Heliyon 10 (2024) e24203

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Nanoparticle-based T cell immunoimaging and immunomodulatory for diagnosing and treating transplant rejection

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ARTICLE INFO

Keywords:
Transplant rejection
T cell
Nanoparticle
Molecular imaging

ABSTRACT

T cells serve a pivotal role in the rejection of transplants, both by directly attacking the graft and by recruiting other immune cells, which intensifies the rejection process. Therefore, monitoring T cells becomes crucial for early detection of transplant rejection, while targeted drug delivery specifically to T cells can significantly enhance the effectiveness of rejection therapy. However, regulating the activity of T cells within transplanted organs is challenging, and the prolonged use of immunosuppressive drugs is associated with notable side effects and complications. Functionalized nanoparticles offer a potential solution by targeting T cells within transplants or lymph nodes, thereby reducing the off-target effects and improving the long-term survival of the graft. In this review, we will provide an overview of recent advancements in T cell-targeted imaging molecular probes for diagnosing transplant rejection and the progress of T cell-regulating nanomedicines for treating transplant rejection. Additionally, we will discuss future directions and the challenges in clinical translation.

1. Introduction

Transplant rejection stands as a formidable obstacle in clinical transplantation, occurring when the immune system recognizes transplanted tissue as foreign, instigating an immune reaction against it [1–3]. T cells identify antigens presented by antigen-presenting cells (APCs) via the major histocompatibility complex (MHC) presented within the peripheral lymph nodes. Subsequently, they undergo activation upon receiving co-stimulatory and cytokine signals [3]. Upon activation, T cells rapidly

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https://doi.org/10.1016/j.heliyon.2024.e24203
Received 8 October 2023; Received in revised form 3 January 2024; Accepted 4 January 2024
Available online 9 January 2024

proliferate and differentiate into effector T cells. Then, effector T cells migrate towards the graft in response to chemokines, instigating and aggravating graft rejection [4]. Within the graft, effector T cells further differentiate into $CD4^+$ effector T cells and $CD8^+$ effector T cells. $CD4^+$ effector T cells secrete a variety of cytokines to recruit immune cells such as macrophages and B cells to assault the graft [5]. In addition, they aid in the differentiation of $CD8^+$ effector T cells to into cytotoxic T cells (CTLs). CTLs secrete granzyme and perforin, inducing apoptosis in the graft and worsening rejection [6]. Consequently, modulating T-cell responses can prevent graft rejection and enhance patient prognosis.

In recent years, molecular imaging and targeted regulation using nanotechnology have emerged as promising approaches for manipulating T cells in the context of transplant rejection [7–9]. Molecular imaging refers to the utilization of non-invasive imaging techniques to visualize molecular and cellular events *in vivo* [10,11]. Probes are the key to molecular imaging and determine the sensitivity and specificity of imaging [12,13]. The advantages of nanoparticles (NPs) applied to molecular imaging are: 1) NPs surface is easy to modify, and can be loaded with ligands to obtain the ability to actively target T cells and improve the sensitivity of imaging [13]; 2) Changing the size, potential, and other characteristics of NPs can reduce the uptake rate by the kidney and the liver, prolonging the circulation time, which is conducive to obtaining a clearer imaging [14]; 3) Nanoparticles can be mapped into the responsive functional groups to achieve a specific controllable release, effectively improving the imaging contrast [15]. Imaging T cells and their interactions with various cells and tissues enables the early detection of transplant rejection and the development of timely, effective immunosuppressive strategies. Furthermore, it enhances our comprehension of the fundamental mechanisms underlying transplant rejection, providing valuable insights into potential targets for intervention.

The current clinical treatment for transplant rejection relies on immunosuppressive drug, which inherently elevate the risk of cancer and infection [16]. Nanotechnology provides multiple advantages in precisely regulating T cells during transplant rejection.

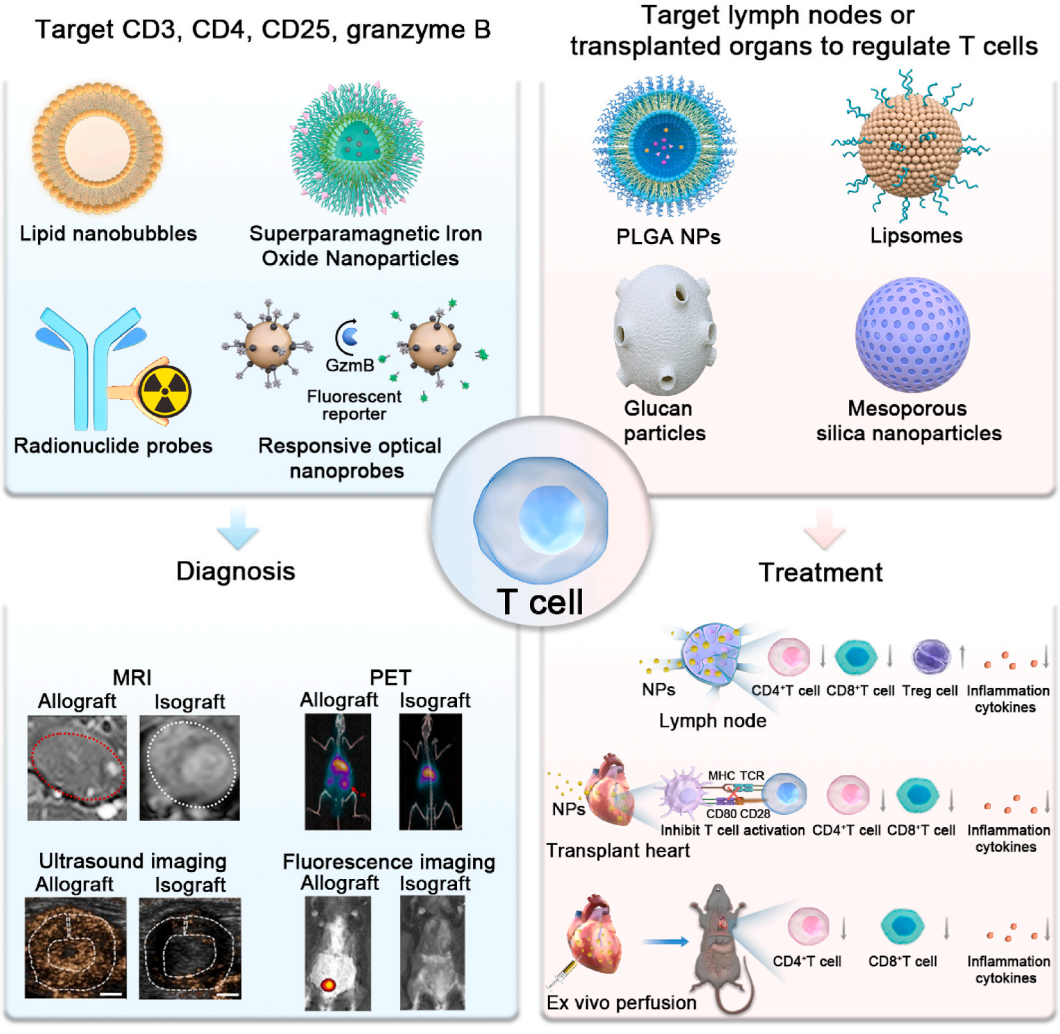

Fig. 1. Presenting various imaging modalities of molecular probes for imaging T cells to diagnose transplant rejection, as well as the application of nanoparticles in modulating T cells for treating transplant rejection in the transplanted heart, lymph nodes, and donor organs. Reproduced with permission from ref 11,32,42,48 Copyright.

Table 1
T cell-targeted imaging molecular probes for diagnosing transplant rejection.

Imaging Target	Imaging Modality	Probe design	Application
$CD3^+$ T cell	US	NB_{CD3}	Heart transplantation [32]
$CD3^+$ T cell	US	NB_{CD3}	Kidney transplantation [33]
$CD3^+$ T cell	US	exogenous NB_{CD3}	Kidney transplantation [10]
$CD4^+$ T cell	US	Cell-MB_{CD4}	Heart transplantation [34]
Activated T cell	US	NB_{CD25}	Heart transplantation [29]
$IL27R^+$ cell	PET	^{125}I-anti-IL-27Rα mAb	Skin transplantation [37]
T cell	PET	^{18}F-FDG	Kidney transplantation [27]
$CD4^+$ T cell	SPECT/CT	^{99m}Tc-HYNIC-mAbCD4	Heart transplantation [11]
Activated T cell	PET	^{89}Zr-OX40 mAb	Heart transplantation [38]
T cell	MRI	IOPC-NH_2	Heart-lung transplantation [28]
$CD3^+$ T cell	MRI	ScAbCD3-PEG-g-PEI-SPION	Heart transplantation [42]
$CD8^+$ T cell	FI	NIR fluorohore(CyOH-Cl)-self-immolative linker-petide(N-acetyl-Ile-Glu-Pro-Asp)	Skin transplantation Heart transplantation [47]
$CD8^+$ T cell	FI	PEG NP -petide (AIEFDsGc) – fluorescent dye (ZW800)	Skin transplantation Islet transplantation [14]
$CD8^+$ T cell	FI	Iron oxide nanoparticles -petide (AIEFDSGc)- fluorescent dye (FITC)	Skin transplantation [48]
$CD8^+$ T cell	FI	gold NP - petide (Biotin-SGSRSGIEFDKGGSGGC-NH_2)	Heart transplantation [49]
$CD8^+$ T cell	FI	fluorescent dye (NH_2CyOH) -self-immolation – petide (Ac-IEFD)	Liver transplantation [30]

Footnotes: US: Ultrasound; NB: Nanobubble; MB: Microbubble; PET: Positron Emission Tomography; SPECT: Single-Photon Emission Computed Tomography; CT: Computed tomography; MRI: Magic Resonance Imaging; IOPC-NH_2: Amination-modified superparamagnetic iron oxide nanoparticles; PEG-bl-PPS: Poly(ethylene glycol)-bl-poly(propylene ulfide); SPION: superparamagnetic iron oxide nanoparticles; FI: Fluorescence imaging; FDG: Fluorodeoxyglucose; NP: nanoparticle; NIR: near-infrared.

Firstly, Nanoparticles enable precise drug delivery to achieve targeted delivery. These particles can be tailored to transport therapeutic agents (immunosuppressive drugs [17,18], nucleic acids [19,20], and immune-modulating agents [21–24]) directly to targeted areas, such as grafts and lymph nodes. This strategy reduces off-target effects and bolsters the therapeutic efficacy of the agents. Secondly, NPs can be administered to the donor organ (pre-transplantation) prior to transplantation [25]. The administration of NPs to the donor organ prior to transplantation (pre-treatment), offering a distinct opportunity to target specific organs [26].

This review presents an overview of the current advancements in molecular imaging and nanotechnology-based targeted regulation of T cells for the treatment of transplant rejection (Fig. 1). It focuses on T-cell molecular imaging for diagnosing transplant rejection

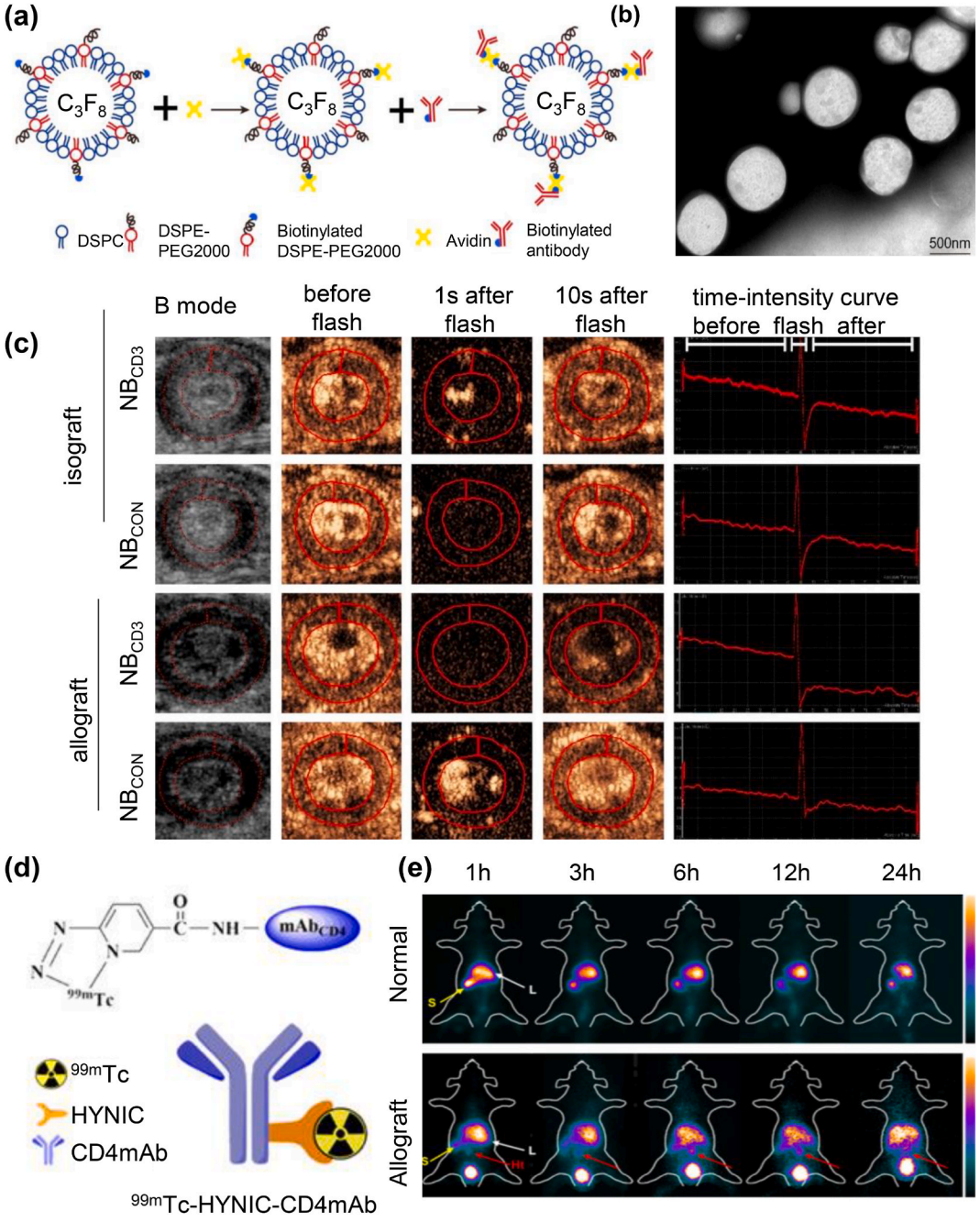

Fig. 2. (a) Schematic flow diagram of the fabrication of CD3-targetd nanobubble (NB_{CD3}). (b) Transmission electron microscope of NB_{CD3} (c) Representative ultrasound images of the allograft and isograft groups. Reproduced with permission from ref 32 Copyright 2018 Elsevier. (d) Structural diagram of the nuclide molecular probe(^{99m}Tc-HYNIC-CD4mAb). (e) Representative SPECT images in the isograft group vs. allograft group. Reproduced with permission from ref 11 Copyright 2021 American Chemical Society.

and the application of T-cell regulatory NPs in the treatment of transplant rejection. The challenges associated with this field are also addressed, along with the future directions that can pave the way for more effective and personalized treatment options for transplant recipients. We attempt to gain insights into the state-of-the-art techniques and speculate on their implications, which we hope can contribute to the advancement of nanotechnology-based approaches in the field of transplant rejection therapy.

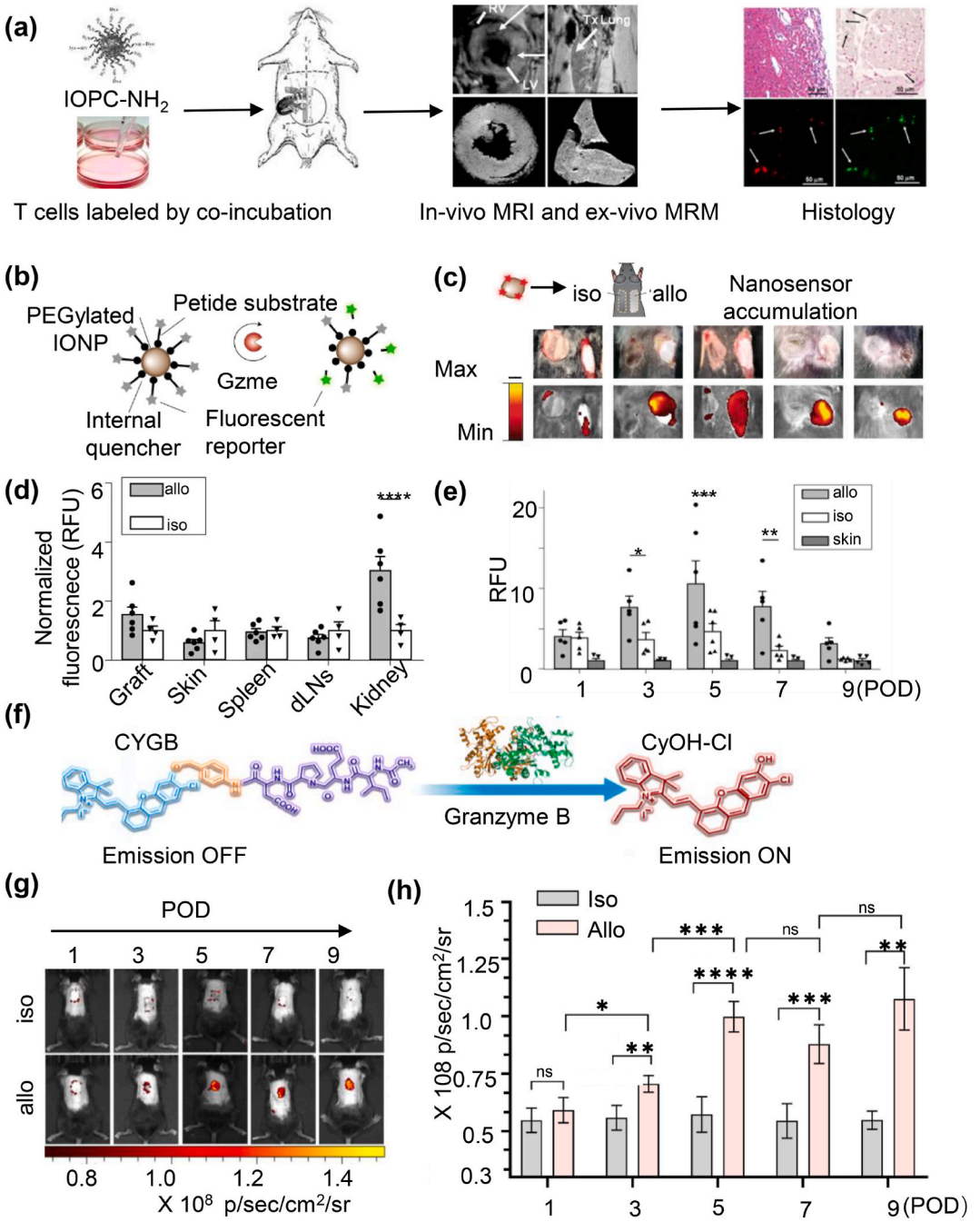

Fig. 3. (a) Schematic of MRI imaging of amine-modified superparamagnetic iron oxide nanoparticles (IOPC–NH_2) to track T cells. Reproduced with permission from ref 28 Copyright 2018 Elsevier (b) Response mechanisms of granzyme B nanosensors. (c) Construction and *in vivo* near-infrared (NIR) fluorescence images of double skin graft rejection mice. (d) *In vivo* metabolic distribution of Granzyme B nanosensors. (e) Quantified fluorescent intensities of excised skin grafts. Reproduced with permission from ref 48 Copyright 2019 nature biomedical engineering. (f) Schematic structure of fluorescent probe, **CYGB**, for granzyme B. (g) *In vivo* imaging of skin transplantation rejection with **CYGB**. (h) Quantification of fluorescence signals in transplanted skin. Reproduced with permission from ref 47 Copyright 2023 Elsevier.

Table 2
T cell-regulating nanomedicines for treating transplant rejection.

Therapeutic Target	Drug	NP composition	Administratio	Application
$CD3^+$ T cell	Prednisolone	LP	iv	Kidney transplantation [55]
$CD43^+$ T cell	Paclitaxel	LP	iv	Aortic transplantation [56]
$CD3^+$ T cell	FK506	LP	UTMD	Heart transplantation [17]
$CD3^+$ T cell	FK506	PLGA NP	iv	Heart transplantation [51]
$CD4^+$ T cell	MPA	PLGA NP	ip	Skin transplantation [62]
$CD3^+$ T cell	FK506	PLGA NP	sc	Heart transplantation [63]
T cell	FK506+RAPA	PEG-bl-PPS	id	Skin transplantation [52]
$CD8^+$ T cell	PP242	docosahexaenoic acid	ip	Heart transplantation [65]
$CD3^+$ T cell	FK506	β-glucan microcapsules	po	Heart transplantation [68]
$CD3^+$T cell	FK506	MP-FK506-MECA79	iv	Heart transplantation [70]
Treg cell	FTY720	CaCO3/CaP/heparin hybrid carrier modified with CCL21#6.10R	iv	Heart transplantation [72]
$CD4^+$ T cell	FK506	MSN@SpAcDEX-CD4Ab	iv	Kidney transplantation [74]
$CD3^+$ T cell	RAPA	PD-L1 NP	iv	Skin transplantation [76]
T cell	MMF	PEG-PLGA NP	ecp	Skin transplantation [78]
T cell	VIVIT peptide	Iron oxide NP	iv	Skin transplantation [22]
T cell	MHCII antibody	Self-assembled peptide	sc	Skin transplantation [82]
$CD4^+$ T cell	Dby peptide	PLGA NP	iv	Bone marrow transplantation [21]
Treg cell	CD3 antibody	MECA79 antibody modified PLGA NP	iv	Heart transplantation [2]
$CD4^+$ T cell	CD40L antibody	MECA79 antibody modified PEG-PLGA	iv	Heart transplantation [85]
$CD3^+$ T cell	DGKα gene	CD3 antibody-modified SPION	iv	Heart transplantation [42]
$CD4^+$ T cell	class II transactivator	PACE NP	ecp	Vascular transplantation [19]
$CD3^+$ T cell	Antagomir-155	lipid microbubble	UTMD	Heart transplantation [9]
$CD3^+$ T cell	Antagomir-155	lipid nanoparticle	GVs+ultrasoun	Heart transplantation [90]
T cell	Cas9/gCD40	lipid nanoparticle	iv	Skin transplantation [20]

Footnotes: LP: liposomes; FK506: Tacrolimus; iv: Intravenous injections; UTMD: Ultrasound-targeted microbubble destruction; ip: Intraperitoneal injections; sc: Subcutaneous injections; id: Intradermal injections; po: Oral administration; regulatory T cells: Treg; ecp: Extracorporeal perfusion; MPA: Mycophenolic acid; PLGA: poly(lactic-*co*-glycolic acid); NP: Nanoparticle; MP: microparticles; RAPA: Rapamycin; PEG-bl-PPS: Poly(ethylene glycol)-bl-poly(propylene ulfide); MSN: mesoporous silica nanoparticles; FTY720: fingolimod hydrochloride; MMF: mycophenolate mofetil; PEG: polyethylene glycol; SPION: superparamagnetic iron oxide nanoparticles; PACE: poly(amine-*co*-ester); MHC: major histocompatibility complex; GVs: gas vesicles.

2. Imaging of T cells for the diagnosis of transplant rejection

T cells are central to the process of transplant rejection. The infiltration of T cell in grafts exhibited a positive correlation with the severity of rejection [3]. Consequently, T cells are viable targets for early diagnosis of transplant rejection. Based on the phagocytic and metabolic mechanisms of T cells, non-specific imaging of T cells was used to monitor transplant rejection [27,28]. To improve the specificity of rejection diagnosis, targeted imaging can be employed towards T cell-specific surface receptors [11,29] or bioactive

macromolecules produced by T cells [30]. In this section, we describe the molecular probes of different modalities, such as ultrasound imaging (US), nuclear imaging, magnetic resonance imaging (MRI), and fluorescence imaging (FI) and so on (Table 1).

2.1. Ultrasound molecular imaging for diagnosis of transplant rejection

Ultrasound molecular imaging has the advantage of being widely used for early diagnosis of graft rejection due to its noninvasive, real-time, reproducible and safe nature [31]. Liu et al. developed a CD3 antibody-modified nanobubble (NB_{CD3}) for T cell imaging with good homogeneity of particle size (Fig. 2a and b) [32].The ultrasound signal of the allograft heart was significantly higher than that of the syngeneic graft, which was attributed to the massive infiltration of T cells into the transplanted heart when rejection occurred (Fig. 2c). Pathological analysis showed that the amount of T cell infiltration in the transplanted heart was positively correlated with the severity of graft rejection. However, targeting the CD3 receptor on the surface of T cells cannot distinguish the activation state of T cells. CD25 is a marker of activated T cells and therefore can distinguish T cell activation status. Wu et al. designed a CD25 antibody-modified nanobubble (NB_{CD25}) to monitor T-cell aggregation in the allograft heart [29]. Quantification of the ultrasound signal into a time-intensity curve can be used as a quantitative indicator for early diagnosis of heart transplant rejection. Moreover, T cell-targeted nanobubbles have demonstrated to distinguish transplant rejection from other related diseases. Grabner et al. designed CD3 antibody-modified nanobubbles (NB_{CD3}) to non-invasively differentiate acute rejection from acute tubular necrosis (ATN) and cyclosporine toxicity [33].

By leveraging the targeted accumulation of T cells in allografts, Grabner et al. employed exogenous CD3 antibody-modified nanobubbles (NB_{CD3}) and exogenous T cells to monitor allogeneic transplanted kidneys [10]. The researchers initially administered human exogenous T cells intravenously, followed by a subsequent injection of specific NB_{CD3} targeting human T cells after 15 min. Based on antigen-antibody specificity, ultrasound exclusively identified aggregated signals of human T cells. The US detected significantly enhanced NB_{CD3} signals in the kidneys of allografted rats, approximately five times higher than those in the control group. Notably, there was a positive correlation observed between ultrasound signal intensity and the presence of rat $CD3^+$ T cells. This outcome confirms the potential utility of tracing exogenous T cells for early diagnosis of transplant rejection. Based on similar design principles, Xie et al. developed the T cell-microbubble complex (cell-MB_{CD4}) [34]. Due to the cascade effect of the lymphocyte-endothelial adhesion cascade effect, the ultrasound signal intensity of cell-MB_{CD4} in allograft heart was approximately four times higher than that of MB_{CD4}. Micron-sized ultrasound contrast agents are unable to penetrate the vascular endothelial barrier. On the contrary, nanosized ultrasound contrast agents (nanobubbles) have the ability to cross the endothelial barrier, enabling specific imaging of T cells within transplants. This holds the potential to improve the specificity of diagnosing graft rejection [35].

2.2. Nuclide molecular imaging for the diagnosis of transplant rejection

Nuclear medicine imaging, with its high sensitivity, is an important method for monitoring transplant rejection [31]. Currently, T cell nuclear molecular imaging is mainly divided into two approaches: ex vivo labeling of T cells followed by reinfusion into the body, and *in vivo* targeting of T cells. *Ex vivo* labeling of T cells commonly uses Fluorodeoxyglucose (FDG), while *in vivo* targeting of T cells involves using radiolabeled specific antibodies or peptides to target T cell surface receptors.

Grabner et al. employed ^{18}F-FDG to pre-label T cells and then reinfused the labeled T cells into rats [27]. The Positron Emission Tomography (PET) imaging revealed a notable increase in the signal intensity of ^{18}F-FDG within the allograft kidneys, indicating that ^{18}F-FDG -labeled T cells can be a novel non-invasive tool for diagnosing graft rejection. The limitation of this study is that the ^{18}F-FDG nuclear molecular probe is directed against all living cells and is not targeted to T cells. It is reported that the upregulation of IL-27 can aggravate graft rejection, which is expressed on the surface of T cells and macrophages [36]. Zhao et al. developed a radioactive molecular probe ^{125}I-IL-27Rα mAb via labeled IL-27Rα monoclonal antibody with ^{125}I [37]. The highest expression of IL-27Rα on allograft skin was observed on postoperative day 10, approximately three times higher than that of the isograft skin. Immunofluorescence analysis confirmed a substantial infiltration of IL-27Rα-positive cells (predominantly $CD3^+$ T cells and $CD68^+$ macrophages) in the allograft skin. IL-27Rα proves to be a promising new molecular marker capable of enhancing the specificity of graft diagnosis. Li et al. developed a radionuclide molecular probe (^{99m}Tc-HYNIC-CD4mAb) to track $CD4^+$ T cells in rat allograft hearts (Fig. 2d) [11]. They observed a significant increase in the radioactive signal within the allograft hearts (Fig. 2e), and immunohistochemistry confirmed the infiltration of $CD4^+$ T cells. Cyclosporine treatment resulted in a reduction in signal intensity within the allograft, corresponding to decreased infiltration of $CD4^+$ T cells. These studies demonstrated that nuclide probes could be used to monitor graft rejection, but could not distinguish the activation state of T cells. To address this tissue, Hirai et al. utilized the radioactive element ^{89}Zr to label OX_{40} (^{89}Zr- OX_{40} mAb) for the first time, enabling the monitoring of activated $CD4^+$ T cells in allograft mouse hearts [38]. As the co-stimulatory molecule OX40 was predominantly expressed on activated $CD4^+$ T cells [39], the probe ^{89}Zr- OX_{40} mAb successfully detected the infiltration of activated $CD4^+$ T cells in allograft hearts at an early rejection time point (d9). This study demonstrated the ability of OX40 immunoPET to noninvasively monitor organ transplant rejection. While targeting specific T cell surface receptors with radiolabeled antibodies or peptides can improve specificity, there may still be some off-target binding, leading to false-positive or false-negative results. Furthermore, due to the limitations of nuclear isotope half-life, nuclear medicine probes are challenging to achieve long-term dynamic monitoring of transplant rejection.

2.3. Magnetic resonance imaging for diagnosis of transplant rejection

MRI which is used to monitor the tracking of T cells *in vivo*,has exhibited superb spatial resolution [40]. Superparamagnetic iron

oxide nanoparticles(SPION) are receiving increasing attention, due to their excellent MRI capability and good biocompatibility [41]. Liu et al. enhanced the internalization of SPION by T cells by modifying these nanoparticles with amination (IOPC–NH_2) [28]. These labeled T cells clustered within allogeneic heart and lung grafts after reinfusion, enabling an early diagnosis of transplant rejection via MRI (Fig. 3a). To boost T cell targetability, Kimberly et al. synthesized CD3 antibody-modified superparamagnetic iron oxide nanoparticles (ScAbCD3-PEG-g-PEI-SPION) [42]. Prussia blue staining showed a significantly more nanoparticles in the allograft skin than in syngeneic skin grafts. Adoptive transfer studies further confirmed that T cells isolated from transplanted skin labeled with ScAbCD3-PEG-g-PEI-SPION can migrate to the site of skin graft rejection after reinfusion into mice. Therefore, CD3-coupled superparamagnetic iron oxide nanoparticles prove to be a promising noninvasive tool for identifying transplant rejection.

Since SPION are easily phagocytosed by macrophages, many scholars have focused on targeting macrophages to diagnose transplant rejection, which has become a hot research topic [41]. Shinichi and colleagues employed superparamagnetic iron oxide nanoparticles to visualize macrophage infiltration in transplanted lungs, enabling the diagnosis of transplant rejection as these particles are easily engulfed by the macrophages [43].

Following the pre-transplantation labeling of islets with SPION, Kriz et al. conducted weekly MR imaging procedures post-transplantation [44]. The MR signal emitted by the islets within allografts gradually declined until complete rejection occurred, indicating the onset of transplant rejection. This decline resulted from macrophage-mediated phagocytosis of SPION. Immunohistochemistry exclusively detected iron nanoparticles in macrophages from allograft mice, further supporting this finding. However, MRI may exhibit lower sensitivity than nuclear medicine imaging, making the early or subtle detection of T cell infiltration during rejection challenging. The precise targeting of T cells with MRI contrast agents poses challenges, potentially leading to nonspecific binding and reduced diagnostic accuracy in rejection cases. Combining MRI with complementary imaging modalities, like positron emission tomography (PET), can offer additional insights and improve overall diagnostic capabilities. Future research should emphasize the design and optimization of targeted contrast agents that specifically bind to T cell surface receptors or rejection-associated biomarkers to enhance diagnostic precision.

2.4. *Fluorescence imaging for diagnosis of transplant rejection*

Fluorescence imaging offers technical simplicity, high sensitivity, safety, and non-invasiveness as distinct advantages. The development of fluorescent probes specifically aimed at monitoring T cell activity holds significant promise for enabling non-invasive and early diagnosis of transplant rejection. Following multiple stimulatory signals, $CD8^+$ T cells undergo differentiation into CTLs. Subsequently, these CTLs migrate to the transplanted organ and release granzyme and perforin, instigating an assault on the graft [3]. Granzyme B, a serine protease, induces cell death mainly through activation of caspase-dependent and independent pathways [6]. Several studies have demonstrated that elevated expression of granzyme B correlates with the severity of graft rejection. This also suggests that granzyme B could be a potential biomarker for monitoring transplant rejection [45,46]. Therefore, detecting granzyme B can be useful in monitoring transplant rejection.

The peptide sequence of N-acetyl-Ile-Glu-Pro-Asp (IEPD), a specific substrate for granzyme B, is usually used to design granzyme-responsive probes. Gao et al. developed a granzyme B-responsive fluorescent probe, **CYGB**, for non-invasive early diagnosis of transplant rejection [47]. Probe CYGB comprises a caged hemicyanine fluorophore and a granzyme B-specifically cleaved peptide-IEPD (Fig. 3f). The fluorophore and peptide are connected through a self-immolating linker *p*-aminobenzyl alcohol. When the granzyme B cleaves the peptide substrate, the self-immolating spacer is triggered, leading to the release of the caged fluorophore and the subsequent emission of fluorescence (Fig. 3g and h). *In vivo* imaging studies confirmed that the probe CYGB enables early diagnosis of mouse skin and heart transplant rejection.

Compared to near-infrared-I (NIR-I) fluorescence imaging, NIR-II fluorescence probes have stronger tissue penetration capability and imaging sensitivity. Based on this, Chen et al. reported a granzyme B responsive nano-sensor (ErGZ) with NIR-II emission for the early detection of allograft rejection [14]. ErGZ comprised a polyethylene glycol (PEG) coated Erbium-doped lanthanide downshifting nanoparticles (ErNPs) with NIR-II emission, a granzyme B cleavable peptide (AIEFDsGc) and a renal clearable fluorophore ZW800 whose emission was quenched by ErNPs. In the allograft rejection site, ZW800 separated from the nanoparticles, leading to the recovery of NIR-II fluorescence signal of ErNPs in the transplanted organ. Subsequently, ZW800 was effectively filtered by the kidneys and excreted through urine. The application of ErGz enables highly sensitive discrimination of early-stage rejection in both the skin and islets allograft mice models.

To overcome the limitations of poor tissue penetration of optical imaging, a new strategy for optical urinalysis was used to detect granzyme B. Quoc D et al. designed an activatable nanoprobe by attaching a FITC-conjugated granzyme B substrate (AIEFDSGc) to the surface of iron oxide nanoparticles containing a quencher on their surface (Fig. 3b) [48]. In the allograft skin, the peptide segment of nanoprobe was specifically cleaved by granzyme B, releasing the fluorescent molecule FITC that filters into urine through glomerular filtration (Fig. 3c and d). Analyzing the fluorescence signals in the urine, allograft rejection could be diagnosed with high sensitivity and specificity before the occurrence of pathological changes in the graft tissue (Fig. 3e). Using similar urine analysis detection methods, Liu et al. synthesized granzyme B-responsive nanosensor (GBRNs) by conjugating a granzyme B substrate (Biotin-SGSRSGIEFDKGGSGGC-NH_2) with gold nanoparticles [49]. In the allogeneic heart, granzyme B cleaves GBRNs, causing the gold nanoparticles to be filtered into urine. The gold nanoparticles exhibit peroxidase-like activity, resulting in a peroxidase substrate (3,3′, 5,5′-tetramethylbenzidine) change color. Granzyme B can be detected under within 1 h through colorimetric urinary readout, allowing for non-invasive detection of transplant rejection.

Instead of in situ cleavage of dye–peptide conjugates from inorganic nanoparticles, Huang et al. proposed a novel strategy for in situ conversion of nanoparticles into small molecule [30]. Activatable polyfluorophore nanosensors(APNs) consist of a granzyme

B-cleavable peptide (Ac-IEFD), a self-immolation linker and a caged fluorescent unit (NH_2CyOH) with a renal clearance moiety hydroxypropyl-β-cyclodextrin. Granzyme B cleaves the peptide, triggering a cascade self-elimination process that breaks down the APNs backbone. This results in the release of renal-clearable fragments of fluorophores, which can be used for near-infrared

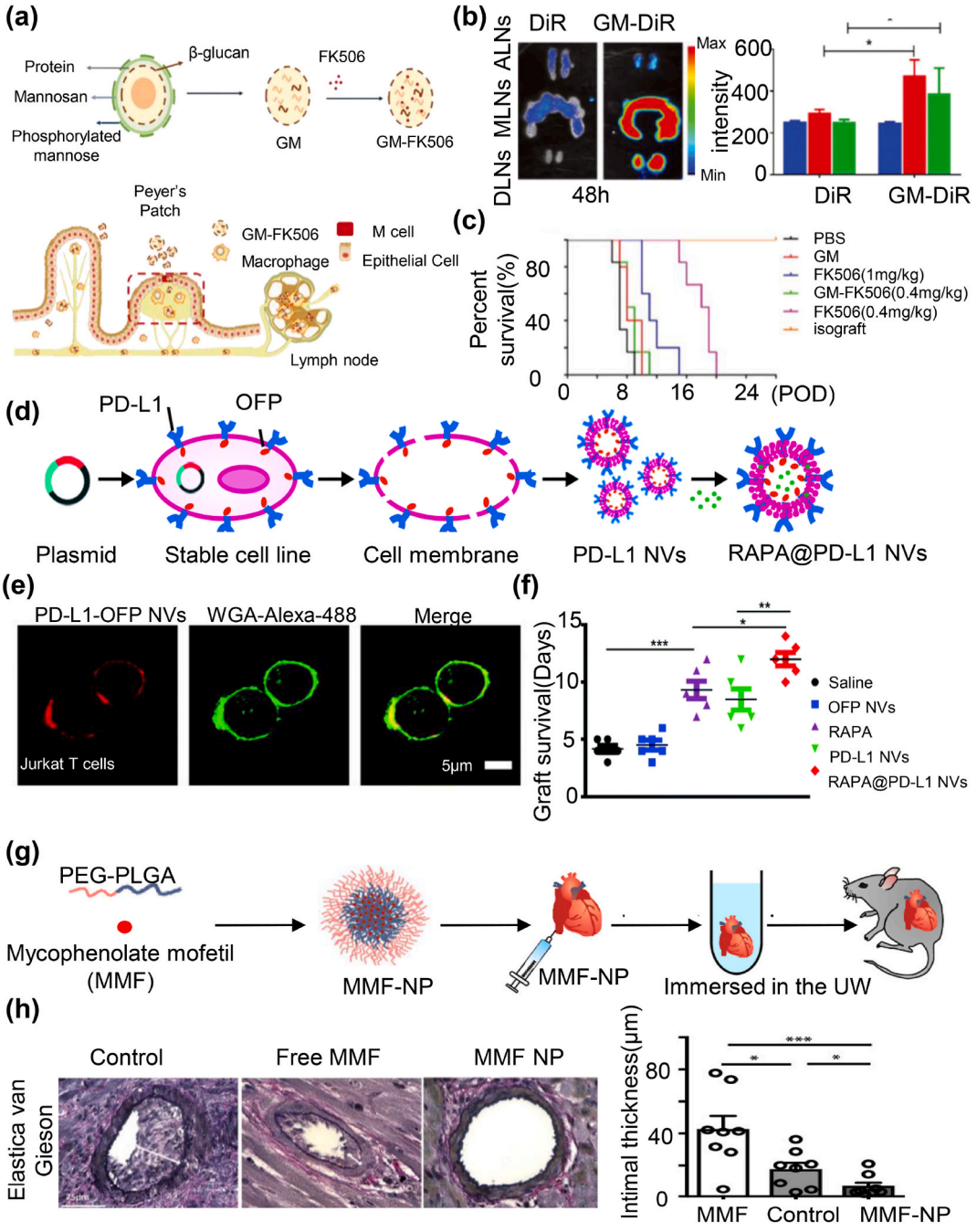

Fig. 4. (a) Schematic representation of β-glucan microcapsules-FK506 (GM-FK506) preparation and lymph node targeting mechanisms. (b) *Ex vivo* fluorescence image of lymph node after oral GM-DiR and quantification analysis. (c) Survival curves of the cardiac allografts and isografts. Reproduced with permission from ref 68 Copyright 2020 The Royal Society of Chemistry. (d) Schematic diagram of the preparation of rapamycin-loaded nanovesicles with high expression of PD-L1 (RAPA@PD-L1 NVs). (e) Representative confocal images of Jurkat cells incubated with PD-L1 NVs. (f) Allograft survival time in different treatment groups. Reproduced with permission from ref 76 Copyright 2021 The Royal Society of Chemistry. (g) MMF-NP was perfused into the donor heart, and the perfused heart was transplanted into recipient mice. (h) Elastica van Gieson staining of the allograft heart and quantitative intimal thickness. Reproduced with permission from ref 78 Copyright 2019 American Chemical Society.

fluorescence (NIRF) imaging and urinalysis. APN-based urinalysis outperforms traditional blood and urine tests. This advanced method allows for the early diagnosis of liver allograft rejection, providing unparalleled accuracy and specificity. It should be noted that, the imaging depth and tissue penetration capability of fluorescent probes may be restricted, hindering accurate visualization of T cells in deep tissues.

3. Regulation of T cells with nanotechnology to mitigate transplant rejection

Upon recognition of the antigen-MHC complex presented by antigen-presenting cells (APCs), T cells undergo activation in response to co-stimulatory and cytokine signals [50]. This activation prompts their differentiation into effector T cells, contributing significantly to graft rejection [4]. Nanoparticles can serve as excellent carriers for delivering small molecule drugs, proteins, genes, and other bioactive substances to either the transplant organ or lymph nodes. This delivery mechanism enables the targeted and controlled release of therapeutic agents, facilitating the modulation of T cell activity. Such targeted delivery holds the promise of augmenting treatment efficacy by precisely regulating T cell responses while minimizing off-target effects. Additionally, it offers the potential to reduce the overall dosage of immunosuppressive drugs, potentially mitigating associated side effects and complications (Table 2).

3.1. Nanoparticles deliver small molecule drugs to treat transplant rejection

The majority of immunosuppressive drugs currently utilized focus on inhibiting T-cell activation and proliferation, such as tacrolimus (FK506) and rapamycin (RAPA) etc. [51,52] Prolonged high-dose administration of these drugs increases the risk of infections and tumors [16]. Furthermore, off-target effects can cause toxic side effects on organs such as hepatotoxicity and nephrotoxicity [53]. To address these issues, NPs are utilized to encapsulate drugs, improving drug accumulation in target organs while reducing off-target effects.

Liposomes (LPs) are widely recognized as safe carriers due to their excellent biocompatibility and degradability [54]. During graft rejection, increased local vascular permeability allows for the passive accumulation of LPs in the transplanted organ [17,55]. Alem et al. demonstrated that glucocorticoids (prednisolone) encapsulated in LPs augment the drug concentration in allograft kidney, effectively reducing kidney graft rejection [55]. Moreover, Pepineli et al. validated that paclitaxel encapsulated in LPs boosts the drug concentration in allograft aortic, thereby attenuating vascular graft rejection [56]. Furthermore, drug-loaded lipid microbubbles have the potential for targeted delivery of immunosuppressants through ultrasound-targeted microbubble destruction (UTMD) technology [9]. Liu et al. employed UTMD to selectively deliver FK506, facilitating drug accumulation in the transplanted heart and reducing cardiac transplant rejection [17].

3.1.1. Nanoparticles target lymph nodes

Lymph nodes are known to be important sites for priming and activation alloreactive T-cells, and targeted drug delivery to lymph nodes proves to be a reliable approach for the regulation of allo-immunity [57]. Shin et al. prepared polyethylene glycol-poly(lactic-*co*-glycolic acid) (PEG-PLGA) and PLGA nanoparticles loaded with FK506 (PEG-PLGA-FK506 and PLGA-FK506) [58]. Compared with free FK506, higher accumulation was observed in rat mesenteric and axillary lymph nodes after intravenous injection of PLGA nanoparticles. This also reconfirms that the use of nanoparticles loaded with small molecules is a promising way to targeted drug delivery to lymph nodes. More surprisingly, modification of PLGA nanoparticles using PEG further increased the drug accumulation in lymph nodes to twice that of PLGA nanoparticles. The possible reasons for PEG-modified nanoparticles to enhance drug accumulation in lymph nodes may be attributed to the surface coating of PEG, a biodegradable polymer, which reduces phagocytosis of the nanoparticles by macrophages, prolongs the *in vivo* circulation time of the drug, and further increases the accumulation of drug in the lymph nodes [59,60]. On this basis, Deng et al. prepared PLGA nanoparticles loaded with FK506 (PLGA-FK506-NP) around 110 nm [51]. Through intravenous injection, PLGA-FK506-NP accumulated 2.9 times higher than free FK506 in lymph nodes and effectively attenuated heart transplant rejection. Unlike intravenous injection, intraperitoneal injection enables NPs to passively aggregate in lymph nodes [61]. Shirali et al. utilized PLGA NPs encapsulated with mycophenolic acid (MPA) and demonstrated that intraperitoneal injection of PLGA NPs also attenuated skin graft rejection [62]. Deng et al. proved that subcutaneous administration of FK506-loaded nanoparticles (PLGA-FK506-NPs) with a diameter of 122.5± nm induced significantly higher accumulation of NPs in lymph nodes than intraperitoneal injection or intravenous administration [63]. Transplanted heart survival analysis showed that subcutaneous injection of PLGA-FK506-NPs prolongs the survival time of transplanted hearts by 50 % compared with intravenous and intraperitoneal injections. Nanoparticles with particle size around 10–100 nm have greater potential in reducing the probability of phagocytosis by mononuclear phagocytes and further improving their passive targeting ability [64]. Based on this, Dane et al. synthesized a 50 nm poly (ethylene glycol)-bl-poly(propylene ulfide) (PEG-bl-PPS) micelles which was used to encapsulate different kinds of drugs [52]. When micelles were used to encapsulate RAPA + FK506, it demonstrated sustained release for 1 week, resulting in a 2-fold increase in the survival time of the graft skin. To target peripheral immune organs lymph nodes and spleen, Zhou et al. modified a rapamycin complex kinase inhibitor PP242 with polyunsaturated fatty acids, which transformed water-insoluble PP242 into a self-assembling nanoparticle (DPNP) [65]. To further improve the aqueous solubility of DPNP nanoparticles, the surface of DPNP was PEGylation using amphiphilic copolymers. After systemic administration, DPNP exhibited the ability to accumulate in lymph nodes and spleen, which effectively prolonged the survival time of the transplanted heart. The underlying mechanism involved in this process includes the inhibition of T cell proliferation, promotion of DPNP accumulation in macrophages, induction of a conversion to anti-inflammatory M2 macrophages, thereby enhancing Type 2 immunity and suppressing Type 1 responses. Additionally, it maintains $CD8^+$ cells in a quiescent state.

Compared to intravenous injection, oral administration has better compliance. However, the liver first-pass effect and

gastrointestinal absorption result in extremely low drug bioavailability [66]. Yeast extract β-glucan particles can be recognized by M cells in the intestinal wall, allowing them to enter Peyer's patches (PP). Once in the PP, they are recognized and phagocytosed by macrophages and subsequently transported to the mesenteric lymph nodes [67]. Wu et al. utilized β-glucan microcapsules (GM) to encapsulate FK506 (GM-FK506) to deliver FK506 to the lymph nodes [68]. The GM microcapsules actively targeted the lymph nodes by recognizing the dectin-1 receptor on the surface of macrophages (Fig. 4a). Compared to free FK506, GM-FK506 exhibited

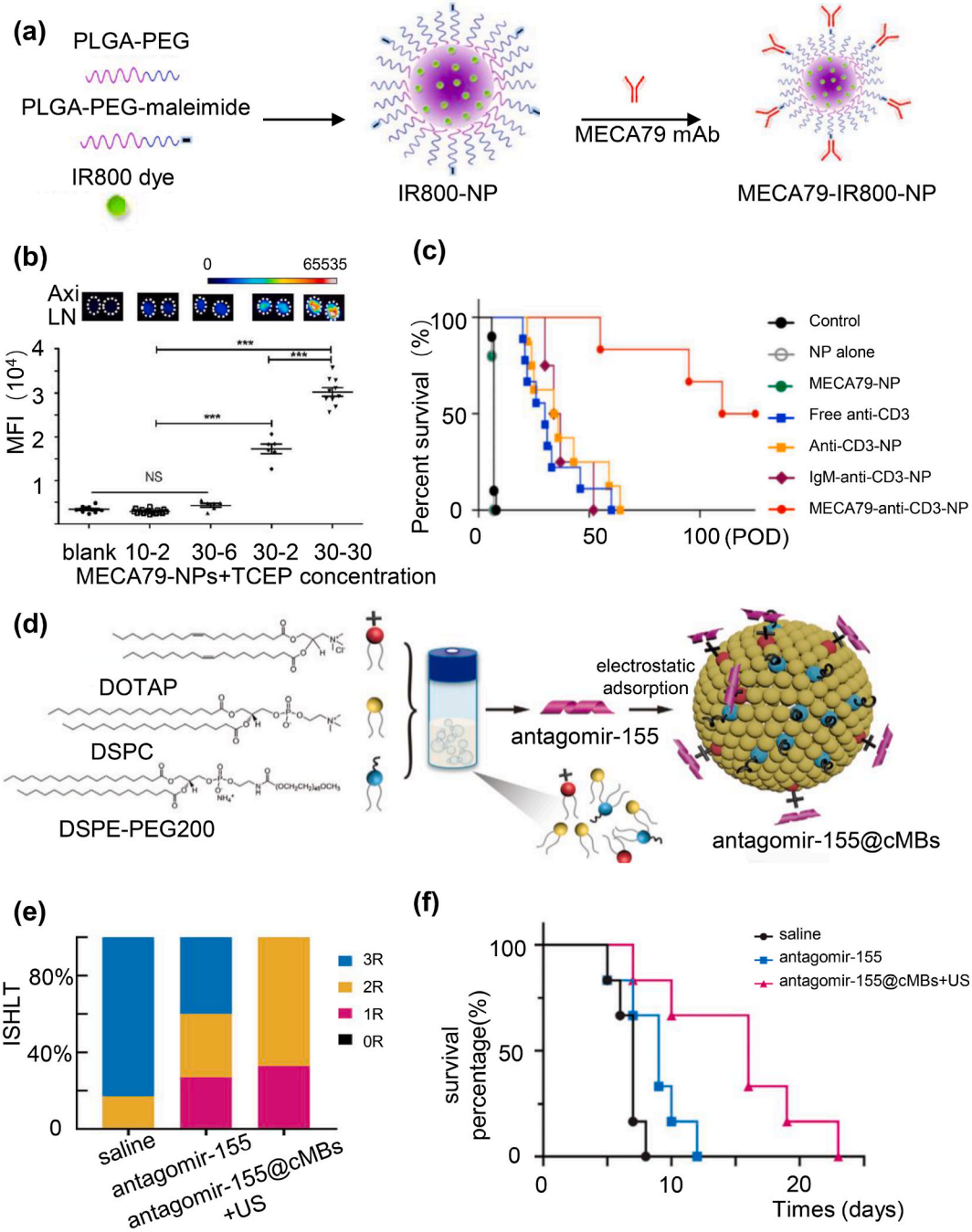

Fig. 5. (a) Schematic diagram of the preparation of MECA79-IR800-NP. (b) Corresponding trafficking of MECA79- NPs to lymph nodes, as assessed by fluorescence imaging. (c) Survival of heart grafts in recipients for different treatment (117.5 days). Reproduced with permission from ref 2 Copyright 2018, American Society for Clinical Investigation. (d) Synthesis of antagomir-155 loaded cationic microbubbles. (e) Rejection grades of different groups valued by the International Society for Heart and Lung Transplantation (ISHLT) score. (f) The allograft heart survival time in different group. Reproduced with permission from ref 9 Copyright 2020 WILEY-VCH.

approximately 27 times higher accumulation in lymph nodes and sustained release for 48 h (Fig. 4b). Furthermore, short-term renal blood urea nitrogen (BUN) and serum creatinine (CR) indices were within the normal range, confirming the potential of GM-FK506 to reduce the nephrotoxicity associated with free FK506. The targeted lymph node strategy shows great potential in reducing drug side effects and prolonging survival (Fig. 4c). However, further clinical trials are needed to confirm its long-term efficacy and safety.

Active-targeted delivery to lymph nodes represents effective treatment of transplant rejection. High endothelial venules (HEVs) are specialized structures within lymph nodes that specifically express peripheral node addressin (PNAd) molecules [69]. Since the MECA79 monoclonal antibody specifically recognizes PNAd, Jamil Azzi et al. used MECA79 monoclonal antibody-modified poly (lactide) (PLA) microparticles to encapsulate FK506 (MP-FK506-MECA79) [70]. MP-FK506-MECA79 increased the accumulation of FK506 in the draining lymph nodes and effectively attenuated cardiac transplant rejection. However, the particle size was too large (2 μm), resulting in inefficient delivery and retention in non-target organs such as the lungs. In addition, CCL21#6.10R is a DNA aptamer. It specifically recognizes CCL21 highly expressed in HEVs and fibroblast reticulocytes (FRC) in lymph nodes, and therefore has the potential to actively target lymph nodes [71]. Che et al. prepared hybrid nanoparticles by using safe and non-toxic materials such as heparin, CaCO3 and CaP. Then modified CCL21#6.10R on the surface of nanoparticles to give it the ability to actively target lymph nodes [72]. The nanoparticles were loaded with fingolimod hydrochloride (FTY720), denoted as FTY720@TNP. After intraperitoneal administration, FTY720@TNP exhibited significant accumulation in lymph nodes. This accumulation effectively inhibited effector T cells in the lymph nodes while promoting the production of regulatory T cells (Treg). Treatment with FTY720@TNP significantly reduced endothelial thickening, luminal stenosis, and interstitial fibrosis, extending the mean survival of the transplanted heart to 100 days. This study provides an effective strategy to prevent chronic allograft vasculopathy after organ transplantation.

3.1.2. Nanoparticles actively target transplant organ

NPs can deliver drugs to the transplanted organ, giving it the ability to actively target T-cells, thus greatly improving the efficiency of targeted delivery of immunosuppressants and effectively reducing the toxic side effects of drugs. T follicular helper cells (Tfh) are crucial in the development of plasma cells (PCs) and donor-specific antibodies (DSA) secretion, making them significant therapeutic targets for addressing antibody-mediated rejection [73]. Shen et al. utilized pH-responsive polysaccharide derivatives to coat mesoporous silica nanoparticles (MSN) loaded with FK506 [74]. They further modified the surface of the nanoparticles with CD4 monoclonal antibodies to obtain MSN@SpAcDEX-CD4Ab, which could enhance the targeting ability to $CD4^+$ T cells. Using the nanocomposite MSN@SpAcDEX-CD4Ab, targeted delivery of FK506 effectively inhibited the functionality of Tfh cells, leading to the termination of B cell activation and maturation into plasma cells. As a result, secretion of DSA was reduced, and the injury caused by antibody-mediated rejection (ABMR) was suppressed.

In order to further enhance the role of nanoparticles in regulating T cell activity, combination therapy has become a new development direction. The programmed cell death protein 1 (PD-1)/Programmed cell death 1 ligand 1(PD-L1) pathway serves as a co-inhibitory pathway that suppresses T-cell activation and negatively regulates immune responses [75]. Yang et al. employed transgenic engineering to obtain PD-L1 nanovesicles (PD-L1 NVs), which were used to encapsulate rapamycin (RAPA@PD-L1 NVs) (Fig. 4d) [76]. RAPA@PD-L1 NVs showed stronger effects on inhibiting T-cell proliferation than PD-L1 NVs or rapamycin alone(Fig. 4e). Furthermore, RAPA@PD-L1 NVs had the ability to induce regulatory T cells in recipient spleens, which effectively extended graft survival (Fig. 4f). This study highlights the role of rapamycin in synergy with PD-L1 in the treatment of transplant rejection.

3.1.3. Ex vivo pretreatment of the organ prior to transplantation

Organ transplantation provides a distinctive clinical setting in which the organ is accessible outside of the body (ex vivo), presenting a valuable opportunity to utilize nanotechnology for targeted delivery of therapeutics directly to the graft [77]. Uehara and colleagues devised a strategy for the direct administration and controlled release of mycophenolate mofetil (MMF) to the donor organ before transplantation [78]. By perfusing MMF-loaded PEG-PLGA nanoparticles (MMF-NPs) into a mouse heart prior to transplantation (Fig. 4g), they effectively prevented cardiac transplant vasculopathy by suppressing pro-inflammatory cytokines and chemokines within the transplanted organ (Fig. 4h). This research group also reported that perfusion of organs with nanocarriers containing anti-IL-6 prior to transplantation exhibited notable enhancements in transplant outcomes in a murine model of cardiac transplantation [25]. In recognition of the therapeutic potential of targeted intra-organ drug delivery prior to transplantation, several research groups have successfully utilized nanotechnology to deliver therapeutics specifically to the endothelium of transplant organs [19,26,79]. By employing ex vivo machine perfusion, therapeutic agents were effectively delivered to human kidneys and blood vessels prior to transplantation, resulting in a notable reduction in activated T cells within the graft. These findings demonstrate that employing a nanocarrier to deliver an immunosuppressive agent to donor organs ex vivo offers a feasible clinical approach to mitigate transplant immunity.

3.2. Nanoparticles deliver biomacromolecules to improve transplant rejection

Therapeutic peptides/proteins have been shown to attenuate transplant rejection by suppressing T-cell function [24]. However, peptides or proteins are susceptible to degradation, resulting in very low bioavailability when directly administered [21]. NPs can protect peptides/proteins from degradation, making them more efficient for delivery to the transplanted organ. On the other hand, functionalized nanoparticles allow them to target lymph nodes and improve their efficacy.

It is known that silencing dendritic cells (DCs) effectively inhibits T-cell activation [80]. Colombo et al. designed composite iron oxide nanoparticles to encapsulate the high-affinity substrate of Calcineurin (CN)-VIVIT peptide [22]. After the nanoparticles were phagocytosed by DCs, the VIVIT peptide inhibited the CN/NFAT pathway in DCs, resulting in the inactivation of DCs. The deactivation

of DCs resulted in the inability of T cells to be activated, effectively alleviating transplant rejection. In a mouse skin graft model, the skin survival was effectively prolonged up to 50 days. More interestingly, the immune tolerance state can still be maintained 20 days after discontinuing the medication, indicating the great potential of this strategy to induce long-term immune tolerance. The self-assembled peptide can form a stable membrane structure in situ to immobilize the protein as a way to avoid protein degradation [81]. Based on this property, the self-assembling peptide can be used to capture APCs so that their contact with T cells is reduced. Wen et al. mixed self-assembled peptides (AEAEAKAKAEEAAKAK) with MHC class II molecules (MHCII) antibodies, resulting in the formation of a latex suspension(aI-Ad) [82]. When injected subcutaneously, aI-Ad spontaneously formed a stable membrane structure that remained intact for 6 days. This formulation effectively captured a large number of APCs and significantly inhibited T cell activation. The significant decrease in IFN-γ expression levels demonstrated that T-cell activation was suppressed. In other study, direct activation of co-inhibitory pathways on the T cell surface can also prevent T cell activation. The Dby peptide, a CD4 epitope encoded by the histocompatibility Y chromosome Ag. It has been proved to inhibit T cell activation by suppressing the expression of the co-stimulatory molecule CD40L [26]. However, high concentrations of the peptide in the bloodstream can lead to associated complications [21,83]. Thus, Martin et al. prepared PLGA nanoparticles encapsulating the Dby peptide [21]. Interestingly, it was demonstrated that the Dby peptide can also inhibit $CD4^+$ T cell activation and proliferation by activating the PD1/PD-L1 pathway, resulting in long-term tolerance of transplanted bone marrow.

Some studies have taken advantage of the special structure of the lymph nodes-HEVs and FRC to functionalize nanoparticles and thus actively target the lymph nodes [70]. The FRC is an important structure of the lymph nodes that secretes chemokines to attract T cells, allowing them to cross the HEVs into the lymph node [84]. Bahmani B synthesized MECA79 monoclonal antibody-modified PLGA nanoparticles encapsulating CD3 monoclonal antibody (MECA79-CD3-NP) targeting HEVs (Fig. 5a) [2]. The particle size was narrowed down to 88 nm as compared to the previously synthesized particles (MP-FK506-MECA79). The nanoparticles MECA79-CD3-NP increased the accumulation of CD3 antibodies in the lymph nodes (Fig. 5b), enhanced their ability to induce the proliferation of Treg, and avoided the side effects associated with the production of inflammatory factors, such as IFN-γ, by free CD3 antibodies. This also effectively prolongs graft survival (Fig. 5c). Therefore, the previous problem of inefficient delivery due to large particle size was solved to some extent. In addition, this team found that FRC enhances the co-stimulatory blocker CD40L antibody the ability of transplant rejection. Zhao et al. modified PEG-PLGA-NPs loaded with CD40L antibody using MECA79, denotated as MECA-79-CD40L-NPs [85]. Firstly, they verified that FRC inhibited T-cell activation in lymph nodes by blocking CD40/CD40L signaling. More surprisingly, FRC also promoted the conversion of $CD4^+$ T cells into Treg. In the allograft heart model, MECA-79-*anti*-CD40L-NPs prolonged the survival of mice up to 1-fold. They also found a synergistic effect of MECA-79-anti CD40L-NP with RAPA. The combination showed better efficacy in inducing long-term induction of tolerance.

3.3. NP-targeted delivery of therapeutic genes to treat transplant rejection

Many studies have demonstrated that modulating key genes can alleviate transplant rejection, indicating that gene therapy holds great promise for the treatment of transplant rejection [9,19,20,86]. Direct delivery of therapeutic genes or the CRISPR/Cas9 system to the graft allows for the regulation of target gene expression, thereby effectively alleviating transplant rejection [87]. Gene suffers from biological instability and lack tissue targeting, so direct delivery is too inefficient. The utilization of NPs for gene delivery overcomes these limitations by improving gene stability and enabling tissue specificity [86].

Guo et al. modified SPION with CD3 monoclonal antibody (ScAbCD3-PEG-g-PEI-SPION) to encapsulate the DGKα gene [42]. The DGKα gene overexpression inhibits T cell proliferation, therefore it can be used as suppressors of the immune response [88]. After whole-body administration, T-cell infiltration in rat allograft hearts were significantly reduced. Moreover, the infiltrated T cells showed DGKα gene overexpression, indicating that the DGKα gene could cause T cell deactivation and effectively reduce heart transplant rejection.

Another type of therapeutic genes are small molecule interfering RNAs (siRNAs) that can inhibit the expression of target genes [9,26]. The siRNA-class II transactivator (CIITA) has the ability to silence MHC II and reduce the recruitment and activation of T cells by endothelial cells [87]. Cui et al. utilized poly(amine-*co*-ester) (PACE) nanoparticles loaded with siRNAs to silence MHCII molecules on the endothelial cell surface [19]. Soaking the transplanted vessels in nanoparticle solution for 6 h before transplantation surgery effectively inhibited the expression of MHC class II molecules on vascular endothelial cells for 6 weeks. Besides, it significantly reduced the levels of $CD4^+$ T cells and $CD8^+$ T cells, effectively prolonging the survival time of the grafts.

Antagomir-155 is a nucleic acid inhibitor, which silences microRNA-155 and reduces rejection of heart transplants. However, when administered by means of intravenous injection, it is prone to degradation caused by nucleases in the blood [89]. Yi et al. used cationic lipid microbubbles to loaded antagomir-155 (antagomir155@cMBs) through electrostatic adsorption (Fig. 5d) [9]. Delivery of microbubbles by means of UTMD resulted in a significant increase in their accumulation in the allograft heart, thereby reducing transplant rejection (Fig. 5e and f). The increased expression level of PU.1 in the graft confirmed that microRNA-155 was inhibited. In order to further reduce the ultrasound intensity required for microbubble rupture and enhance safety, Wang et al. explored a new method to break gas vesicles (GVs), and then used low-intensity pulsed ultrasound to deliver antagomir-155 to the transplanted heart [90]. To improve stability of antagomir-155, lipid nanoparticles were used to encapsulate antagomir-155. GVs and low-frequency ultrasound were used to produce a cavitation effect that allowed the nanoparticles to penetrate the vessel wall and reach the allograft heart. Inhibition of microRNA-155 upregulated the expression of cytokine signal transduction suppressor 1 (SOSC1), reducing infiltration of inflammatory cells and effectively decreasing graft rejection. The combination of ultrasound and nanoparticles shows promising potential for treating graft rejection.

The CRISPR/Cas9 system is a promising gene-editing tool that utilizes a single RNA-directed Cas9 nuclease to effectively eliminate

disease-causing genes [91]. Zhang et al. employed Cas9 mRNA (mCas9) and a guide RNA to block the co-stimulatory molecule CD40 [20]. mMCas9 and guide RNA were encapsulated into PEG-b-PLGA-based cationic lipid nanoparticles, denoted as $CLAN_{mCas9/gCD40}$. $CLAN_{mCas9/gCD40}$ was phagocytosed by DCs, silencing CD40 on the surface of DCs. As a result, T-cell activation was inhibited, and the survival time of allogeneic skin was prolonged. Surprisingly, the treatment with $CLAN_{mCas9/gCD4}$ exhibited an efficacy superior to RAPA. This research presents a promising approach to attenuating transplant rejection, i.e. reprogramming DCs by means of nanoparticles loaded with the CRISPR/Cas9 system.

4. Challenges to clinical transition of nanoparticles

NPs have multiple advantages in the field of transplantation, as proven by preclinical studies discussed above. However, there are still many obstacles to the clinical translation of NPs. Firstly, differences between humans and animal models may affect the distribution and efficacy of NPs [92,93].Secondly, properties of nanoparticles such as shape, size, and charge determine their stability, interaction with the body, biodistribution, and bioavailability [94]. These are all factors that need careful consideration in the clinical translation of nanoparticles. Additionally, there are still significant challenges in designing targeted nanoparticles for different transplant organs. Moreover, the goals in developing nanomedicines still need clarification, whether it aims to reduce ischemia-reperfusion injury, induce long-term transplant tolerance, or both. These are crucial aspects that researchers need to define. Most importantly, ensuring the reproducibility and scalability of nanoparticle synthesis is key. In addition, how to ensure the reproducibility and scalability of nanoparticle synthesis is also a key issue that must be solved for the clinical translation of nanoparticles.

From a regulatory perspective, the transformation of nanoparticles also faces some barriers. Food and Drug Administration (FDA) approval for a "new drug" (certain nanoparticles are considered new drugs) requires a substantial amount of cost and time. Nanotechnology platforms using existing FDA-approved treatment methods take about 3–4 years and cost $20–50 million to commercialize [95]. The translation of some "new" delivery systems, such as nanoparticles containing genetic materials, biomimetic proteins, or artificial antigen-presenting cells, might be more challenging as FDA approval for these new nanotherapeutic products may cost over $500 million and take more than 10 years to bring to the make [95]. We believe that the clinical translation of nanoparticles in the field of transplantation should focus on designing nano-carriers for existing approved drugs.

Organ ex vivo perfusion is one of the potential directions for the clinical translation of nanoparticles. Organ ex vivo perfusion aims to alleviate ischemic damage during organ preservation. Organ ex vivo perfusion represents a specific "therapeutic window" in transplantation, directing immunosuppression or anti-inflammatory agents to the graft before transplantation, controlling drug release, and immediately protecting the graft from injury post-transplantation, promoting transplant tolerance by altering the allograft. Therefore, ex vivo delivery may represent a primary strategy to promote the clinical translation of nanoparticles in transplantation and pave the way for their use in other medical fields.

5. Conclusion

In summary, this review summarizes the research progress of nanoparticle-based targeted molecular imaging and immunomodulation strategies for diagnosing and treating transplant rejection. Targeted T cell imaging strategies primarily involve targeting surface receptors on T cells and the active molecules secreted by T cells. Furthermore, we also summarize the unique advantages of nanoparticles as carriers for immunosuppressive drugs. Nanoparticles can efficiently deliver drugs to lymph nodes or transplanted organs, effectively inhibiting the activation and proliferation of T cells, thereby alleviating transplant rejection reactions. The targeted delivery advantage of nanoparticles enhances drug utilization and reduces drug side effects. We believe this review will inspire researchers dedicated to developing novel methods for early diagnosis and treatment of organ transplant rejection.

6. Future directions of nanotechnology in transplantation

The vigorous development of nanotechnology presents new opportunities for precise diagnosis and treatment of rejection reactions. Nevertheless, challenges persist in the application of nanotechnology in transplant medicine. Among various imaging modalities, Computed tomography (CT) imaging is characterized by high spatial and temporal resolution and widespread clinical application. However, currently, there are no CT imaging probes for diagnosing transplant rejection. The development of CT probes with superior imaging performance, enabling long-term dynamic monitoring of immune cells, will provide new means for non-invasive diagnosis of transplant rejection [96–99]. Moreover, current probes for imaging T cells are mostly adapted for unimodal imaging. Each imaging modality has its own advantages and disadvantages. Combination of different imaging techniques to achieve multimodality imaging can enhance the sensitivity and specificity of diagnosing graft rejection. For instance, the combination of fluorescence imaging with ultrasound, PET, or MRI can enhance imaging penetration depth, providing a more comprehensive and accurate analysis of the immune response status. The sensitivity and specificity of rejection diagnosis are two indispensable requirements for ensuring diagnostic accuracy. Combining the response characteristics of multiple biomarkers associated with rejection into a smart probe (e.g., AND-gate probes, dual-lock probes) significantly enhances diagnostic specificity and helps avoid "false-positive" results. Moreover, prior to the clinical translation of nanoparticles, it is crucial to comprehensively evaluate their short-term and long-term toxicity, particularly in terms of their interactions with various biological molecules within the human body.

The development of multifunctional nanoparticles capable of diagnostic imaging and immunotherapy holds the promise of integrating diagnosis and treatment of rejection reactions. Advancements in the development of delivery carriers with enhanced

biocompatibility and targeted delivery capabilities, such as cell membrane-modified carriers, bioinspired biomimetic carriers, and live cell carriers, can significantly augment targeting precision, thereby amplifying therapeutic efficacy. Additionally, the utilization of nanocarriers to deliver gene editing systems like CRISPR/Cas9 into the body enables precise and targeted modulation of T cell immune responses, paving new pathways for inducing immune tolerance. Through advancements in molecular imaging and targeted T cell regulation using nanotechnology, we are nearing the resolution of challenges linked to transplant rejection. Further research and innovation in nanotechnology present significant potential to revolutionize the management of transplant rejection, enhance patient outcomes, and propel advancements in organ transplantation.

Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Mengdan Ding: Writing – original draft. **Tang Gao:** Writing – review & editing. **Yishu Song:** Writing – review & editing, Writing – original draft. **Luyang Yi:** Writing – review & editing, Writing – original draft. **Wenqu Li:** Writing – original draft. **Cheng Deng:** Writing – original draft. **Wuqi Zhou:** Writing – original draft. **Mingxing Xie:** Writing – review & editing, Writing – original draft. **Li Zhang:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

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.

Acknowledgements

This study was supported by the Natural Science Foundation of China (8215316, 82171964 and 82202234), the Natural Science Foundation of Hubei Province (2021CFA046 and 2023AFB753), and the Natural Science Foundation of Hubei under Grant JCYJ20210324141216040.

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