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Review article

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Biomaterials to enhance adoptive cell therapy

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Abstract

Adoptive cell therapy (ACT) harnesses the capabilities of immune cells to fight complex diseases such as cancer. Treatment with adoptive transfer of engineered cells has led to impressive remission rates in patients with haematological malignancies. Despite these advances, ACT remains limited by treatment-related adverse effects, scaling challenges, limited access of immune cells to some disease sites, and the immunosuppressive milieu of solid tumours. New biomaterials technologies for improving cell-manufacturing techniques and the controlled delivery of engineered cells into the body are proving capable of overcoming these limitations. Tunable biomaterials can be used to mitigate the high cost and time-intensive manufacturing of ACT. Further, numerous biomaterials platforms, ranging from nanoparticles to hydrogels, have been engineered to enable spatial and temporal control of the expansion and release of engineered cells while limiting their propensity to develop exhaustion phenotypes. This Review describes the fundamental roles of biomaterials as both manufacturing platforms and delivery vehicles for enhancing ACT, and also highlights current and future applications of these materials-based approaches that could lead to improved therapeutic outcomes.

Sections

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Key points

- Biomaterials have important applications in the production, engineering and delivery of multiple immune-cell types used for adoptive cell therapy (ACT).
- Three-dimensional scaffolds, artificial antigen-presenting cells and in vivo production are expected to improve the reliability and scalability of ACT, as well as reducing its cost and production time.
- Localized delivery and slow-release formulations that incorporate stimulatory cofactors reduce the risks associated with currently approved ACTs.
- The applications of biomaterials-enabled ACT could be expanded to non-oncological settings such as autoimmune disorders and infectious diseases.

Introduction

Adoptive cell therapy (ACT) has shown remarkable effectiveness in the treatment of advanced haematological malignancies[1]. ACT is a form of immunotherapy that involves in vitro expansion of the patient's own (or an immunologically compatible donor's) immune cells, which undergo genetic engineering and are then infused back into the patient[1–4] (Fig. 1). Chimeric antigen receptor (CAR) T cell therapy is a form of ACT that has achieved high rates of complete recovery in patients with treatment-resistant, relapsed or refractory B cell malignancies, sometimes in combination with other therapies[5–10] (Box 1). These unprecedented successes have resulted in the approval of six CAR T cell cancer therapies by the US Food and Drug Administration so far, and many promising clinical trials of similar strategies are ongoing[11]. Unfortunately, distinct challenges to the widespread implementation of ACT remain (such as the need for adoptive transfer of exceptionally large doses of immune cells, concerns about treatment safety and ensuring effective delivery of transferred cells to the tissue of interest). These obstacles have restricted the use of ACT to haematological malignancies. Researchers are thus using a range of biomaterials-based solutions to improve the production, delivery and effectiveness of immune cells for use in ACT.

This Review describes applications of biomaterials in the manufacture and delivery of ACT and also highlights current and future materials-based approaches that could improve the clinical outcomes of ACT. Biomaterials-enabled systems for the expansion of T cells are not discussed in detail but have been authoritatively reviewed elsewhere[12].

Obstacles to adoptive cell therapy

Costly and complex manufacture

Despite the successes of ACT in the treatment of haematological malignancies, current ACT paradigms face a range of clinical and engineering issues (Fig. 2). Each patient's immune cells must first be harvested by leukapheresis and then be subjected to a complex manufacturing process entailing purification, activation, engineering (for engineered ACTs), population expansion to the numbers needed for clinical dosing, formulation, quality control and infusion into the patient[13]. In many patients, collection of the requisite number of lymphocytes is difficult, as immune-cell counts are often reduced by prior malignancy treatments and/or the presence of advanced disease. To reach the number of cells required for transplantation (typically around 10^9 cells)[14] as well as to perform the genetic engineering steps, cell culture is required to expand and/or differentiate the collected cells.

Commonly used cell-culture systems for ACT include rocking bed, stirred tank and perfusion bioreactors[15]. These platforms might not recapitulate the natural environment of T cells or other immune cells, leading to undesirable phenotypic changes that limit their usefulness for ACT. Under current paradigms, the process of ACT manufacture typically takes around 3–5 weeks, during which time the patient's health status often deteriorates[16,17]. Furthermore, the complexity of treatment manufacture, logistics and administration results in high costs: a course of CAR T cell therapy for lymphoma incurs an estimated total cost of more than US$400,000 (ref. 18). In consequence, ACT is difficult to access and implement on a large scale. By reducing the manufacturing timescale and/or the cell counts required for ACT, costs could be greatly reduced for these life-saving treatments.

Safety concerns

Most ACTs on the market incur substantial risks of serious adverse effects. Of particular concern are systemic inflammatory response syndromes such as cytokine release syndrome (CRS), in which high levels of T cell activation result in high levels of inflammatory cytokine release[19]. At least 70% of patients receiving approved CAR T cell therapies experience CRS, and treatment protocols often require prophylactic administration of anti-CRS drugs, such as tocilizumab, a monoclonal antibody directed against interleukin-6 (IL-6), before CAR T cell treatment. CRS is characterized by persistent fever, hypotension, hyperferritinaemia and organ dysfunction[19,20]. As CAR T cell therapy increases the number of immune effector cells, it can also cause immune-effector-cell-associated neurotoxicity syndrome (ICANS), a poorly understood condition characterized by expressive dysphasia, tremor, confusion and headache[20,21]. Although both CRS and ICANS can be effectively treated if caught early, they pose substantial and ongoing risks to patients. CAR T cell treatments directed against B cell targets such as CD19 can also induce B cell aplasia owing to CAR T cell-mediated killing of healthy B cells, which reduce B cell counts to dangerously low levels[22]. Furthermore, potential target antigens on solid tumours are often expressed on healthy cells as well, leading to potentially life-threatening 'on-target, off-tumour' (OTOT) toxic effects, in which CAR T cells target and lyse healthy cells as well as tumour cells[23]. Technologies that enable the localized delivery of modified cells and anti-inflammatory cytokines to a specific immunological niche can potentially prevent undesirable systemic reactions to ACT.

Limited efficacy in solid tumours

The exceptional success of CAR T cells in treating B cell malignancies has generated great interest in using CAR T cells (and other forms of ACT) to treat solid tumours. Unfortunately, the diversity of antigens on solid tumour cells makes it difficult to design a CAR that can effectively target most tumour cells of a given type, which makes solid tumours difficult to target and treat immunologically and also leads to poor efficacy of ACT in clinical trials[24]. Limited lymphocyte trafficking to the site of solid tumours also poses a considerable challenge to the efficacy of ACT, as the current paradigm for ACT delivery relies on systemic administration of transferred cells (Fig. 2). Once delivered into the bloodstream, transferred immune cells can easily access and kill (largely blood-borne) haematological cancer cells; however, immune-cell access to solid tumours is difficult for several reasons, including trafficking from

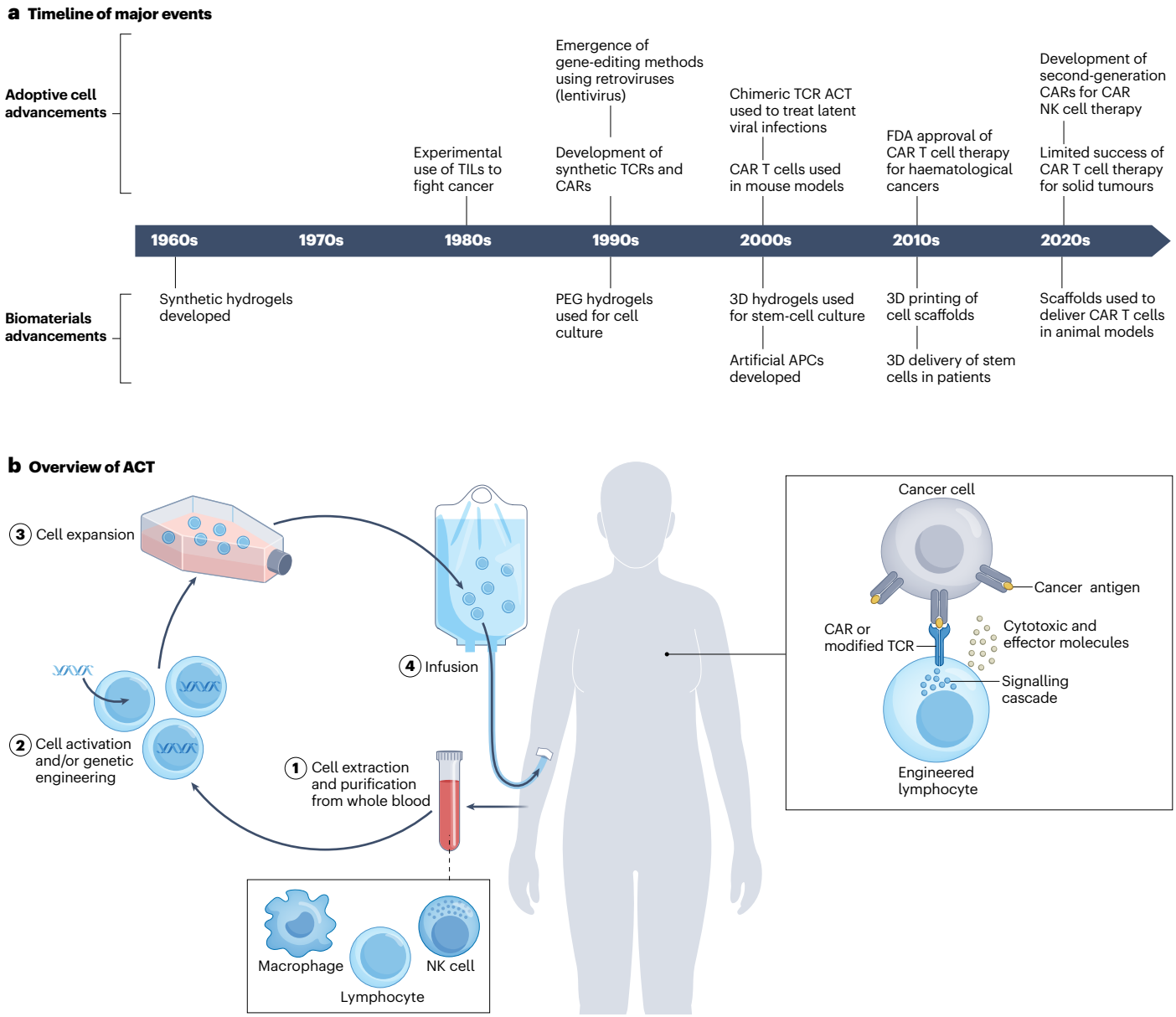

Fig. 1 | Biomaterials and the development of adoptive cell therapies. **a**, Timeline of important events in (top) the development of adoptive cell therapy (ACT)[1,14,173–176] and (bottom) development of biomaterials for use in therapeutic cell delivery[36,51,63,117,177–181]. **b**, Manufacture and applications of ACT. Autologous ACT begins with extraction and purification of a patient's own immune cells. Subpopulations of T cells that recognize specific antigen(s) of interest are isolated, or the cells are engineered to express a chimeric antigen receptor (CAR). The T cells are then activated and expanded before being reinfused into the same patient. Once inside the patient, the transferred T cells recognize the target antigen using either their native T cell receptor (TCR) or a CAR. These lymphocytes can then carry out cytotoxic functions, such as killing tumour cells by using perforin and granzymes to induce apoptosis. Other cell types used in ACT carry out functions specific to their phenotype. APC, antigen-presenting cell; NK, natural killer; PEG, polyethylene glycol; TIL, tumour-infiltrating lymphocyte.

the bloodstream into tissues, poor immune-cell infiltration and the immunosuppressive tumour milieu[25,26].

The immunosuppressive tumour microenvironment is a major barrier to effective immunotherapy, as solid tumours contain an abundance of immunosuppressive cells and proteins that induce lymphocyte anergy, inhibition and a marked reduction in proliferation[27,28]. Lymphocytes can also be deterred from entering solid tumours by chemical signals such as IL-10, vascular endothelial growth factor and transforming growth factor-β that are produced by various immunosuppressive cells, such as T regulatory (T_{reg}) cells, myeloid-derived suppressor cells, tumour-associated dendritic cells (DCs) and tumour-associated macrophages[17,29]. Physical obstacles posed by increased tissue stiffness resulting from the increased collagen content of tumours also prevent immune-cell infiltration[30]. Scale-up and interpatient clinical

Box 1

Types of adoptive cell therapy

Autologous therapies

All forms of adoptive cell therapy (ACT) currently on the market are autologous, meaning that the infused lymphocytes are sourced from the same patient to whom they are delivered. By expanding and engineering a patient's own cells, autologous therapies avoid immune incompatibility with the host and leverage the patient's antitumour immune response.

Tumour-infiltrating lymphocytes (TILs) are a mixture of immune cells isolated from a patient, usually during surgical resection of a solid tumour[182]. TILs, which typically include T cells, B cells and natural killer (NK) cells, are screened for tumour-antigen specificity, expanded ex vivo and reinfused into the patient. Despite some promising results, persistent obstacles to TIL ACT include tumour-cell immune escape, the immunosuppressive tumour microenvironment and the toxicity of adjuvant interleukin-2 (IL-2) treatment[183].

In ACT based on chimeric T cell receptors (TCRs), T cells isolated from a patient's blood are expanded and engineered to express TCRs that recognize peptides derived from tumour antigens[184,185]. For these T cells to effectively target tumour cells bearing the target antigens, the engineered gene sequences must encode a complete TCR complex that recognizes specific combinations of human leukocyte antigens and tumour-associated antigens[184]. Antigen binding to these TCRs activates the T cells and induces target cell killing[186–190]. Importantly, effective TCR-based ACT requires tumour antigens to be presented by the major histocompatibility complex (MHC), expression of which can be downregulated or lost by many cancer cell types[186].

ACT based on chimeric antigen receptor (CAR) T cells is the most successful form of ACT so far, and several CAR T cell therapies have obtained FDA approval[175]. CAR T cells are engineered to express a CAR that can bind to an intact tumour antigen independently of MHC presentation[175,191]. This property of CAR T cells avoids some of the obstacles experienced by other ACT types, including loss of MHC expression and human leukocyte antigen mismatch leading to graft-versus-host disease[191]. Lentiviral or γ-retroviral vectors are the most common method of gene transduction in clinical trials and in approved CAR T cell ACTs[82] but tend to have limited transduction efficiency[192]. Nanomaterial-based transduction approaches such as lipid nanoparticles might improve transduction efficacy. Gene editing methods such as CRISPR–Cas9[11,193] can precisely tailor lymphocyte genomes to incorporate CARs or knock out specific genes, and CRISPR–Cas9-edited CAR T cells have undergone several successful clinical trials with promising results[194–197].

NK cell-based ACT has received substantial attention. NK cells are innate effector lymphocytes that respond to a range of inhibitory and activating signals beyond specific antigen recognition[198,199]. One primary motivation for pursuing NK cell-based ACT is its potential for development of generic therapies that rely on infusion of unmodified NK cells, although this approach has achieved only moderate efficacy[198,200–202]. By contrast, CAR-modified NK cells have shown substantial efficacy in both haematological cancers and some solid tumours, in some cases exhibiting superior efficacy to CAR T cells. CAR NK cells might have a decreased propensity to elicit cytokine release syndrome, improving the safety of these treatments[198,199,203,204]. These advantages have led to growing interest in this cell type, and over 60 CAR NK clinical trials are currently ongoing[204].

Non-autologous therapies

The high cost and lengthy manufacturing times of autologous ACT have led to growing interest in allogeneic ACT. However, allogeneic ACT presents its own challenges as the receptors and antigens of allogeneic cells are distinct from those of the patient, which can lead to immune rejection and autoimmune dysfunction[205]. Induced pluripotent stem cells (iPSCs) can be manipulated to produce lymphocytes for ACT. Using iPSCs for ACT avoids autoimmunity and rejection issues while shaving weeks off current timelines for autologous cell production, and iPSCs are easier to modify than patient-derived lymphocytes[204,206]. Moreover, iPSC-derived NK cells have shown an antitumour efficacy similar to that of autologous engineered NK cells[204,206].

Other immune-cell types

Macrophages engineered to express CARs have shown promising antitumor cytotoxicity in preclinical studies and are currently being assessed in clinical trials as a potential allogeneic ACT[143,207]. T regulatory (T_{reg}) cells are a $CD4^+$ T cell subset that maintains immune homeostasis and prevents autoimmunity; T_{reg} cell-based ACT has been used to treat some autoimmune diseases, including systemic lupus erythematosus, type 1 diabetes mellitus and multiple sclerosis. B cell ACT, which can provide a long-term source of antibodies, has been investigated for the treatment of chronic infections and might also have a role in virus neutralization[208]. In addition, γδ T cell ACT has been investigated in the treatment of haematological and solid malignancies owing to the capacity of these cells to differentiate into either proinflammatory or anti-inflammatory phenotypes and to recognize tumour stress antigens, and their lack of reliance on MHC expression[209].

variability[31] also present major obstacles to the expansion of ACT to different kinds of cancers[29]. These challenges have not been amenable to being addressed with cellular engineering approaches alone, and they highlight an unmet need to develop technologies that can not only restrict transferred cells to a specific immunological niche but also maintain their tumour-reactive phenotypes.

Biomaterials for ACT manufacture

Biomaterials have emerged as important tools in the manufacture of cell-based therapies, and biomaterial-based cell-culture and transduction strategies hold promise to improve the scalability, throughput, reliability and flexibility of ACT. For example, use of 3D porous scaffolds for cell culture can more closely replicate the mechanics of the physiological

microenvironment than is achievable using standard cell-culture systems, and therefore results in cell phenotypes that are closer to those observed in vivo[32–34]. Biomaterials-enabled culture systems can also promote the development of T cell populations that more readily infiltrate solid tumours[35]. Development of new cell activation and culture protocols that take advantage of mechanical and biochemical stimuli holds promise for the generation of more-effective cell-based therapies.

Biomaterial properties and platforms

The mechanical properties of biomaterials used in cell-culture systems can modulate interactions between the biomaterial and immune cells. For example, viscoelastic biomaterial properties specifically alter cell motility, phenotype and activity. Viscoelasticity refers to a material's solid-like (elastic) and liquid-like (viscous) properties, which often operate on distinct timeframes[36]. These properties can be determined by rheological techniques that measure the dynamic shear moduli of a material, including the storage modulus G' (which represents solid-like behaviour), the loss modulus G'' (which represents liquid-like behaviour) and the ratio G''/G', also known as $\tan(\delta)$. Other characteristic time-dependent viscoelastic parameters that affect how a biomaterial performs in cell-culture systems include stress relaxation half-life and thixotropic viscosity recovery time (Table 1). Yield stress (the value of

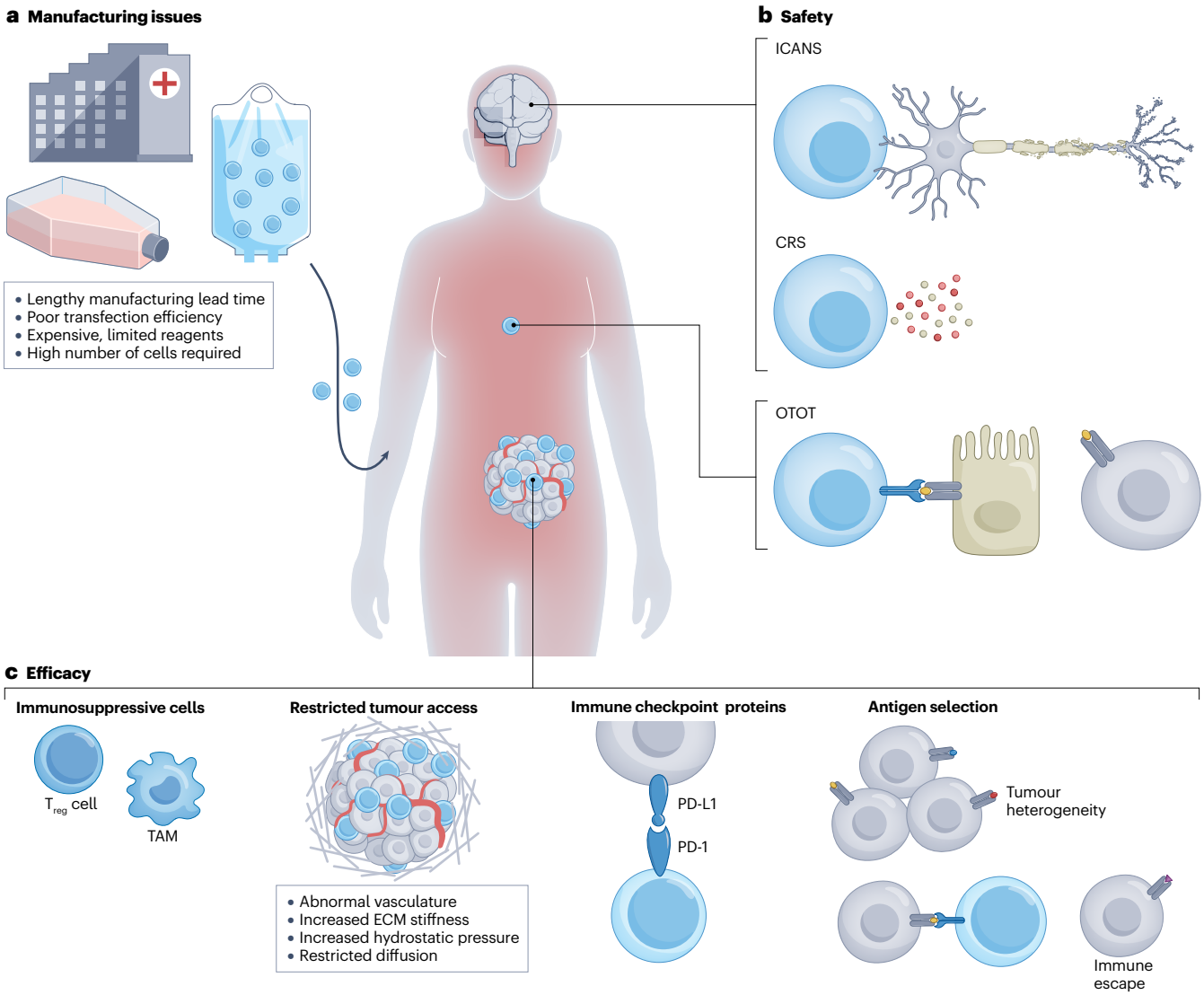

Fig. 2 | Current challenges in adoptive cell therapy that can be addressed using biomaterials. a–c, Use of adoptive cell therapy (ACT) is limited by issues relating to (**a**) manufacturing processes, (**b**) safety and (**c**) efficacy. The manufacture of immune cells for use in ACT currently takes multiple weeks, owing to the large number of cells required for systemic infusion. Moreover, bottlenecks in the manufacturing process are related to the availability and high cost of reagents, which could prevent a patient from receiving this treatment. Serious safety concerns include immune-effector-cell-associated neurotoxicity syndrome (ICANS), cytokine release syndrome (CRS) and 'on-target, off-tumour' (OTOT) toxicity. Issues that limit the efficacy of ACT include the presence of immunosuppressive cells, such as T regulatory cells (T_{reg} cells) and tumour-associated macrophages (TAMs), in the tumour microenvironment; restricted access of ACT to the tumour owing to its altered physiological and mechanical state; the presence of immune checkpoint proteins such as PD-L1 on tumour cells; and difficulties with antigen selection due to tumour-cell heterogeneity, which potentially leads to immune escape. ECM, extracellular matrix.

Table 1 | Biomaterials used in adoptive cell therapy

Type of biomaterial	Characteristics	Composition	Material property measurement	Biochemical properties	Advantages	Disadvantages	Refs.
Injectable materials	Provide protection from shear forces during injection Enable the control of cargo diffusion through tunable mesh size Undergo thermal gelation, radical crosslinking or shear thinning	Chitosan or PEG Modified cellulose Polymers or nanoparticles Alginate and M^{2+} Synthetic peptides	Shear moduli G', G'', tan(δ); yield stress; stress relaxation time; thixotropy and/or self-healing time; shear thinning and/or viscosity	Cellular locomotion: free motion through dynamic crosslinks; integrin receptor engagement by RGD peptides or biopolymers Cytokines: increase local concentrations through bonding or encapsulation; prevent systemic toxicity; drive local cell proliferation and cytotoxicity Activation and antigen presentation: activating receptor agonists (anti-CD3 or anti-CD28 antibodies)	Minimally invasive administration to locations accessible by needle Mouldability ensures a tissue-conforming interface Soft materials mimic tissue	Difficult to control depot morphology in vivo	51,58,94,123, 124,132,136, 138,139,142, 143
Implantable materials	Cryogelation to form macropores Multistep processing to harbour multiple cofactors Pore size aids movement of cells, nutrients and other cargo Hydration of scaffold drives convection 3D printing or patterning to fit complex geometry, or implanted microneedles	Alginate and M^{2+} Modified hyaluronic acid Fibrin or fibrinogen Crosslinked PLGA	Young's modulus; tensile strength; bulk and/or compressive modulus; stress relaxation time	Cellular locomotion: free motion through microporosity; integrin receptor engagement by RGD peptides or biopolymers Cytokines: increase local concentrations through bonding or encapsulation; prevent systemic toxicity; drive local cell proliferation and cytotoxicity Activation and antigen presentation: activating receptor agonists (anti-CD3 or anti-CD28 antibodies); cell-mimetic antigen presentation	Robust quality control Ease of manufacturing	Requires an extra surgical procedure Poor or uncontrolled interface with host tissue	60–63, 93,120, 125–129, 133–135
Nanomaterials	Mimic cell–cell interactions with artificial antigen-presenting cells Merge with cell membrane to deliver genetic material in vitro or in vivo Attach to cells to colocalize stimulatory cues	Liposomes, lipids Human serum albumin Silica rods or macroparticles PEI or PLGA	Aspect ratio Particle size Surface charge	Cytokines: tethering to increase local concentration; prevent systemic toxicity Activation and antigen presentation: activating receptor agonists (anti-CD3 or anti-CD28 antibodies); cell-mimetic antigen presentation Targeting to specific cells and tissues	Transfection can be implemented in vitro or in vivo	High complexity and cost are barriers to translation	64–70, 76–80, 83,84,86–90

CD, cluster of differentiation (a cell surface marker or antigen); M^{2+}, any divalent cation; PEG, polyethylene glycol; PEI, polyethyleneimine; PLGA, poly(lactic-co-glycolic acid); RGD, arginine-glycine–aspartic acid peptide motif.

stress beneath which a material resists deformation) also affects cell migration and phenotype[37,38]. The elastic properties of stiffer materials are measured by Young's modulus, which describes elastic resistance to uniaxial stress, or compressive or bulk moduli, which describe a material's resistance to compressive forces.

For many cell types, including immune cells, cell-culture matrices based on stiffer materials (those with an increased G' or Young's modulus) promote cell proliferation and reduce apoptosis; most cell types tend to migrate away from softer areas and towards stiffer ones[39–41]. However, matrices that are too stiff to allow nutrient diffusion and cellular locomotion cause cell death[39,42]. For example, despite not being adherent cells, T cells recognize mechanical forces imposed by their external environment, and their motility is affected by the chemical identity of the substrate[43–46]. Indeed, extracellular matrix (ECM) stiffness affects both the phenotype and proliferation of T cells. As a result, precisely tuning the mechanical environment of T cells that are being cultured for use in ACT can increase their antitumour efficacy[47]. More work is needed to fully characterize the effects of matrix viscoelasticity on the phenotype of immune cells, but tuning viscoelasticity independently from bulk stiffness enables the regulation of T cell phenotype and function[48].

Biomaterials exhibit structural hierarchy, and their polymer mesh size and pore size can greatly affect their performance as cell scaffolds. In hydrogels, the polymer mesh size refers to the average diameter of the gaps between polymer chains making up the material and typically lies in the range of 1–100 nm[49]. In general, molecules smaller than the mesh size can freely diffuse through the network, whereas those larger than the mesh size are trapped within it[50]. Small mesh sizes might also

restrict cellular locomotion through a hydrogel, as smaller mesh sizes result in slower overall motion due to the increased density of crosslinks surrounding the cell[51]. Lymphocytes, which are 7–10 µm in diameter, are highly mobile in vivo and capable of migrating between blood and tissue. However, these cells cannot typically freely traverse a polymer network unless the polymer chains are crosslinked by biochemically responsive moieties (such as peptide linkages, which can be degraded by matrix metalloproteases secreted by entrapped cells) or are dynamic (that is, composed of physical interactions that can be rearranged by the entrapped cells). In static networks, void spaces (pores) that are sufficiently large to allow cellular motility can be introduced through cryogelation or other methods, such as 3D printing, salt leaching or emulsion templating[52]. In many scaffold materials, the optimal pore size range for cell culture is 100–500 µm (ref. 53). By tuning pore and mesh size, both the movement of cells within (and egress out of) a scaffold and the diffusion of nutrients, gases and waste required for cell proliferation and activation can be optimized for a given application[54–56]. Particularly for T cell-based ACTs, culture systems must maintain a physical environment in which T cells can not only bind to antigen-presenting cells (APCs), an interaction termed the immunological synapse, but also interact with other co-stimulatory factors.

In addition to mechanical cues, both soluble and insoluble biochemical cues provide the signals required for control and manipulation of immune-cell phenotypes. Common ECM-related insoluble biochemical cues include cell adhesion ligands, such as RGD (arginine–glycine–aspartic acid) peptides, which enable integrin engagement; common soluble cues include stimulatory cytokines that promote T cell activation and proliferation[43]. Scaffolds can be designed to incorporate reactive moieties (such as strained alkynes, which facilitate copper-free click reactions) that enable the biorthogonal conjugation of biomolecules such as cytokines, antibodies or Toll-like receptor agonists. These biomolecules provide opportunities to shift cell signalling towards maximizing T cell survival or function, or altering DC activity[57,58]. Alternatively, proteins (such as heparin) that can bind to cytokines can be incorporated into scaffolds to improve cytokine retention and presentation to T cells[54].

Cell culture and genetic engineering

Scaffold materials that contribute to the modification and genetic engineering of cells for use in ACT (Fig. 3) range from soft hydrogels to rigid macroporous scaffolds. Hydrogel scaffolds that have reversible thermal gelation aid the harvesting of cells from a gel after proliferation[59]. The rehydration of a cryogelated material can drive bulk fluid flow by creating fluid convection, which improves the efficacy of viral transfection compared with traditional cell-culture methods[60,61]. Inclusion of cell-adhesive RGD motifs into a scaffold can enhance mechanotransduction, which (along with other biochemical cues) greatly accelerates the expansion of CAR T cells[62]. Alginate scaffolds enable cell proliferation and transfection stages to be completed in a single step by incorporating retroviral vector particles along with human blood mononuclear cells[63]. Moreover, alginate scaffolds can be engineered to harbour diverse chemical signals (such as anti-CD3 and anti-CD28 antibodies bound to the scaffold using click chemistry) that promote T cell activation and enhance retroviral gene transfer. Cytokines such as IL-2 can also be encapsulated into the scaffold to drive cell proliferation. Through their ability to precisely control physical and biochemical cues, biomaterials platforms provide both the flexibility and control necessary to manipulate patient-derived immune cells.

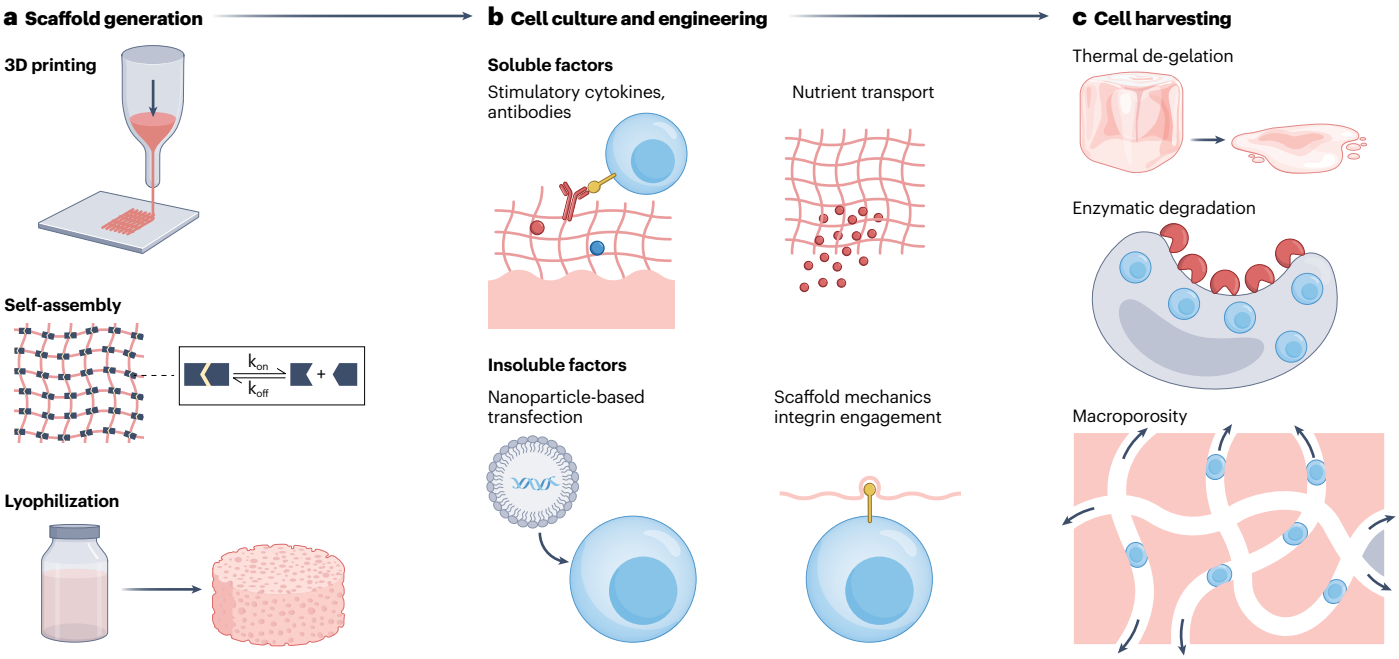

Fig. 3 | Uses of biomaterials in the manufacture of adoptive cell therapy. **a**, Scaffolds for culturing immune cells can be constructed by 3D printing, rehydration of a lyophilized scaffold or dynamic self-assembly. **b**, Cell growth, differentiation and engineering can be controlled by both soluble factors (such as stimulatory cytokines, antibodies or nanoparticle-based nucleic acid delivery) and insoluble factors (including scaffold mechanics and the biochemical addition of cell-adhesive peptide ligands). **c**, Harvesting the cells from the material can be done by taking advantage of temperature-dependent de-gelation in materials that have a critical solution temperature, by enzymatic degradation of the material or by washout of the cells through the material's macropores.

Antigen presentation

Scaffolds can also be modified to replicate the natural activation of T cells by mimicking the function of APCs. In adaptive immune responses, interactions between APCs and T cells are highly ordered: APCs present antigens on their surface in conjunction with the protein complex MHC class II which, along with co-stimulatory proteins, then binds to and activates naive T cells. Thus, a scaffold that recapitulates antigen presentation by APCs (known as an artificial APC) can optimize immune-cell responses[64]. Importantly, the aspect ratio and size of artificial APCs can alter T cell activation[65]. In the traditional manufacture of CAR T cells, magnetized polymeric microparticles known as Dynabeads conjugated to CD3 and CD28 molecules are frequently used to both activate and isolate modified T cells. Development of artificial APCs is therefore of great interest, but their incorporation into a complete culture system for ACT applications remains difficult. However, silica rod or microparticle-based scaffolds coated with a lipid bilayer mimicking that of a naturally activated T cell drive greater expansion of T cells than is possible using conventional cell-culture techniques, while also preferentially promoting the desired $CD8^+$ cytotoxic phenotype[66–68]. Polymer-based scaffolds that tether anti-CD3 and anti-CD28 antibodies can also bind to and activate T cells[69]. The semi-flexible nature of scaffold-tethered antibody binding, which mimics that of antigen presentation by natural APCs, activates T cells more efficiently than do the same antibodies attached to rigid beads. Flow-based devices incorporating hydrogel membranes that present activating antibodies to T cells have also been developed and shown to rapidly and efficiently activate T cells[70].

Cell differentiation

In regenerative medicine, directing the differentiation of stem cells using biomechanical and chemical cues delivered by biomaterials has been widely studied, and such approaches could also be useful in immunotherapy[71]. ACTs based on allogeneic differentiated stem cells might obviate the need for extraction and purification of the patient's own immune cells, thereby minimizing delay and making such 'off-the-shelf' treatment more scalable[72,73]. The ability to expand, validate and store populations of immune cells in advance provides more flexibility, speed and ease of treatment than is possible with current syngeneic methods of ACT production[72].

Biomaterials have been used to help direct the differentiation of various stem-cell lineages towards T cells or natural killer (NK) cells. Dual alginate and gelatin encapsulation of $CD117^+$ haematopoietic stem cells directed them towards an NK phenotype[74]. Physical encapsulation of these cells also led to increased cytokine secretion, which promoted their differentiation more effectively than did traditional cell-culture conditions. Three-dimensional organoid culture conditions have also been used to direct the differentiation of CAR-engineered induced pluripotent stem cells (iPSCs) into CAR T cells[75]. The 3D organoid-derived iPSC CD19-CAR T cells displayed conventional αβ T cell phenotypes, albeit with lower CAR expression than conventionally produced CD19-CAR T cells. When co-delivered with IL-15, the iPSC CD19-CAR T cells displayed antitumour effects that were more potent in immunodeficient mice bearing $CD19^+$ human tumour xenografts than were those achieved by conventional CD19-CAR T treatment.

Nanoparticle-based transduction

Nanoparticles have been used in the transfection of CAR T cells, to overcome the challenges associated with retrovirus-based methods. For example, liposomes or lipid nanoparticles can be used to encapsulate messenger RNA molecules encoding a CAR or other relevant protein[76–79]. Liposomal transfection has a more-robust transfection efficiency and a lower safety risk than retroviral vector techniques[80–82]. Other approaches use polymeric or magnetic nanoparticles containing DNA or RNA. These nanoparticles are endocytosed into the cell and must undergo endosomal escape to release their nucleic acid cargo[83–86]. Polyethyleneimine (PEI) and other polymers that can cross the cell membrane have been used to encapsulate and deliver genetic material to T cells[86–89]. Structuring of such polymers into self-assembled nanoparticles, ring-shaped or loop-shaped structures enhances the efficacy of delivery owing to their increased ability to condense DNA and their reduced cytotoxicity; however, only moderate transfection success has been achieved, owing to limited T cell endosomal escape[88,90,91]. Nanoparticle-based techniques also enable the engineering of cell types (such as macrophages) that have resistance to specific viral vectors[92]. Expanding ACT to use a greater diversity of immune-cell lineages by using non-viral, nanoparticle-based transfection is a promising approach that could improve the safety and efficacy of such treatments.

Manufacture in vivo

Moving the cell-manufacturing process for ACT in vivo holds promise to accelerate and simplify this treatment. Biomaterials facilitate the in vivo generation of cells for ACT by colocalizing cells, transfection agents and activation signals at the treatment site. For example, alginate-based cryogelated scaffolds can be implanted into the body to serve as a local niche for the transduction and expansion of T cells[63]. The alginate scaffold can retain extracted mononuclear cells as well as the requisite signals and reagents for retroviral gene transfer and T cell expansion, including CD3 agonists, CD28 agonists and cytokines such as IL-2. Nanomaterials such as lipid nanoparticles that carry modified genes can transport this genetic material directly into endogenous immune cells such as T cells and macrophages[93]. This approach has been used to generate CAR T cells that specifically lyse activated fibroblasts in heart tissue, thereby reducing fibrosis and restoring post-injury cardiac function[93]. Hydrogels can also be used to deliver genetic material. For example, a chitosan-based hydrogel loaded with engineered exosome mimetics has been used to recruit and reprogramme endogenous macrophages, thereby promoting phagocytosis and inhibiting tumour recurrence and metastasis, in immunocompetent mice[94]. By taking advantage of injectable or implantable materials, investigators could continue to accelerate and improve ACT.

Biomaterials for delivery of ACT

Fundamental biomaterial properties

In the case of implanted materials that form a long-lasting depot in vivo, material degradation determines the rates at which modified cells are released and/or traffic to the target site. Importantly, implanted biomaterials must withstand the compression forces, interactive dynamics and the complex, heterogeneous environment of the body. In the case of biomaterials used to generate cells for ACT outside the body, biochemical cues that can interact with the generated cells can also be incorporated into biomaterials to enhance the delivery or efficacy of ACT. Here, we consider the properties of biomaterials used for the delivery of ACT.

Biomaterials for use in vivo. The physical and mechanical properties of cell-laden biomaterials enable control over the rate of cellular egress into the body. The material's degradation rate determines both how quickly transferred T cells (and/or other cargo) reach the target site, as well as how long they are retained in the host. This slow-release property

of materials offers an advantage over traditional systemic administration of ACT via infusion because slow and prolonged cell delivery can help mitigate the CRS associated with systemic delivery[95–97]. Certain rheological properties have been found to be predictive of material depot persistence. For example, increased storage modulus, yield stress and zero-shear viscosity are indicative of increased in vivo persistence times in physically crosslinked and covalently crosslinked hydrogels[98].

The chemical nature of crosslinks also affects material degradation. Alginate gels depend on the addition of calcium or other divalent cations to initiate crosslinking; however, calcium is a critical second messenger in many cell types, including lymphocytes, and consequently increased levels of calcium can be intentionally or unintentionally immunostimulatory[99]. Pure collagen-based biomaterials offer low immunogenicity; however, once exposed to moisture, they lose their mechanical strength and structural stability. Collagen-based biomaterials for in vivo use must therefore be combined with other polymers or crosslinked independently[100]. Hyaluronic-acid-based biomaterials strongly promote cellular migration and proliferation but tend to undergo relatively rapid clearance from the body (half-life ~1 day in the subcutaneous space)[101]. By contrast, synthetic materials such as polyethylene glycol (PEG) resist clearance because they are not subject to proteolysis and have slow kinetics of hydrolysis[102]. It is important to analyse and consider the contributions of these polymers' chemical properties when determining what kind of materials to use for the delivery of ACT, as they can greatly alter retention of the transferred T cells as well as the pharmacokinetics and outcome of ACT. The immunogenicity of biomaterial scaffolds is also an underappreciated design criterion, as most mouse models used in preclinical studies of ACT lack immune systems[103].

The pharmacokinetics of transferred cells, material components and associated biomolecules is an underappreciated factor in ACT. The pharmacokinetics of a single infusion of CAR T cell ACT comprises several phases: an initial drop in cell numbers due to redistribution, followed by a population expansion period of around 2 weeks, after which CAR T cell numbers fall sharply. Only a small number of CAR T cells persist after 1 month[104,105]. Use of a slow-release material platform could provide flexible control over the pharmacokinetics of ACT. Studies that simultaneously measure the pharmacokinetics of scaffold-delivered CAR T cells and cytokines can also provide insight into the efficacy of ACT. The rate of egress of encapsulated cells might differ substantially from that of co-encapsulated cytokines or cofactors; biomaterials that retain stimulatory cytokines could help to drive the proliferation of cells in a local immune niche[51]. The pharmacokinetic profiles of therapeutic cofactors such as cytokines should be measured alongside those of transferred cells, individually as well as in concert, to fine-tune the expansion and growth curves of cell types used in ACT.

Biochemical cues. Major challenges for ACT include generating large enough numbers of autologous engineered cells, achieving long-term functional persistence of those cells in vivo, and enabling their infiltration into the immunosuppressive tumour microenvironment. Biochemical cues are often harnessed to address these challenges, and as such, the co-encapsulation of suitable molecules in these biomaterials is necessary for the success of ACT.

Cytokines and other immune signalling molecules are often harnessed for co-delivery in biomaterials-assisted ACT. Cytokines have a wide spectrum of functions, including modulation of antitumour immune responses through paracrine and autocrine signalling. For instance, cytokines can cause cells to generate reactive oxygen species, secrete other proinflammatory cytokines and mediate cytotoxicity, among other effects[106,107]. In the production of T cell-based therapies, cytokines assist with the activation, expansion and differentiation of T cells and their developing subpopulations. Cytokines can also be co-delivered with materials-based ACT to enhance T cell proliferation and activity in vivo. Interleukins are cytokines that have potent immune effects (including on the development and progression of cancer) that can be exploited for tumour prevention and treatment[108]. The therapeutic potential of interleukins has been widely studied; however, clinical trials of these agents in patients with cancer achieved only modest efficacy and reported serious adverse effects due to systemic toxicity and CRS[109]. Biomaterials have been used to improve the delivery of interleukin therapies[110,111] but show particular promise for the co-delivery of immune cells and cytokines (including interleukins). For example, IL-2, IL-7 and IL-15 might benefit a wide repertoire of ACTs depending on their functions. IL-2 acts as a growth factor for $CD4^+$ T cells and NK cells and, similarly to IL-15 and IL-7, also promotes the clonal expansion of activated $CD8^+$ T cells[109], whereas IL-15 and IL-7 increase the differentiation of activated T cells into effector T cell subsets and are required for the generation and expansion, respectively, of memory lymphocyte subsets such as long-lived stem-cell-like memory T (T_{SCM}) cells[106,112]. Thus, cytokines, and in particular interleukins, are an important biochemical cue to incorporate into biomaterials for ACT delivery.

Stimulatory and inhibitory antibodies play important roles as biochemical cues. Like cytokines, stimulatory antibodies aid in the in vitro differentiation and expansion of patient-derived T cells; for instance, naive T cells bearing a modified TCR or CAR become activated by exposure to anti-CD3 and anti-CD28 antibodies[63,113]. When delivering ACT, other stimulatory antibodies can be used to regulate the activity of the transferred cells as well as that of surrounding immune cells. For instance, co-stimulatory pathways can be triggered by the delivery of agonistic antibodies that enhance the expansion and function of adoptively transferred T cells. For example, treatment with anti-4-1BB antibody prolongs the survival of T cells after adoptive transfer by preventing activation-induced cell death, thereby aiding tumour regression[114]. Conversely, inhibitory pathways can also be blocked in a similar manner alongside ACT delivery. Cytotoxic lymphocyte-associated antigen 4 (CTLA4) and PD-1 are inhibitory molecules on the surface of activated T cells that are critical in halting cell cycle progression and inhibiting the production of cytotoxic cytokines such as IL-2[115]. These inhibitory molecules can be potently blocked by using antagonistic antibodies delivered in combination with ACT, which counteracts T cell suppression and thereby improves T cell activity[116,117].

Co-delivery of immunomodulatory drugs can also enhance the antitumour response of ACT. For instance, components of the stimulator of IFN genes (STING) pathway, which are often used as adjuvants in vaccination strategies, can play an important role in the detection and eradication of tumour cells by the immune system. Co-delivery of cyclic nucleotides such as cGAMP (2′3′-cyclic GMP-AMP) or DMXAA (5,6-dimethylxanthenone-4-acetic acid) that activate the STING pathway alongside ACT promotes the trafficking of modified T cells to the tumour site and drives an aggressive antitumour response[118–120]. Other co-delivered drugs that modulate the tumour microenvironment promote immune-cell infiltration, proliferation and survival[121,122].

Current approaches

Various implantable, injectable and biomimetic biomaterials are currently under development for use in the delivery of ACT. Materials-based

ACT technologies offer the advantage of localizing transferred cells to either the tumour location itself[123,124] or the post-resected tumour site[125,126]. This method assists transplanted cells with trafficking to tumours and overcomes some of the challenges seen with systemic infusion[23]. Biomaterials have even afforded researchers the ability to successfully deliver engineered immune cells to an area distal to the target site, creating a transient inflammatory niche that still achieved tumour clearance despite being away from the draining lymph node[51] (Fig. 4a).

Implantable scaffolds. Implantable scaffolds typically use strong and stiff macroporous materials such as alginate[63,120,126–128], other natural polymers[126,129] or synthetic polymers[125] adapted to harbour cells and biochemical cofactors. These materials are attractive for biological applications because of their facile degradation, easy customization and lack of immunogenicity once formulated. Some implantable scaffolds can be frozen and lyophilized to obtain pore sizes large enough to enable preloading of drugs, proteins and cells[130]. The advantages of implantable scaffolds include robust quality control and ease of manufacturing. However, they must be surgically implanted, and the complex geometry of in vivo implantation sites can result in a poor interface with host tissue (Table 1).

Natural polymers that promote the natural migration of lymphocytes have been used in implantable scaffolds to improve their biocompatibility and enhance the activity of transferred cells. Hyaluronic acid is an important ECM-mimicking material but requires structural modification or crosslinking to maintain its mechanical integrity[129,131,132]. Cryogelation and free-radical crosslinking of hyaluronic acid scaffolds has been used for the delivery of NK and CAR T cell ACT[117,129]. In a study of NK ACT, such implanted scaffolds enhanced the trafficking ability and tumour-infiltrative capacity of transferred cells in immunodeficient mice bearing breast cancer tumours[133]. Use of a 3D-printed macroporous alginate–gelatin scaffold led to improved antitumour effects of NK cells in several in vitro tumour models[133]. Fibrin scaffolds are produced by mixing soluble ECM-derived fibrinogen with thrombin in the presence of calcium. This same process is involved in blood clotting, wound healing and tissue regeneration; accordingly, fibrin scaffolds provide a biodegradable niche that does not cause inflammation, tissue necrosis or fibrosis[134,135]. Fibrin gels have been formed in situ in the resection cavity of surgically removed glioblastoma multiforme tumours to enable local delivery of CAR T cells in mice[134]. This scaffold material enabled the CAR T cells to benefit from nutrients within the fibrin gel that improved their viability and function once released, which improved the antitumour activity of these cells compared with a simple infusion of CAR T cells only into the resection cavity.

Implantable scaffolds can also modulate the activity of transferred cells via the co-delivery of other therapeutic agents. Migration-promoting macromolecules (such as the hexapeptide GFOGER, which is derived from type 1 collagen) as well as activation-stimulating anti-CD3, anti-CD28 and anti-CD137 antibodies can be incorporated into a 3D scaffold[120]. To assist in complete tumour clearance, high concentrations of the immunostimulatory STING agonist cyclic di-GMP were added to an alginate scaffold, which promoted T cell priming by recruiting and stimulating APCs. The release of both cyclic di-GMP and CAR T cells from the implanted biomaterial produced a synergistic activation of host DCs, resulting in substantial T cell activation, elimination of local tumours, and abscopal effects (namely, triggering of a global antitumour immune response powerful enough to also prevent metastases and efficiently treat distant and heterogeneous tumours). Small-molecule drugs, such as metformin, co-delivered with a scaffold can also modulate immune-cell responses[128]. Therefore, implantable scaffolds can serve as platforms for the delivery of both engineered cells and other co-delivered therapeutic agents. However, as scaffolds must be implanted at or near the tumour site, the use of this technology is limited to surgically accessible locations. Implantable scaffolds are particularly useful in the post-resection setting owing to the availability of a pre-existing surgical site; however, care must be taken to select a material with an appropriate degradation rate because long-term retention of implanted scaffold material can impair normal organ function[136].

Injectable hydrogels. Injectable materials offer several advantages as ACT delivery systems. Hydrogels composed of swollen hydrophilic polymer networks are attractive scaffold material owing to their biocompatibility, tunable properties and ability to remain insoluble in water due to their crosslinked structure[137]. Like implantable scaffolds, injectable hydrogels used for ACT delivery must retain mesh sizes and crosslink types that are amenable to cell transport; however, unlike implantable scaffolds, they must also have physical and chemical properties that enable the transient changes in viscosity needed for injection through a needle and subsequent reformation of the gel[136].

Injectability of hydrogels is promoted by various material properties that can be leveraged in minimally invasive delivery of ACT[51,58,123,124,138,139] (Table 1). Some injectable materials undergo shear thinning — a reduction in viscosity under the high strain-rate conditions of injection. Others exist in a sol phase during injection and then undergo crosslinking in vivo (for example by thermal gelation or fibrin formation on exposure of fibrinogen to thrombin and calcium). Injectability also enables biomaterial delivery to surgically inaccessible or irregularly shaped sites[137,139]. The disadvantages of injectable materials include the difficulty of controlling depot formation in vivo, which results in irregular or amorphous structures, and the risk of needle clogging if gelation occurs too soon (Fig. 4b).

Synthetic polymers such as polyamides, PEG[123,124,140] and its variants[51,58] are commonly used in injectable biomaterials owing to their highly controllable physical and chemical properties[141]. Synthetic hydrogels based on triethylene glycol-substituted polyisocyanopeptides (PICs) have been used to deliver pre-activated T cells[58]. PIC polymers enable fine-tuning of hydrogel morphology and mechanics to create microscopic pores and strain-induced stiffening that resembles the mechanical behaviour of natural tissue and allows straightforward cell encapsulation and delivery by needle injection[58]. The highly controllable nature of synthetic polymers enable RGD peptides, attached using azide click moieties, to be incorporated into the material[58]. A synthetic polymer platform allowed large-scale in vitro expansion and sustained in vivo delivery of T cell ACT over a 4-week timespan[58].

Natural polymers used in injectable hydrogels include polysaccharides such as chitosan[123,124], alginate[142], and peptides such as collagen and its derivatives[132,138], which have ECM-mimicking properties. Some natural polymers intrinsically form a gel under physiological conditions whereas others require functional modification or mixing with synthetic polymers to form a gel. Chitosan is commonly used for injectable gels because of its biocompatibility and ease of functionalization. When modified with PEG, chitosan can form thermally reversible hydrogels that have been used to locally deliver T lymphocyte ACT to in vitro models of glioblastoma[123]. Low-viscosity hydrogels have also

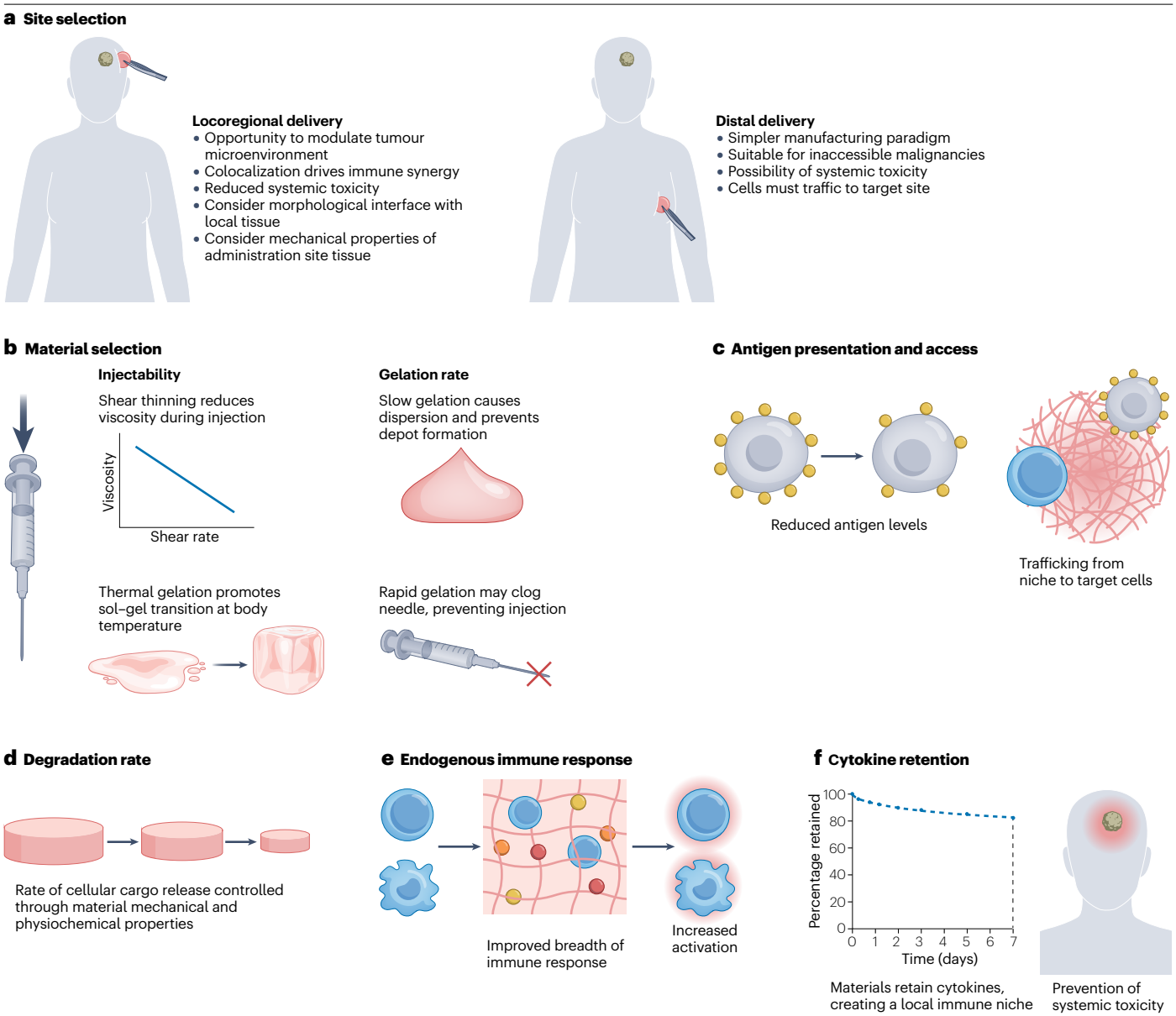

Fig. 4 | Applications of biomaterials in the delivery of adoptive cell therapy. Biomaterials contribute to adoptive cell therapy (ACT) through delivery modality and location, inherent biomaterial properties, the pharmacokinetics of cellular and non-cellular ACT components, and interactions with the endogenous immune system. **a**, 'Locoregional' delivery of ACT (administration proximal to the tumour or target site) can directly modulate the tumour microenvironment and drive a synergistic host immune response mediated by draining of both the tumour and ACT depot to the same lymph node. Locoregional delivery also reduces systemic toxic effects by decreasing systemic exposure to cytokines. Both the mechanical properties of tissues at the administration site and the morphological interface between the biomaterial and local tissue must be considered in the design of locoregional ACT. Distal delivery refers to ACT administration away from the target site, which involves a simpler ('one size fits all') manufacturing approach. Although distal delivery might be desirable for surgically inaccessible malignancies, important challenges include systemic toxic effects and the requirement for cell trafficking to the target site. **b**, Injectable materials often use shear-thinning properties (that is, a reduced viscosity at the high shear rates typical of injection) or stimuli-responsive crosslinking (meaning that they undergo a sol–gel transition at physiological temperatures). The gelation rate of injectable biomaterials affects not only its injectability but also scaffold formation and cargo delivery characteristics. **c**, Biological issues inherent to solid tumours pose issues for the success of ACT. Tumour antigens might be downregulated or absent in subpopulations of cancer cells, leading to immune escape, and access to target cells might be limited by chemical or structural obstacles in the tumour microenvironment. **d**, The rate at which a biomaterial degrades in vivo controls the timescale over which cells and other molecules are released from an implanted scaffold. The degradation rate is determined by interactions between the host immune system and the material's mechanical and chemical properties (such as the presence of enzymatically degradable or hydrolytically labile crosslinks). **e**, Endogenous innate immune cells such as macrophages, dendritic cells and neutrophils can also be recruited to and activated by implanted scaffolds, creating an increased breadth of immune response. **f**, The retention of cytokines by implanted biomaterials creates a local inflammatory environment that activates the transferred lymphocytes while reducing systemic cytokine exposure, thereby reducing the systemic toxic effects of ACT.

been used to aid in the intracranial infusion of tumour-specific CAR T cells in mouse models of glioblastoma[132]. The injectable hydrogel carrier, which was composed of thiolized hyaluronic acid and gelatin, enabled continuous, high-rate infusion of CAR T cells into the brain without causing mass effects or damaging the cells' migration capacity or cytotoxicity. The hydrogel carrier also increased the efficiency of this treatment by preventing cell sedimentation within the delivery system, which occurred when using a saline carrier[132].

Injectable hydrogel platforms are an effective means to both deliver ACT and enhance its efficacy against solid tumours in vivo. For instance, hydrogel platforms based on self-assembling peptides can use ECM-derived and immunostimulatory moieties in their structure[143]. In mice, such a hydrogel platform successfully delivered CAR gene-laden nanomicelles to macrophages and/or microglia in the resection cavity after surgical removal of a glioblastoma, thereby creating CAR macrophages with tumoricidal activity against residual glioblastoma stem cells (which are associated with disease recurrence)[143]. The in situ generation of CAR macrophages stimulated an adaptive antitumour immune response that prevented postoperative recurrence of glioma. Chitosan-PEG hydrogels have been used to locally deliver ganglioside 2 tumour-antigen-specific, IL-15-releasing CAR T cells to retinoblastoma tumours, a cancer that is difficult to treat surgically[124]. Non-covalent crosslinking of polymer nanoparticle hydrogels was used to co-encapsulate the CAR T cells and cytokines such as IL-15[51]. The dynamic crosslinks of polymer nanoparticle hydrogels afford control over mesh size and aid injection via shear thinning. Co-encapsulation of CAR T cells and IL-15 mitigates the toxic effects associated with systemic cytokine administration while enhancing CAR T cell activation and expansion in immunodeficient mice. Further, this polymer nanoparticle hydrogel resulted in increased CAR T cell expression of CD39, a marker of tumour reactivity, which was associated with improved treatment efficacy against subcutaneously implanted human medulloblastoma tumours in a mouse xenograft model irrespective of whether CAR T cell ACT was delivered either proximal or distal to the tumour site[78]. Other crosslinking strategies include injectable gelatin methacryloyl gels with dual cytokine and CAR T cell co-encapsulation, which can be photocured in situ to create a depot for sustained release of ACT. This system promoted melanoma tumour regression in immunocompetent mice[138].

Work is ongoing to develop biomaterials for various forms of ACT using CAR NK cells, CAR T_{reg} cells, CAR macrophages and DCs[144,145] (Box 1). So far, DC ACT has been limited by the fragility and short lifespan of transplanted cells. A self-assembled peptide hydrogel has been used to encapsulate modified DCs and thereby improve the delivery of ACT. This gel incorporates exogenous DCs, antigens and biomolecular cofactors that effectively preserve the viability, retention and function of the transplanted DCs[139]. In vitro, this hydrogel aided both antigen uptake by DCs and DC maturation. In vivo, the hydrogel depot recruited host DCs and also promoted transport of activated DCs to draining lymph nodes, resulting in increased cellular proliferation and activation of a potent immune response that slowed tumour growth[139]. These studies highlight the advantages of using injectable hydrogels as a platform for ACT delivery, as well as their potential to enhance the activity of transplanted cells in vivo while also, in some cases, simultaneously activating the host's immune system.

Nanomaterials. Nanomaterials, in particular nanoparticles, have been widely explored to enhance the efficacy of ACT. These materials improve the survival and engraftment of transplanted cells and can be used to deliver therapeutic agents to sites in the body that would otherwise be hard to reach, such as beyond the blood–brain barrier[146]. Nanoparticles can also tether proteins or other biomolecules that enhance the survival and function of transplanted immune cells[110].

Liposomal nanoparticles are frequently used to deliver therapeutic agents, drugs and imaging agents into target cells. Liposomes are spherical structures composed of a phospholipid bilayer that can encapsulate both hydrophilic and hydrophobic molecules. The cell-membrane-like outer layer of liposome nanoparticles easily integrates into cell membranes, which aids their cellular uptake[147]. PEGylated liposomes engineered to directly modulate immune-cell function have been used to prevent the loss of lymphocyte effector function and aid cell expansion in vivo[148]. PEGylated liposomes can additionally be targeted to ACT cell-specific antigens by conjugated anti-CD90 or anti-CD45 antibodies or recombinant IL-2, and can carry a cargo of co-encapsulated immunostimulatory molecules[111,148]. Liposomal nanoparticles have also been used to pre-treat the tumour microenvironment before ACT[149]. These liposomes contained a phosphoinositide 3-kinase inhibitor (to inhibit immunosuppressive tumour cells) and the synthetic immunostimulatory glycolipid α-galactosylceramide (to activate NK T cells). The liposome surface was decorated with iRGD, a cyclic RGD-containing nonapeptide. Binding of the included RGD sequence to integrins on tumour cells exposed a second epitope that triggered liposome endocytosis. Subsequent CAR T cell ACT resulted in robust expansion of the T cells as well as improved antitumour efficacy in glioblastoma-bearing immunocompetent mice pretreated with liposomal nanoparticles compared with those treated with CAR T cell ACT alone[149].

Nanoparticles have been used to both modulate and improve the delivery of ACT. Polymeric nanoparticles are useful for their biocompatibility and their ability to be functionalized with molecules that specifically target certain cells or tissues. Nanoparticles also enable the controlled release of therapeutic cytokines or drugs, which mitigates the toxicity associated with their systemic delivery[110,150]. Polymeric nanoparticles can also tether specific proteins or drugs to adoptively transferred T cells, which directly enhances their function in vivo. Tethering of human serum albumin to nanoparticles increases the bioavailability of drugs while decreasing their toxic effects and evading host immune responses. For instance, an IL-12 nanostimulant was assembled with human serum albumin, then bound using click chemistry to azide-labelled CAR T cells. The resulting CAR T cell–cytokine complex promoted the plentiful secretion of additional cytokines as well as further recruitment and expansion of CAR T cells in an immunodeficient mouse model of lymphoma[151]. Click chemistry can likewise be used to tether magnetic nanoparticles to CAR T cells that enable these cells to be guided magneto-acoustically to the tumour site, thereby avoiding obstacles and increasing tumour penetration[152]. Nanoparticles can also indirectly improve treatment efficacy; gold nanoparticles have been used to disperse thermal energy and thereby remodel the tumour microenvironment, which reduces the stiffness of the ECM and enables increased infiltration of CAR T cells and other immune cells[28]. These studies demonstrate the utility of nanomaterial-based methods to enhance the delivery of ACT.

Biomimetic biomaterials. Biomaterials that mimic the natural properties of living tissues can be used to develop biomimetic products for enhancing the delivery of ACT. Nanomaterials, when used in this context, can occasionally have the disadvantages of poor tumour targeting, rapid clearance from the body, and instability[153]. Biomimetics,

however, are engineered to directly mimic the natural functions of living cells and offer various means of resolving those shortcomings.

Co-delivery of artificial APCs with ACT leads to improved treatment efficacy. Endogenous APCs contribute to ACT by migrating to secondary lymphoid organs where T cells are activated. Accordingly, artificial APCs must be similarly able to migrate to these organs and present antigens to T cells to ensure efficient T cell activation and immune response. Amphiphile ligands (also known as amph-ligands) consisting of CARs conjugated to an amphiphilic polymer-lipid tail have been developed that can function similarly to endogenous APCs in vivo[154,155]. These amph-ligands traffic to lymph nodes and present CAR T cell ligands to endogenous immune cells, thereby prompting expansion of the transferred T cell population as well as promoting increased donor cell polyfunctionality and antitumour efficacy in various immunocompetent mouse tumour models[154]. Because amph-ligands promote immunological crosstalk, they have also been successfully used as a tumour vaccine-boosting strategy in the treatment of glioblastoma tumours with substantial antigen heterogeneity in immunocompetent mice[156].

Nanostructured artificial APCs, including nanoparticles, have been used for the in vivo stimulation of $CD8^+$ T cells. For example, ellipsoidal particles made of poly(lactic-*co*-glycolic acid (PLGA) and shaped by thin film stretching have been used as T cell activators. These particles enable high cell attachment and low internalization rates, as well as reduced non-specific uptake and a large surface area of contact[64,157]. PLGA has also been used to develop artificial APCs that incorporate an anti-PD-1 monoclonal antibody to prevent the suppression of $CD8^+$ T cell effector function[158]. This artificial APC technology resulted in substantial antigen-specific proliferation of $CD8^+$ T cells within the tumour microenvironment and spleen in immunocompetent mice. Artificial APCs are an exceptionally promising biomimetic therapy that can enhance the delivery of ACT and even be used to engineer transferred T cells in vivo.

Future outlook

Improved treatment safety and efficacy

Biomaterials have the potential to solve some of the most pressing challenges that currently face ACT. Through use of biomaterials, new therapies can be designed to have reduced adverse effects, to provide close control of cellular phenotypes during ACT manufacture and delivery, and to synergize with existing immunotherapies.

CRS and ICANS are common adverse effects of many ACTs used in the clinic[19,20]. These adverse effects are due to the high concentrations of inflammatory cytokines released during the rapid expansion of activated adoptively transferred cells[159]. Alleviating these adverse effects is an important current challenge. Materials-based solutions have the potential to control the rate of cellular egress out of the material depot and into the body, which could potentially prevent such a rapid surge in T cell numbers in the blood. The material properties can be tuned to finely control cellular pharmacokinetics, thereby reducing this surge and attenuating the cytokine response.

OTOT toxic effects are another common obstacle to the design of effective ACTs[23]. Particularly for TCR-based ACTs, many tumour antigens must be completely avoided because of toxicities associated with low levels of antigen expression in healthy tissues. Locally delivered materials-based solutions have the potential to prevent or mitigate OTOT toxicity by efficiently shepherding transferred cells to the target location (such as a solid tumour) and reducing exposure of the transferred cells to other bodily tissues, for example by controlling the biodistribution of the transferred cells.

Future biomaterial applications

Materials-based solutions might be particularly suited for the treatment of solid tumours in confined or immune-privileged body locations, such as the brain or eye[124]. T cells and other immune cells have a limited ability to traffic into these tissues, so intravenous delivery of ACT can be ineffective. In particular, clinical trials have demonstrated promising results for local injection of ACT into the brain[160]. This success implies that use of a slow-release depot to precisely control the biodistribution of transferred T cells and create a localized proliferative niche could improve tumour-cell clearance in a confined area. Additionally, other therapeutic agents could be co-delivered into the same niche to stimulate local T cell proliferation.

The phenotype of the delivered T cells is a highly important determinant of effective ACT[161]. In particular, formation of T_{SCM} cells, the least-differentiated memory T cell subset) promotes the persistence of adoptively transferred cells in the body and leads to improved treatment efficacy[162]. Materials that aid the co-delivery of other therapeutic molecules, such as IL-15 or IL-7, can enrich the transferred T cell population in T_{SCM} cells[106,112]. By co-manufacture or co-delivery of cells and therapeutic agents in the same material, the transferred cells can be highly exposed to the therapeutic agent of interest. Other co-delivered agents could be added to reduce T cell exhaustion or enhance their tumour-infiltrative capacity[54,163]. The effects of transferred cell phenotypes on the safety and efficacy of ACT are complex and still not fully understood[164]. As we move toward the increased use of personalized medicine, some cell phenotypes might be discovered to be more (or less) desirable for a particular patient's tumour burden or immune status, and therefore the ability to precisely modulate cell phenotypes will be crucial. Biomaterials could act as a modular platform to co-deliver selected therapeutic agents to guide the phenotypes of transferred cells towards those most likely to provide effective treatment for a given patient while diminishing the need for additional cellular engineering.

Efforts are underway to develop materials-based ACT and immunotherapies that can coordinate with the endogenous immune system[120] (Fig. 4e). Co-delivery of therapeutic agents, including antibodies that activate particular immune-cell subsets or drugs that target specific immunological cascades, could spatially or temporally synergize with ACT and improve its efficacy. Further investigation is needed to develop materials that can co-deliver all these agents without inducing CRS, ICANS or any of the other adverse effects associated with ACT. Additionally, transferred immune cells could be co-delivered with other cells that produce tunable and continuous biomolecular cues depending on endogenous or exogenous stimuli[165]. Such living ('smart') co-therapies could provide increased flexibility and robustness of ACT.

ACT modalities are also being developed for non-oncological indications, such as autoimmune disorders, infectious diseases and fibrosis[93,166,167]. These applications could be enabled or improved by using biomaterials to both engineer and deliver cells for use in ACT. Localization of immune cells at a specific injury site might improve the efficacy of a treatment and reduce off-target adverse effects. The ability of materials to recruit and/or engineer endogenous immune cells might also assist in the recapitulation of a natural immune response to disease. The sustained treatment exposure allowed by controlled-release platforms increases the persistence of ACT and enables the treatment of diseases that normally require chronic dosing[51,63]. Biochemical cues released by a material can prevent the development of exhaustion phenotypes in transferred cells, thereby potentially extending ACT to the treatment of diseases caused by latent viral infections[167]. As an example,

T_{reg} ACT demonstrated efficacy in animal models of type 1 diabetes mellitus[168], although this approach showed only moderate success in human trials, probably owing to rapid clearance of the transferred T_{reg} cells (NCT01210664)[169]. Materials platforms could extend and increase the release of T_{reg} and other therapeutic cells in this scenario; tethering PLGA nanoparticles decorated with IL-2 to adoptively transferred T_{reg} cells increased their ability to slow or stop the onset of type 1 diabetes mellitus in non-obese diabetic mice[170].

Future challenges

Although biomaterials strategies show promise for increasing the potential of ACT, important challenges that remain to be overcome include safety, scalability and regulatory approval. Thus far, biomaterials for ACT have been assessed only in preclinical studies. As these technologies advance, only scalable and safe materials will be viable for human use in the clinic. Many materials currently under development require lengthy and costly synthetic pathways that require production scaling up and optimization; even after these processes, the cost of biomaterials-enhanced ACT could exceed that of traditional cell-only treatments. Treatment complexity might also increase; for instance, ACTs involving multicomponent formulations or loading of cells might need to be prepared at the bedside directly before treatment. Off-the-shelf ACT formulations are already difficult to produce; adding biomaterials components will surely increase this complexity. Nonetheless, emerging research in this field suggests that ACTs with off-the-shelf capabilities will soon be available[171].

The incorporation of new materials and modalities of treatment is likely to require additional regulatory requirements, as good manufacturing practices must be defined for new biomaterials[172]. Rigorous safety testing will be required to assess the toxicity of ACT components and their metabolites, as well as for surveillance of potential foreign-body immune responses and/or graft-versus-host disease induced by implanted materials. Additionally, new materials-based treatments must demonstrate their superiority over existing ACTs for the same malignancy, which represents a higher barrier than that faced by the first ACTs. Careful consideration will have to be given to the diseases chosen for the development of new forms of ACT; in diseases for which successful therapies already exist (such as B cell malignancies). new ACTs face higher regulatory barriers than they do for diseases that are currently largely untreatable, (such as glioblastoma multiforme). Currently, few biomaterials technologies have been commercialized, but many more are on the horizon[36].

Citation diversity statement

We acknowledge that papers authored by scholars from historically excluded groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

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E.A.A. and A.K.G. are listed as inventors on a patent application (PCT/US2021/055897) that covers some of the technologies described in this manuscript. The other authors declare no competing interests.

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