6% of pericapsular growth and fibrosis in AlgXO transplants, which was significantly lower than control transplants ($p < 0.0001$, Fig. 12c). Also, the significant reduction in chemokines and cytokines, including MCP-1, IL-4, and IL-12p70 was observed in the pericapsular area of AlgXO transplants compared to the control group, indicating the immunoprotective effect of XO on the pericapsular environment (Fig. 12d). Moreover, XO released from platform inhibited the proliferation of splenocytes and $CD3^{+}$ T cells *in*

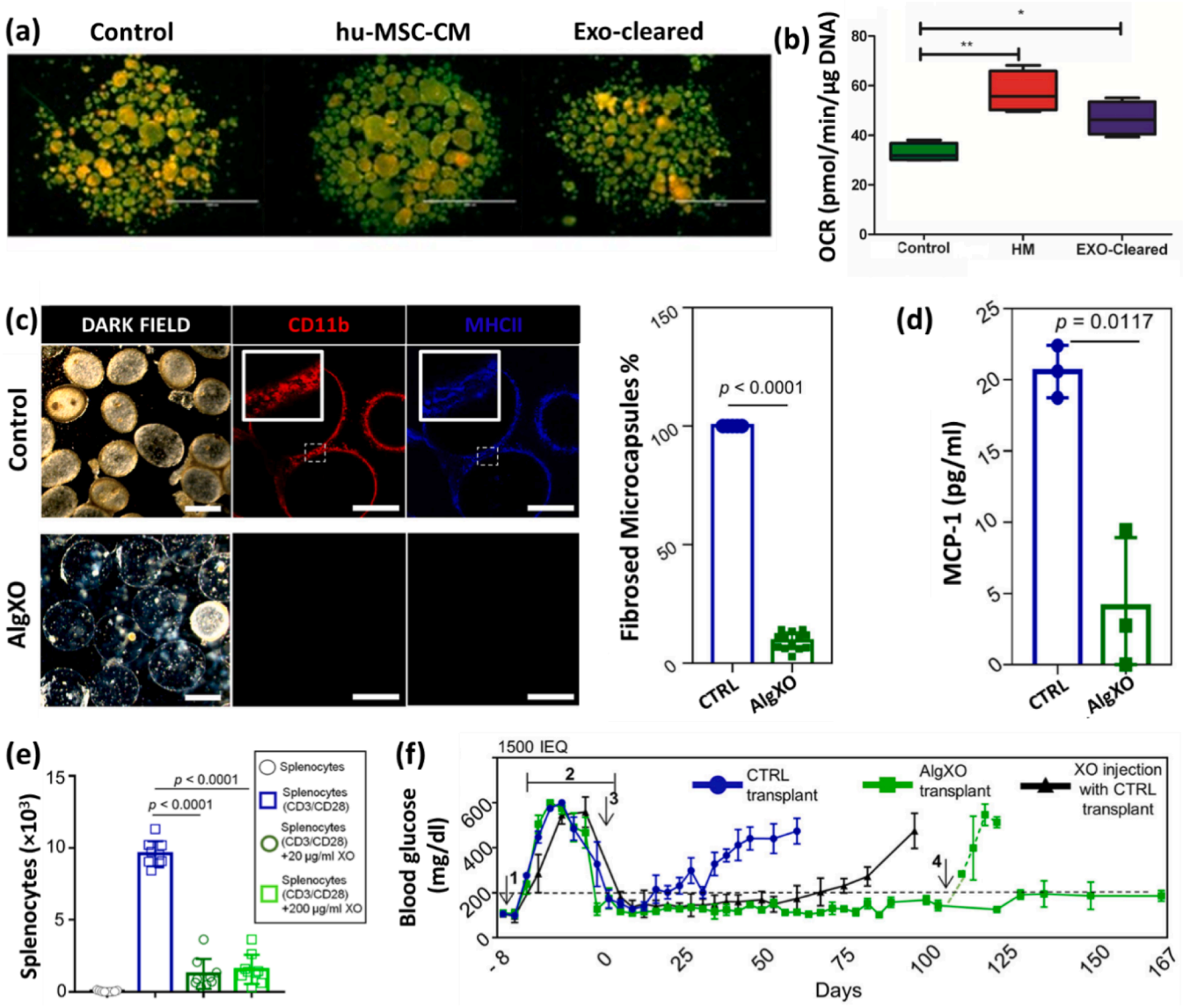

Fig. 12. (a) Acridine orange and propidium iodide staining of islets after 3 days of co-culture with three different mediums under normoxic or hypoxic conditions. (b) Extracellular flux analysis by assessing cumulative average mean baseline oxygen consumption rate (OCR) of islets co-cultured with three different mediums (*$p <$ 0.05, **$p < 0.01$) (HM: hu-MSC-CM). Reprinted with permission from ref. [217]; Copyright© 2018, Wiley. (c) Laser-scanning confocal microscopy analysis of AlgXO and control group 1 month after transplantation. (d) The MCP-1 was released in the pericapsular area of implants in control and AlgXO-treated groups. (e) Splenocyte counts for CD3/CD28 activated cells treated without and with different concentrations of XO. (f) Non-fasting blood glucose levels in diabetic mice (N = 5) transplanted with islets within CTRL microcapsules, CTRL microcapsules with non-encapsulated XO, and AlgXO. Reprinted with permission from ref. [27]; Copyright© 2021, Springer Nature.

vitro (Fig. 12e). Overall, the reduced inflammatory response by the AlgXO led to greater than 170 days of euglycemia in the T1D immunocompetent mouse model; however, this index was failed in 30 and 60 days for groups treated with alginate microcapsules (CTRL) and CTRL microcapsules with non-encapsulated XO, respectively (Fig. 12f).

Attempts to improve the viability and function of islets have also led to the design of a system for the co-delivery of islets with human bone marrow mesenchymal stem cells (hBMSCs) and their exosomes containing miR-375 inhibitor (anti-miR-375) and siRNA against Fas receptor (siFas) to overcome the downregulation of insulin secretion and β-cell apoptosis in a T1D model [204]. Plasmid encoding siFas and anti-miR-375 transfected hBMSCs showed higher viability and 3.88 times accumulative insulin release compared to hBMSCs co-cultured islets due to immunomodulatory effects of bioactive agents. Moreover, the immunomodulatory effect of platforms was further improved by the intravenous injection of hBMSC and PBMC co-cultured exosomes, which significantly inhibited the apoptosis of islets by 90%, while non-transfected hBMSC led to acute apoptosis of islets after transplantation. Collectively, these exosomes were able to improve the outcome of transplantation by enhancing the population of Tregs and suppressing PBMC proliferation, which enabled the reversal of diabetes without a need for insulin.

Despite all these recent advances achieved by exosomes, it is essential to note that using exosomes for graft rejection is a new and active area of research, which many of its aspects and potential are still being explored. Therefore, further research is needed to understand more about the optimal type of exosomes and the most effective delivery methods that can improve the outcome of transplantation.

5.3. Co-transfer of engineered regulatory immune cells

Tregs are a lymphocyte immunosuppressive heterogeneous population of $CD4^+CD25^+FoxP3^+$ T helper cells that are either thymic or periphery differentiated [218,219]. Tregs suppress effector T cells and APCs through modulating DCs and secretion of anti-inflammatory cytokines (*e.g.*, IL-10 and TGF-β) [220,221]. Therefore, locally induced or co-transplanted Treg is a potent therapeutic approach for promoting the survival and function of grafts. Graham *et al.* studied the protective effect of co-transplanted Tregs on islet-loaded microporous poly (lactide-co-glycolide) (PLG) scaffold in the diabetic mouse model [222]. Results showed extended graft survival and restored normoglycemia due to the replacement of recipient Tregs by transplanted Tregs, indicating the tolerance induction of islet antigen-specific Tregs. The protective effect of Tregs was also observed for the second islet transplantation on a systemic level, indicating their potential for controlling both islet-antigen specific naïve and effector T-cell activity. Nevertheless, in spite of targeted immunosuppression by antigen-specific Tregs, there are ongoing hurdles that need further attention, including the expansion of the population of rare antigen-specific Tregs, understanding transplant-specific immune responses, and refining antigen-specific approaches, which are crucial to fully harness the potential of this strategy in preventing graft rejection in the future [223,224]. A report also showed the co-transplantation of Tregs and islet cells in an agarose hydrogel to the liver [225]. In another study, Tregs were transferred to a poly(ethylene glycol) norbornene degradable hydrogel around 2 cm-branched peripheral nerves (PN) allografts to locally suppress the immune system. Results showed that the Treg-loaded hydrogel within PN allografts promoted the regeneration of branched PN defect as well as suppressing the immune response [226]. Despite the promising potential of co-transplantation of Tregs with cells for the inhibition of graft rejection, there are some hurdles in clinical translation, including the limited source, the requirement for good manufacturing process facilities for the Tregs expansion, instability of the Tregs phenotype, and their short-term function [224,227–230].

5.4. Co-loading of regulatory cytokines with the transplants

Due to the challenges of *ex-vivo* Treg expansion followed by local administration that was discussed in the previous section, in situ Treg expansion and/or induction through manipulation of immune cells is preferable for the suppression of transplant rejection. This can be achieved by the development of a specific Treg-inducing formulation via biomaterials-mediated cytokine delivery, which does not induce severe side effects. For example, TGF-β plays an essential role in the promotion of naïve $CD4^+$ T cell differentiation to Treg [231]. Thus, localized release of TGF-β within scaffolds was studied to alleviate inflammation and improve graft acceptance [71]. Recombinant TGF-β1 was locally delivered along with islets into the diabetic mice by a scaffold to modulate immune response. For this aim, PLG microparticles were first formed by dissolving PLG polymer in dichloromethane, followed by emulsification in 1% poly(vinyl alcohol). After washing and lyophilization, 2 mg of microspheres were reconstituted in deionized water containing 1 mg of mannitol and recombinant murine TGF-β1, lyophilized, and then compressed to a central disk with 3 mm diameter and 100 μm of height. This protein containing non-porous layer was then sandwiched between two layers containing lyophilized PLG microspheres and NaCl particles and pressed together into a 5 mm diameter disk with a height of 2 mm. Next, the obtained structure was gas-foamed under CO_2 gas and salt particles were removed by immersion in deionized water to obtain the final scaffold with outer porous layer (Fig. 13a). As shown in Fig. 13b, 83% of TGF- β1 was released on the first day, the next 10% was released between days 1 to 3, and the remaining 7% accounted for 30 days from the center layer of the scaffold. Flow cytometric analysis of CD45 expression indicated reduced leukocyte infiltration into TGF-β1-contained scaffold and allogeneic islet transplants seven days post-implantation (Fig. 13c,d). In addition, the reduced expression of inflammatory cytokine compared to the control scaffold was observed due to the delivery of TGF-β1, which resulted in longer graft survival in the TGF-β1-contained scaffold and long-term effect of TGF-β1 (Fig. 13e). Also, the number of leukocyte population and MHCII expression of F4/80 cells and CD11c cells within the scaffold loaded with 2 μg of TGF-β1 was decreased more compared to the 0.2 μg of TGF-β1 loaded and control scaffold, indicating the role of TGF-β1 amount in the extend of inflammatory responses and tolerogenic phenotypes of APCs. Immunofluorescence imaging of the TGF-β1 releasing scaffold confirmed the prevention of macrophages and NK cell infiltration into the scaffolds and these immune cells were primarily localized in the exterior surface of the scaffold and far from the islets. However, TGF-b1 delivery failed to completely hinder CD8 T cell infiltration into the scaffold and amongst the islets (Fig. 13f-h). The same PLG scaffold has also been utilized for the delivery of IL-33 immunomodulatory cytokine within a protein carrier in the adipose tissue to prevent graft rejection [232]. *In vivo* results showed that IL-33 expanded local $CD4^+$ $Foxp3^+$ Tregs in scaffold containing islets and decreased graft destructive $CD8^+$ T cells response, which resulted in extended allograft survival from 14 to 33 days in comparison to the control scaffold.

Porous PLGA microparticles fabricated via the double emulsion method are also utilized for the controlled release of TGF-β1 [230]. The results showed a burst release on the first day via diffusion, followed by a lag phase, which was controlled by polymer degradation. The released TGF-β1 could convert naïve $CD4^+$ T cells to Tregs to prolong immune tolerance. The effect was similar to soluble TGF-β1 in a dose-dependent manner while overcoming the short half-life and off-target delivery of soluble TGF-β1. Nevertheless, while *in vitro* studies showed the immunoregulatory effect of the particles, the *in vivo* study did not result in a significant difference in rejection rate and also glycemic control of TGF-β1/PLGA microparticles-treated and control groups. This could be due to the multi-dimensional aspects and complexity of immunological responses *in vitro*. In another attempt, PLGA microparticles consisting of IL-2, TGF-β, or rapamycin, as a controlled release vehicle, were separately tested in a rat hindlimb vascularized composite

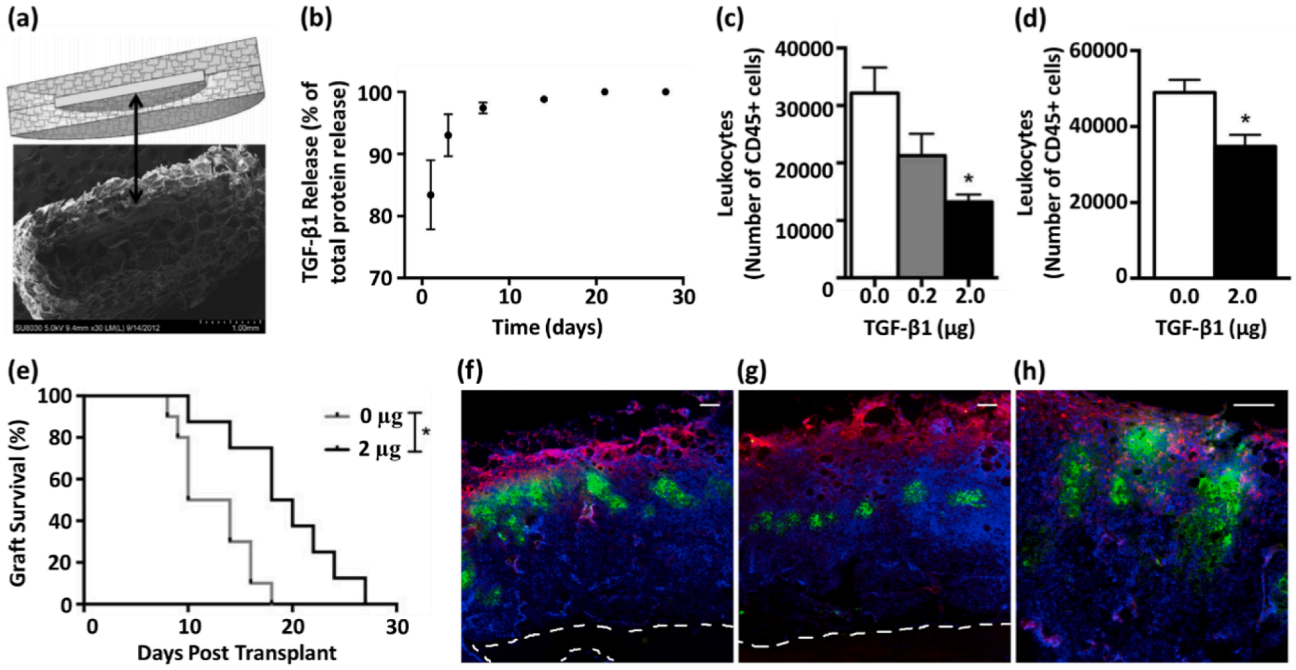

Fig. 13. (a) The PLG scaffold microstructure. (b) In vitro release profile of 2 μg of TGF-β1 loaded scaffold. (c) Infiltration of leukocytes to the scaffolds containing different amounts of TGF-β1. (d) Infiltration of leukocyte to allogenic islets seeded into the scaffolds with and without TGF-β1 (*$P < 0.05$). (e) Kaplan-Meyer survival of Graft ($N = 8$). Immunofluorescence images of (f) F4/80 (red), (g) NK1.1 (red), and (h) CD8 (red) within histological sections of islets seeded in scaffolds containing 2 μg of TGF-β1 at day 7. Insulin and nuclei are visible in green and blue, respectively (Scale bar:100 μm). Both F4/80 and NK1.1 are just detected on the surface of the scaffold without penetration and close vicinity to the internal islets. CD8 was partially detected within the scaffold. Reprinted with permission from ref. [71]; Copyright© 2016, Elsevier B.V.

allotransplantation model to induce Treg differentiation from naïve T cells [233,234]. The study showed that the Treg-inducing microparticles enriched Treg and reduced inflammatory Th1 populations in allograft draining lymph nodes, leading to increased allograft survival for more than 300 days. Despite all these advances, future studies on biomaterial-based delivery of immunoregulating agents with the aim of graft survival needs more innovations towards multifunctional carriers or multi-delivery of reagents to guarantee effective *in vivo* responses and promote the capability of formulations for clinical translation and long-term survival of transplants.

6. Surface engineering strategies for immunomodulation of transplants

To date, several studies have demonstrated the importance of surface engineering in exploiting the immunomodulatory responses by biomaterial-based platforms in transplantation settings to render long-term survival to the grafts [28,235,236]. One strategy is the chemical modification of polymer structure to alter immune responses. For example, Hue *et al.* created pancreatic islets-encapsulated pectin/alginate microcapsules and assessed the attenuation of immune response by three variable degrees of methyl esterification (DM) of immunomodulatory polymer pectin (DM18, DM55, and DM69 pectins) [235]. Pectin families on the surface of biomaterials mitigated DAMP induced-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) immune activation, which subsequently could decrease the secretion of pro-inflammatory cytokines and chemokines (Fig. 14a). In this study, DM18 pectin/alginate microcapsules revealed less DAMP-induced NF-κB activation in THP-1 cells compared to microcapsules containing either DM55-or DM69-pectin under low nutrients and/or hypoxia (Fig. 14b). Also, islets encapsulated in DM18 pectin/alginate microcapsules demonstrated reduced amount of pro-inflammatory GRO-α, and TNF-α, and IL-6, as well as increased levels of anti-inflammatory IL-10 in comparison to microcapsules containing DM69-pectin/alginate and alginate microcapsules (Fig. 14c). Among all, DM18 pectin/alginate microcapsules was able to support the long-term survival of xeno-transplanted rat islets in a diabetic mice model. Also, Bochenek *et al.* showed the encapsulation of viable glucose-responsive allogeneic islets into alginate derivatives chemically modified with three anti-fibrotic triazole rings enabled the correction of insulin deficiency without the need for immunosuppression in NHP models [109]. The chemical modification of alginate microparticles effectively decreased macrophage activation by providing a distinct surface that prevents the adherence of $CD68^{+}$/$CD11b^{+}$ macrophages and reduces fibrotic overgrowth, which subsequently resulted in increased cell viability to about 90.0% after 4 months. In contrast, non-modified and non-encapsulated β-cells were recognized by the immune system after a short time and also elicited significant macrophage activation, causing the formation of fibrosis tissue.

In addition to chemical modification of polymers used for particle formation and transplantation, a wide variety of ligands, such as FasL, programmed cell death-1 (PD-L1), TGF-β, etc., can be directly immobilized on the surface of formed particles for localized immunomodulation to enhance the viability rate of transplants [57,221]. For example, localized immunomodulation and prolonged survival of pancreatic allogeneic islets in diabetic mice was achieved by microgels conjugated with an apoptotic form of the Fas ligand with streptavidin (SA-FasL) on their surface [29,237]. Although a previous study showed indefinite survival of pancreatic islets after direct chemical modification of their surface with SA-FasL without chronic immunosuppression in an animal model [238], using biomaterials to present SA-FasL on their surface eliminates the need for islet chemical modification, which is associated with substantial technical and regulatory challenges potentially. In addition, it can be more efficient and improve the safety profile of islets. In this study, biotinylated microgels were fabricated by reacting biotin-PEG-thiol with maleimide-terminated four-arm PEG (PEG-4MAL)

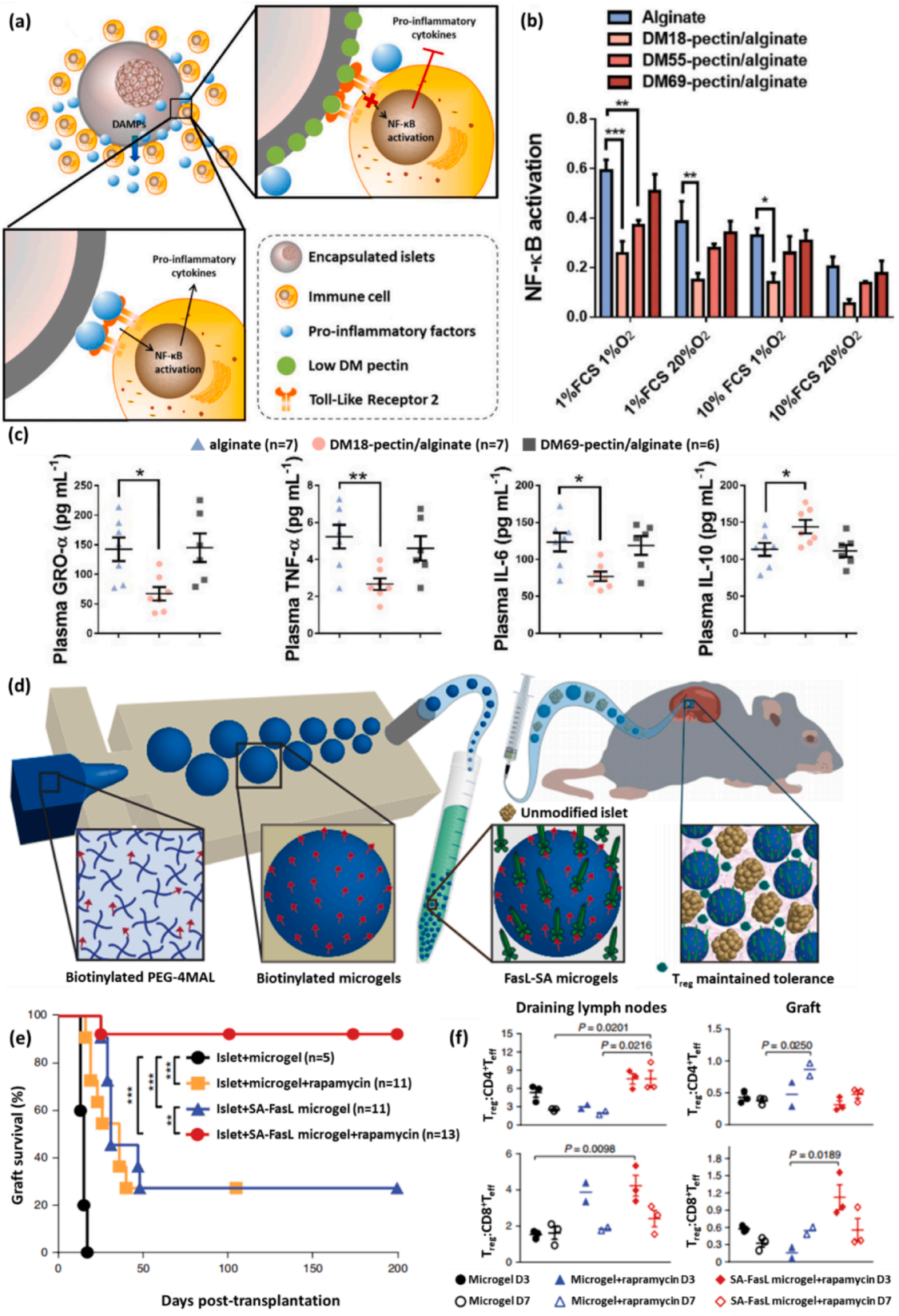

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Fig. 14. (a) Schematic illustration of pectin/alginate microcapsules' inhibitory effect on the secretion of pro-inflammatory cytokines and DAMP-induced inflammatory responses in panceratic islets. (b) The level of NF-κB activation in THP-1 cells 5 days after culturing with islets encapsulated in alginate, DM18-, DM55-, and DM69-pectin/alginate under different culture conditions. Data is presented as Mean ± SEM (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). To mimic the relatively low oxygen tensions, islets were cultured under hypoxic conditions (1% O_2). To simulate reduced nutrient availability, the islets were cultured using 1% fetal calf serum (FCS). (c) Plasma levels of GRO-α, TNF-α, IL-6, and IL-10 eight weeks after the implantation of encapsulated islets (*$p < 0.05$, **$p < 0.01$). Reprinted with permission from ref. [235]; Copyright© 2021, Elsevier B.V. (d) Schematic illustration of fabrication of biotinylated microgel from PEG-4MAL macromers utilizing flow-focusing microfluidics, followed by immobilization of SA-FasL on the surface of microgel. (e) Survival of allogeneic pancreatic islets co-transplanted with SA-FasL-presenting microgels in different treated groups. Transplantation was conducted under the kidney capsule of diabetic mice (f) Immune response monitoring and the ratios of Treg to $CD4^+$ and $CD8^+$ Teff cells in kidney-draining lymph nodes and kidney of indicated groups on days 3 and 7 of the study. Reprinted with permission from ref. [237]; Copyright© 2018, Springer Nature.

macromers utilizing microfluidics polymerization (Fig. 14d) [237]. Covalently tethered biotin on microgels was capable of capturing SA-FasL on the surface due to the high affinity of biotin to SA. The co-transplantation of engineered microgels with islets, when treated by a short course of rapamycin (0.2 mg kg^{-1} daily for 15 days post-transplantation), demonstrated greater than 90% islets survival in 200 days (Fig. 14e). In contrast, control biotinylated microgels led to the rejection of whole allografts in 15 days. The enhanced survival rate was attributed to the sustained presentation of SA-FasL within the islet graft microenvironment and FasL-mediated inhibition of T effector cells that are responsible for islet allograft rejection. In addition, rapamycin could enhance the acceptance rate and function of pancreatic allografts by boosting the immunomodulatory efficacy of SA-FasL microgels via the enhanced ratio of Treg cell to $CD4^+$ and $CD8^+$ Teff cells in the graft and draining lymph nodes in the group receiving SA-FasL-engineered microgels plus rapamycin compared to the unmodified microgels plus rapamycin and control groups (Fig. 14f). Another study on the same platform also showed that the massive population of $FoxP3^+$ (a marker of Tregs) cells was responsible for the long-term survival of the islets co-transplanted with the SA-FasL-presenting microgels [239]. The effect of SA-FasL on prolonging the islet survival was further studied by seeding allogenic islets into PLG microgels with temporary rapamycin administration (daily up to 15 days), which resulted in 200 days of normoglycemia and graft survival [240].

In another study, targeted delivery of tacrolimus to the lymph nodes following systemic administration was studied using engineered MECA79 antibody-coated microparticles to prolong the survival of heart allograft [241]. The monoclonal antibody MECA79 binds to peripheral node addressins in lymph nodes as the primary site for the activation of immune cells, which inhibits T cell priming. Moreover, using MECA79 coating provides targeted delivery of tacrolimus as an immunosuppressant via intravenous injection by a noticeable accumulation of microparticles in the draining lymph nodes of transplanted mice, resulting in the suppressed inflammatory cytokines production by T cells and increased survival rate of the allograft.

To circumvent the need for systemic immunosuppression, a versatile coating layer was also introduced for pancreatic islet allograft using 3,4–dihydroxyphenethylamine (DOPA) conjugated PLGA–PEG NPs, which were loaded with immunosuppressant FK506 [242]. DOPA–functionalized polymeric NPs, by decorating the islets in a multi-layer manner, could create a protective shield against immune recognition and did not interfere with the function or viability of islets. In particular, the survival rate of allografts significantly increased in the xeno-transplantation model, owing to surface camouflage and localized controlled release of FK506.

The next strategy is the utilization of the PD-1/PD-L1 pathway, which has demonstrated efficacy in managing immune responses in transplant settings since the delivery of PD-L1 ligand has the capacity to promote self-tolerance and regulate immune responses. For example, by conjugating streptavidin/programmed cell death-1 (SA-PD-L1) protein to biotinylated PEG microgels, Coronel *et al.* achieved a regulatory immune response after pancreatic islet transplantation [243]. The controlled presentation of SA-PD-L1 on the surface of microgels led to a higher local retention time of the immunomodulatory agent over 3 weeks *in vivo* compared to the free SA-PD-L1. Also, the administration of modified microgels enhanced the population of $CD4^+$ Tregs followed by an increase in the $CD4^+$ T anergic cell population compared to control microgels, resulting in immune tolerance and long-term survival of islet allografts without exploiting chronic systemic immunosuppressive agents. Similarly, the surface engineering of macroporous alginate scaffold with TGF-β resulted in the enhanced viability of allofibroblasts within the scaffold [244]. TGF-β leads to a greater population of immature DCs and Tregs as well as reduced effector functions of CD4 and CD8 cytotoxic T cells by enhancing the IL-10 signals.

Full coating of the islets/cells loaded particles is another tested strategy for the improvement of graft survival. As an evidence, rapamycin-containing PEG coating on islet cells-laden alginate microcapsules could prevent the damage of transplanted cells by the immune system and led to significant inhibition of fibrotic cell infiltration compared to the alginate microcapsule group without coating [128]. Hume *et al.* also developed cell-laden PEG hydrogel and fully coated it with a PEG layer via dip-coating method while this layer was enriched with anti-Fas antibody and intercellular adhesion molecule-1 (ICAM-1) to induce T cell apoptosis and (Fig. 15a) [245]. For polymer coating, PEG hydrogel was swallon in glucose solution and dipped then into a glucose-free pre-polymer solution of PEG diacrylate, resulting in the diffusion of glucose from hydrogel matrix, which subsequently reacted with glucose oxidase (GOx) at the hydrogel surface. Thiolated proteins, including IgG, anti-Fas, and ICAM-1 were incorporated with the coating solution prior to polymerization. Studies confirmed that the coating on the PEG hydrogel led to apoptosis or death of over 60% of T cells compared to only 18% of T cell death in non-functionalized hydrogel group after 48 h, which can lead to reduced post-transplantation inflammation and increased tissue acceptance (Fig. 15b). There is also a report on using tetrahydropyran phenyl triazole (THPT) as an anti-fibrotic coating on the surface of a device comprises of a cell reservoir attached to a thin porous polymeric immune isolation membrane to enhance the biocompatibility and mitigate FBR [236]. The membrane optimum pore size was investigated to allow oxygen and nutrients to diffuse, while preventing the immune cells' access to encapsulated graft cells (Fig. 15c). Devices with pore sizes ≤ 0.8 μm did not allow macrophage or T cell infiltration to the device. However, high infiltration of macrophages and T cells, in addition to the loss of the graft, was occurred inside the devices with the pore size of 3-μm (Fig. 15d). The THPT-coated device containing the HEK293T cells caused enhancement in the amount of erythropoietin (EPO) and haematocrit in serum (Fig. 15e), indicating the ability of THPT coating in maintaining the viability and function of xenografts for at least 130 days by providing durable protection against fibrosis. The effect of THPT-coated devices on FBR was further confirmed by H&E and Masson's trichrome staining, which showed the biocompatibility of the proposed device with only a thin fibrotic capsule, while the uncoated device revealed a thick fibrosis on its surface (Fig. 15f). Overall, all these examples demonstrate that the surface functionalization of biomaterials/islets via different strategies has opened new avenues to enhance the survival rate of allografts through different mechanisms.

7. Barriers, future opportunities, and concluding remarks

Despite all the novel and exciting studies conducted on biomaterial-

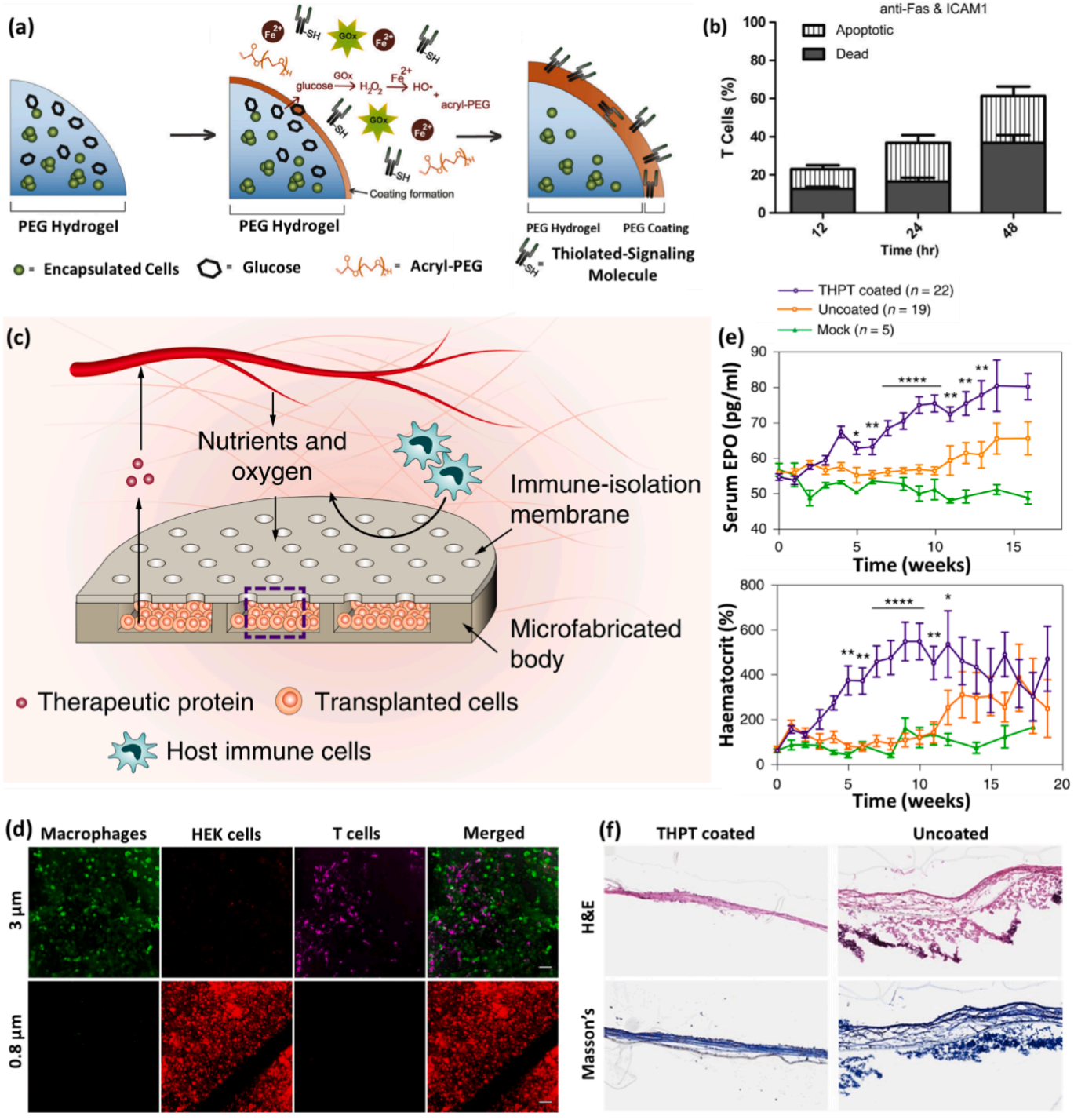

Fig. 15. (a) Schematic illustrating the formation of polymer coatings and covalently incorporation of multiple antibodies on cell-laden PEG hydrogels. (b) Flow cytometry analysis of T cells seeded up to dually functionalized coating for 12, 24, and 48 h. Reprinted with permission from ref. [245]; Copyright© 2011, Elsevier B. V. (c) The schematic illustration of the device design, consisted of a microfabricated body sealed to a polymeric membrane with a controlled pore size, which allows the exchange of oxygen and nutrients but prevents infiltration of immune cells. (d) Immunostained devices after 35 days assessing the presence of macrophages, T cells, and HEK cells in the reservoir space of the devices with pores of 0.8 and 3 µm. (e) Efficacy of THPT-coated devices in the long-term sustained delivery of EPO, by analyzing the serum concentrations of EPO and hematocrit. Data are presented as Mean ± SEM, *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$. (f) H&E staining and Masson's trichrome staining of the coated and uncoated devices after 130 d. Reprinted with permission from ref. [236]; Copyright© 2020 Springer Nature.

based immune engineering for organ/cell transplantation, moving from bench to clinic is still a challenging process due to the FBR formation by many biomaterial systems. Therefore, there is a consensus on the need for novel effective strategies for the long-term prevention of FBR, which is only possible by understanding the complex mechanisms at bio-interfaces that rule these processes.

The mechanisms that govern the effectiveness of novel immuno-modulatory strategies to limit inflammatory responses to biomaterials are highly investigated both *in vitro* and *in vivo* [246–249]. However, extrapolating the obtained results from these models to human patients is a major challenge that limits the translation of novel approaches. This is mainly due to the highly responsive nature of immune cells to their

microenvironment, the evolved state of the human immune system, the dependence of immune cell behavior on the medical history of patients with different pathophysiological backgrounds [250], and significant differences between human and murine species [251]. For example, in the presence of IL-4, murine macrophages polarize towards the M2 phenotype *in vitro* [252], while this response has not been observed in human macrophages [253]. Also, the cell culture environment and microenvironments of small animals do not fully mimic the biology of the human body. Therefore, developing novel 3D models or studies on big animals needs further attention for faster movement toward clinical trials and commercialization.

Another issue that should be considered is patient-specific factors that animal models may need to represent adequately during animal studies. For example, the effect of common disorders, including diabetes and obesity on the immune system and transplant rejection needs further attention and is not considered yet in animal models [254–257]. The heterogenicity of the human immune response is another issue to be considered for the development of biomaterial-based immunoengineering products for transplantation. Moreover, novel biomaterials-based immune engineering strategies should be approved by regulatory organizations to find their way to clinics and markets. Therefore, using new biomaterials requires approval for all aspects of safety considerations and performances, which increases the cost of clinical trials. That is the reason companies with an interest in islet transplantation systems prefer already-tested biomaterials compared to new materials like self-assembling biopolymers. All these challenges, along with long development timelines, funding shortages, and regulatory uncertainty have made the fast clinical translation and commercialization of biomaterial-based immunoengineering products a convoluted path. Nevertheless, the development of these biomaterial-based systems to improve the outcome of graft transplantation is considerably of interest because of the versatility of using different strategies, such as creating physical barriers, drug delivery systems, co-transplantation platforms, and localized delivery of immunomodulatory agents by surface functionalization to hinder immune attack and improve the survival of transplanted cells. To do so, a precise understanding of the whole mechanisms involved in immune rejection as well as the biological environment of the graft transplantation is essential and future designs need multifunctionality to simultaneously manipulate multiple mechanisms involved in immune rejection to support the life-long survival and function of transplanted cells or tissues. Immune engineering is a rapidly evolving field and there are currently significant explorations beyond the bench in preclinical and clinical stages. One noteworthy example is Sigilon Therapeutics, focusing on the development of Shielded Living Therapeutics™, a platform that integrates advanced cell engineering techniques with biomaterials to improve the functionality and durability of therapeutic cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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