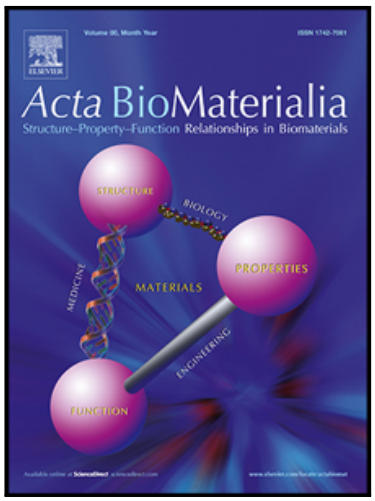

Biomaterial-Driven 3D Scaffolds for Immune Cell Expansion toward Personalized Immunotherapy

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Biomaterial-Driven 3D Scaffolds for Immune Cell Expansion toward Personalized Immunotherapy

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Abstract

Immunotherapy has emerged as a transformative medical approach in recent years, providing novel treatments for cancer eradication, autoimmune disorders, and infectious diseases. Fundamental to the success of therapy is the enrichment of the immune cell population, particularly T cells, natural killer cells, and dendritic cells. However, achieving a robust and long-term proliferation of immune cells is still challenging both *in vivo* and *ex vivo*. *In vivo* expansion leverages the patient's natural microenvironment and regulatory mechanisms through therapeutic interventions like immune checkpoint inhibitors, cytokine therapy, and targeted antibodies. This approach fosters long-term immune memory and sustained protection. In contrast, *ex vivo* expansion involves isolation, manipulation, and expansion of the immune cells under controlled conditions before reinfusion, allowing for precise control over the process and generating potent immune cell populations. Hydrogels, due to their tunable biomechanical properties, high biocompatibility, and ability to mimic the extracellular matrix, provide an ideal platform for both *in vivo* and *ex vivo* immune cell expansion. For instance, hydrogel-based scaffolds or beads can facilitate a controlled and efficient expansion of immune cells *ex vivo*, whereas injectable and implantable hydrogels can provide innovative solutions for enhancing immune cell activity within the patient supporting prolonged immune cell activity. This review aims to elucidate the importance of hydrogel-based strategies in immune cell expansion, advancing the development of effective, personalized immunotherapies to improve patient outcomes.

Keywords

Immune cell expansion, 3D scaffolds, Hydrogels, Immunotherapy, T cell activation

1. Introduction

In the last few years, immunotherapy has emerged as a transformative medical approach, offering novel avenues for the treatment of cancer, autoimmune disorders, and infectious diseases [1–5]. Crucial to the efficacy of immunotherapy is the expansion of immune cells, whose activation and proliferation are pivotal for setting up an effective immune response [6–8]. Immune cells, including T cells, natural killer (NK) cells, and dendritic cells (DCs), constitute the frontline of the immune system. However, achieving a robust immune response poses a not-trivial challenge, particularly in the context of adoptive cell transfer therapies and other immunotherapeutic modalities [9–11]. *In situ* and *ex vivo* strategies play essential roles in overcoming this hurdle, offering complementary approaches to bolster immune cell population and enhance therapeutic outcomes [12–15].

In vivo expansion refers to the activation and proliferation of immune cells within the patient, stimulated by therapeutic interventions such as immune checkpoint inhibitors, cytokine therapy, or targeted antibodies [16–19]. This approach capitalizes on the natural microenvironment and regulatory mechanisms within the host, promoting the expansion of specific immune cell subsets. Additionally, *in vivo* expansion fosters long-term immune memory, facilitating sustained protection against recurrent threats [20]. In contrast, *ex vivo* expansion involves isolation and manipulation of immune cells extracted from the patient, followed by their expansion under controlled conditions before their reinfusion [21]. This approach allows for precise control over the expansion process, enabling the generation of large quantities of highly potent and specific immune cell populations. *Ex vivo* expansion techniques encompass a diverse array of methodologies, including cytokine stimulation, co-culture systems, and genetic engineering, each tailored to optimize immune cell proliferation, functionality, and persistence [22]. Moreover, *ex vivo* strategies help in overcoming tumor immune evasion mechanisms by recognizing and targeting tumor-specific antigens more effectively [23]. Importantly, both the strategies can rely on patient's immune cells thereby (i) dramatically reducing the risk of rejection or adverse reactions such as graft-versus-host disease compared to donor-derived T cells and (ii) enabling personalized therapies tailored on patient's immune profile [24–27].

In this regard, given their biomechanical and physical tunability, high biocompatibility and capacity to mimic the extracellular matrix, hydrogels offer a versatile platform for hosting, activating, and stimulating immune cells, providing an ideal biomimetic environment that supports cell expansion and functionality both *in vivo* and *ex vivo* [28–31]. Additionally, hydrogel matrix and texture can be adapted on demand to specific applications. For instance, hydrogel scaffolds provide a three-dimensional (3D) environment that closely mimics the natural extracellular matrix (ECM), offering support for cell attachment, growth, and differentiation [12,30,32]. These scaffolds are particularly beneficial for the *ex vivo* expansion of immune cells such as T cells and DCs [31]. The porous structure of hydrogel scaffolds facilitates efficient nutrient and oxygen diffusion, essential for sustaining high cell densities. This structure also allows for the removal of metabolic waste products, creating a more conducive environment. Moreover, their mechanical stiffness can be easily adjusted to match the requirements of different immune cells, influencing cell signaling and function [33,34]. Another approach to obtain *ex vivo* expansion relies on using artificial antigen presenting cells (aAPCs), which provide a unique platform for the microencapsulation of activators and co-stimulatory factors. This encapsulation creates a highly controlled microenvironment that can be optimized for specific cell types by adjusting the aAPC size, composition, and functionalization [12]. These aAPCs can be made of different materials, for example biomimetic or biological components such as liposomes or cell membranes, inorganic such as iron oxide or carbon nanotubes, polymeric such as polystyrene or poly(lactic-co-glycolic acid) (PLGA) and can be produced in large quantities, making them suitable for scaling up the *ex vivo* expansion process [12].

As it concerns *in vivo* expansion strategy, injectable hydrogels are designed to be delivered minimally invasively as liquid and undergo a sol-gel transition at the target site to form a stable matrix [13]. Therefore, these hydrogels can be administered by injection, reducing the need for surgical interventions, a tremendous

advantage for patient comfort and recovery. In addition, the in-situ gelation ensures that the hydrogel conforms to the tissue contours. Injectable hydrogels are usually loaded with immune cells, antibodies for cell activation and cytokines to promote localized anti-tumor response [35]. Less common due to their greater invasiveness are the implantable hydrogels consisting of pre-formed structures that are surgically placed at specific sites within the body to support immune cell expansion and functionality [13]. These hydrogels can be engineered to release cytokines and growth factors in a controlled manner, sustaining immune cells and enhancing their functionality over time. This strategy is critical for therapies requiring prolonged immune cell activity. Implantable hydrogels can be customized in terms of size, shape, and mechanical properties to fit specific anatomical sites and therapeutic needs [12,13]. Although most research to date has demonstrated effectiveness only in preclinical animal models, hydrogels show significant promise for clinical translation to humans. However, no completed clinical trials involving hydrogels-based strategies are currently listed in the National Library of Medicine at the US Institutes of Health. To fully assess hydrogel potential, further studies are needed to evaluate their safety and effectiveness in patients.

As illustrated in **Figure 1**, the classification of hydrogels used for immune cell expansion is based on their structural features and functional application rather than the production method. Specifically, hydrogels are categorized according to their physical form and mode of delivery, distinguishing between scaffold-based, bead-based, injectable, and implantable hydrogels. This classification helps contextualize their role in either *ex vivo* or *in vivo* expansion strategies, highlighting how different hydrogel structures can be tailored to support immune cell growth and activation effectively. As we embark on the exploration of immune cell expansion for immunotherapy, we aim to elucidate the multifaceted importance of both *in situ* and *ex vivo* hydrogel-based approaches. Through an in-depth analysis of current research, clinical applications, and future perspectives, this review endeavors to highlight the critical role of immune cell expansion in shaping the future of immune-based therapies. By advancing our understanding of these strategies, we aspire to accelerate the development of more effective, personalized, and accessible treatments, ultimately improving outcomes for patients worldwide [27].

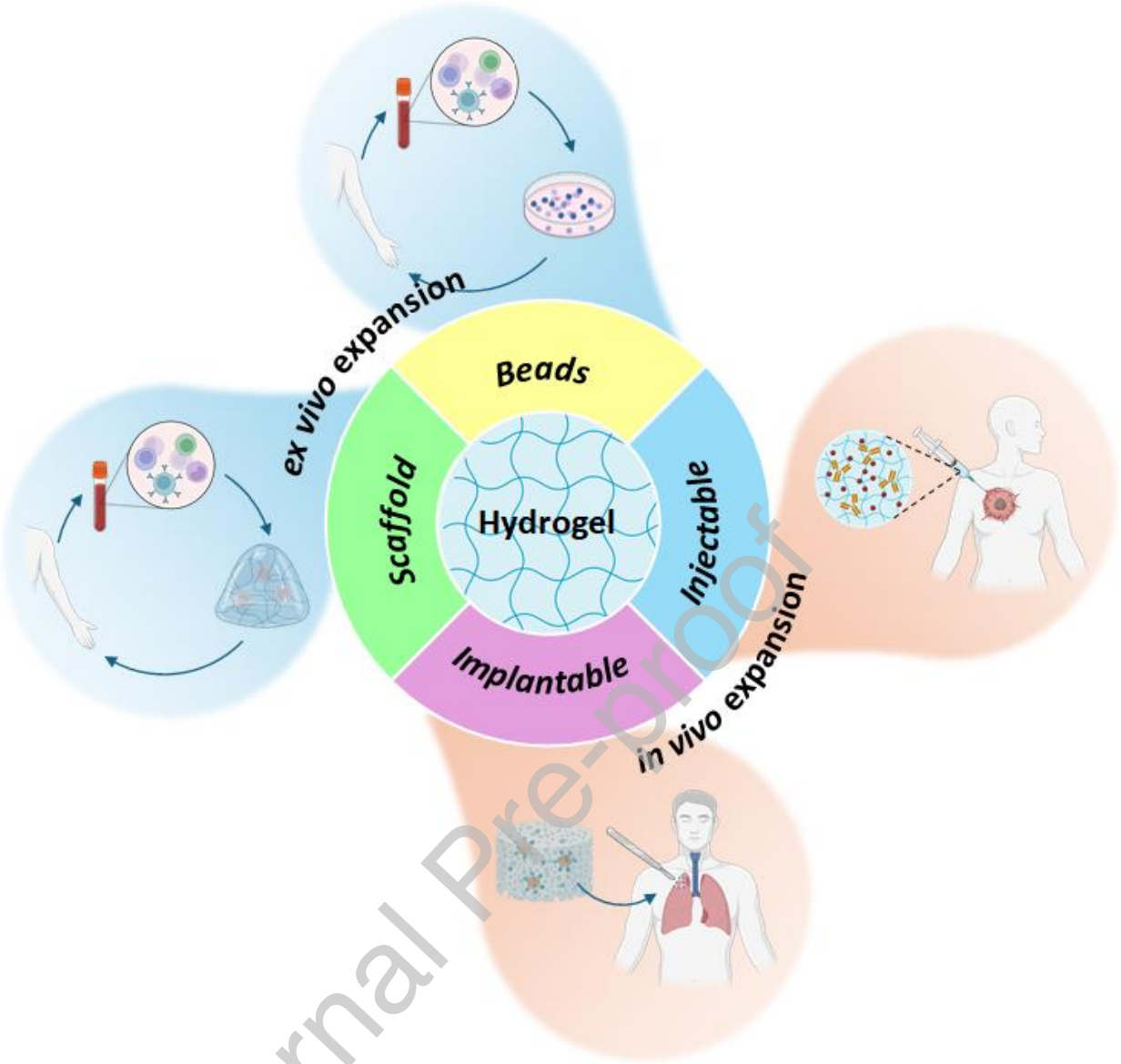

Figure 1. Classification of hydrogel-based strategies suitable for *ex vivo* and *in vivo* activation and expansion of immune cells for immunotherapy treatments. Created with BioRender.com.

2. Lymph node architecture

The immune system is organized into lymphoid organs that guide the maturation, migration, and activation of immune cells [8]. Primary lymphoid organs, including the bone marrow, thymus, and fetal liver, provide environments for lymphocyte development. Secondary lymphoid organs, such as lymph nodes, spleen, and tonsils, gather immune cells to efficiently detect antigens and activate lymphocytes. Tertiary lymphoid structures, which resemble secondary lymphoid organs, form in abnormal locations during chronic inflammation and are linked to immune responses in conditions like cancer. In this review, we will focus on lymph node architecture and biomechanics, as our interest in delving into bioengineering strategies to mimic their microenvironment and function for enhancing immune response [3,36,37].

Lymph nodes (LNs) play a vital role in immune responses by regulating fluid flow and cell migration, facilitating antigen detection by lymphocytes. Structurally, LNs are encapsulated organs composed of lymphoid tissue that beside immune cells (including lymphocytes, macrophages, and DCs), feature a complex ECM composed

of collagen-rich reticular fibers that provide mechanical support and spatial organization for immune cell interactions, lymphatic sinuses and vessels [38]. The different LN compartments exhibit distinct mechanical properties that influence immune cell behavior. The LN cortex, rich in densely packed lymphocytes, has a relatively high stiffness (ranging from 9 to 12 kPa) [39,40], which helps confine B cells within follicles. In contrast, the inner region, composed of paracortex and medulla, is softer, having a stiffness around 6 kPa [39]. In the paracortex, T cells interact with antigen-presenting cells (APCs), and the lower stiffness facilitates cell migration and interactions. Furthermore, the medulla, composed of interconnected sinuses and fewer lymphocytes, has a lower stiffness when compared to the cortex, allowing fluid drainage and cell egress. Lymphatic vessels in tissues maintain fluid balance by transporting excess fluid, antigens, and immune cells to the local LN. Under inflammation, these vessels expand to increase fluid transport. Once in the LN, antigens and immune cells are deposited into the subcapsular sinus, where T and B lymphocytes are guided by chemokine gradients to interact with antigens in specific locations. For example, chemokine CXCL13 directs B cells to follicles [41], while chemokines CCL19 and CCL21 recruit T cells to the paracortex [42]. Lymphocytes also enter the LN through high endothelial venules, which express chemokines and other molecules to guide them. An overview of lymph node architecture and lymphocyte migration is depicted in **Figure 2**.

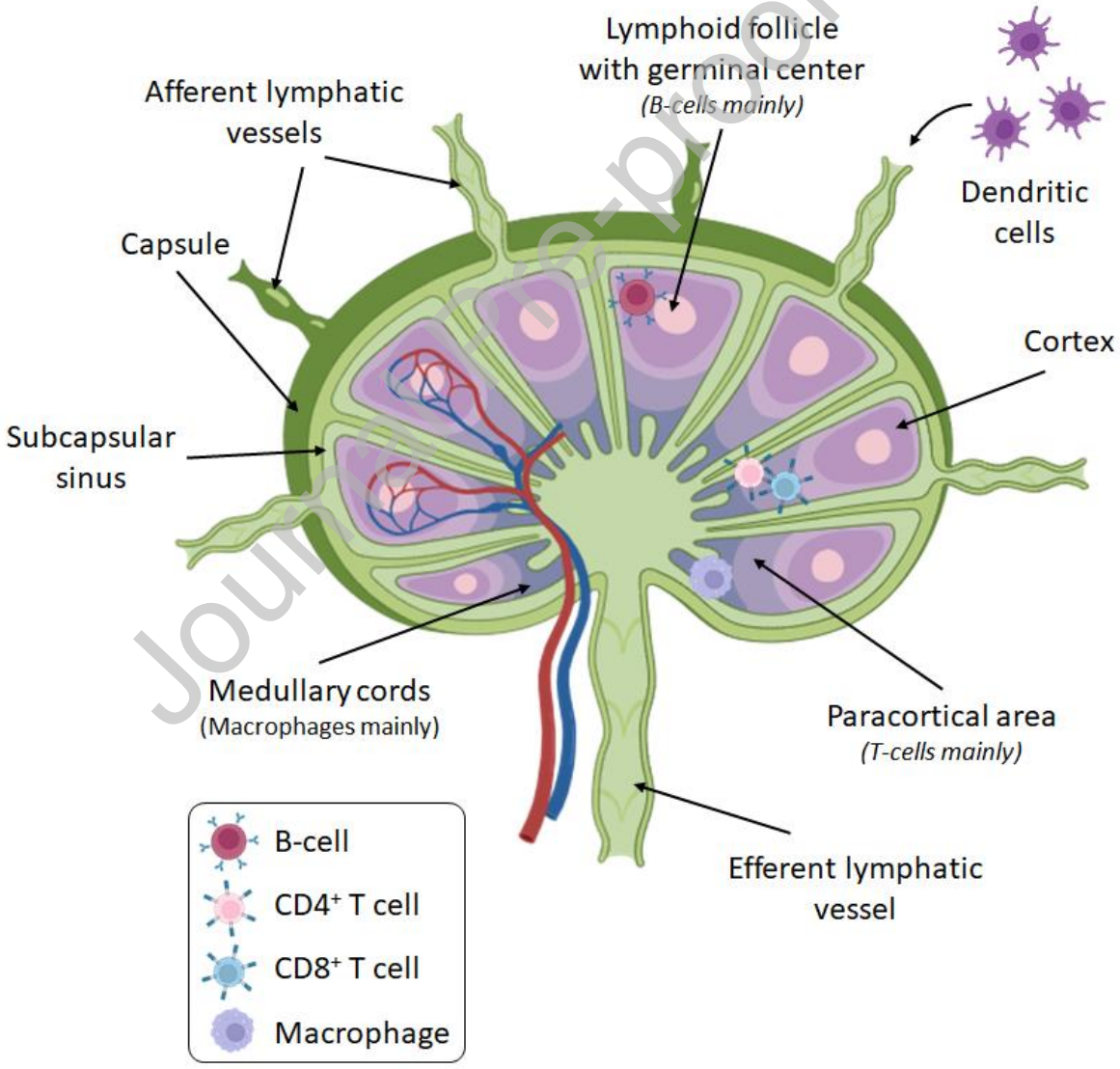

Figure 2. Scheme of lymph node architecture and lymphocyte migration. In lymph nodes, activated dendritic cells interact with both $CD8^+$ and $CD4^+$ T cells, leading to the proliferation of antigen-specific cytotoxic and helper T cells. In turn, helper T cells interact with B cells to promote the formation of plasma cells that produce high-affinity antibodies. Created using BioRender.com.

Antigen processing within LNs follows distinct pathways for T and B lymphocytes. While small antigens diffuse through collagen conduits, larger antigens require active transport by APCs, such as DCs and macrophages. APCs either present these antigens directly to B cells or process them into peptides displayed on major histocompatibility complex (MHC) molecules to prime T cells. Activated T cells differentiate into effector or memory cells and proliferate to fight infections. $CD8^+$ T cells target infected cells, while $CD4^+$ T follicular helper cells assist B cells in producing high-affinity antibodies. This intricate network of cellular interactions, mediated by chemokines and adhesion molecules, dictates LN organization and function. Given the pivotal role of LNs in immune activation, replicating their structure and signaling environment *in vitro* is crucial for optimizing T cell activation and expansion strategies. However, achieving biomimetic LN models that fully recapitulates their unique mechanical properties—such as stiffness gradients, ECM porosity, and dynamic fluid flow—while incorporating biochemical signals that regulate immune cell behavior remains a major challenge in tissue bioengineering [28].

Recent advances have attempted to address these limitations by designing structures that mimic LN-specific ECM properties. For instance, Ribezzi *et al*. [43] developed 3D bioprinted scaffolds using tailored hydrogel formulations to support lymphoid cell expansion. The study explored various bioinks composed of alginate, methylcellulose, and type A gelatin to fine-tune hydrogel stiffness and structural stability (**Figure 3a**). While the rheological analyses confirmed that methylcellulose enhanced viscosity and that alginate improved long-term structural integrity, the findings also revealed a significant decrease in stiffness over time, with Young's modulus reducing by half within a week (**Figure 3b**). This rapid softening may raise concerns about scaffold longevity and mechanical consistency, which are critical for sustained immune cell culture. Furthermore, while MEC1 chronic lymphocytic leukemia cells and primary peripheral mononuclear cells demonstrated sustained proliferation when loaded in these scaffolds, it remains unclear whether these formulations can effectively support the expansion of naïve or antigen-specific T cells.

Beyond hydrogel scaffolds, lympho-organoids (LOs) offer an alternative strategy to replicate LN function *ex vivo*. For example, Lenti *et al*. [44] introduced synthetic LOs composed of LN stromal progenitors and decellularized ECM-based scaffolds (**Figure 3c**). After transplantation in mice, these constructs successfully integrated with native lymphatic vasculature and facilitated antigen-specific immune responses post-immunization (**Figure 3d**). While these results are promising, several challenges remain. The reliance on decellularized ECM introduces variability in composition and mechanical properties, which could affect reproducibility and scalability. Additionally, while LOs partially restored LN function, their ability to fully recapitulate the complex spatial organization and dynamic antigen presentation of native LNs has not been established.

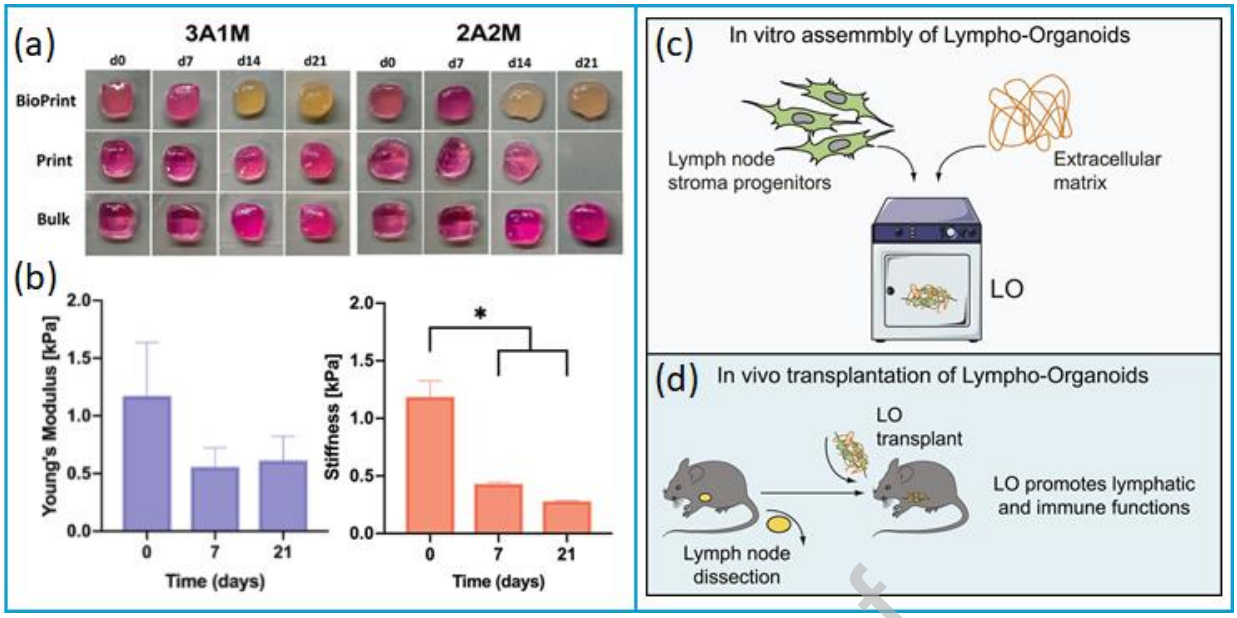

Figure 3. (a) Optical images over three weeks of bioprinted, printed, and bulk scaffolds made of alginate (2% w/v), methylcellulose (2% w/v), gelatin (10% w/v) at different blend composition; (b) Young's modulus and stiffness of bioprinted scaffolds evaluated over 3 weeks. Adapted from Ref. [43], this article is distributed under a Creative Commons Attribution (CC-BY) license. (c) *In vitro* assembly and (d) *in vivo* transplantation of lympho-organoids (LOs). Reproduced from Ref. [44] © 2019, The Authors. This article is distributed under a Creative Commons Attribution (CC-BY-NC-ND) license.

3. *Ex vivo* activation and expansion of immune cells

Isolating immune cells from patients and expanding them *ex vivo* in a controlled environment is vital in immunotherapy to enhance patient's immune response against specific targets like cancer cells or pathogens [37,45,46]. Additionally, *ex vivo* approaches provide large flexibility in engineering immune cells [47,48]. For instance, chimeric antigen receptors (CARs) can be genetically encoded into T cells (CAR-T cells) to target specific molecules on cancer cells [49,50]. However, current technologies for *ex vivo* T cell activation and expansion suffer from high costs and long preparation time before their re-inoculation in the patient [51–54].

Typically, *ex vivo* expansion of T cells involves four key steps [55]: (i) collection, (ii) isolation, (iii) activation, and (iv) expansion. First, T cells are generally collected from the patient's blood or occasionally from tumor-infiltrating lymphocytes (TILs) extracted from cancer tissue. Then, peripheral blood mononuclear cells (PBMCs) comprising any blood cells with a round nucleus (i.e. lymphocytes, monocytes, NK cells or DCs) are separated from blood using density gradient centrifugation or functionalized magnetic beads. Next, T cells activation is typically achieved by exposing them to specific antigens, such as tumor antigens or pathogens, presented by APCs like DCs. Alternatively, T cells can be activated using antibodies targeting T cell surface receptors, such as anti-CD3/anti-CD28 or Dynabeads, which are commercially available aAPCs consisting of polystyrene beads coated with anti-CD3/anti-CD28. Another method for T-cell activation and expansion involves using engineered silica rods functionalized with anti-CD3/anti-CD28. These rods provide a physical surface for T cells to bind to, mimicking the interaction between T cells and APCs. The interaction triggers T cell activation and facilitates their expansion, particularly when combined with cytokines. For example, Cheung *et al*. [37] developed mesoporous silica microrods, which support a fluid lipid bilayer that presents membrane-bound cues for T cell receptor stimulation and co-stimulation. Additionally, the microrods enable the sustained release of soluble paracrine signals, enhancing the T cell activation process. This system has

demonstrated up to ten-fold enhanced polyclonal expansion of human T cells compared to traditional Dynabeads, showcasing its efficiency and potential for improving *ex vivo* T cell expansion.

After activation, T cells are cultured *in vitro* with a culture medium supplemented with cytokines such as interleukin (IL) 2, 7, and 15 to promote their viability, proliferation, and long-term survival [56,57]. Methods using Dynabeads [55] and silica rods [37] provide strong activation signals, leading to substantial increases in T cell numbers, typically within 7–14 days. Additionally, these protocols allow for easy retrieval of activated T cells, ensuring a high yield of purified cells, which is crucial for clinical applications [58]. Furthermore, the use of cytokines such as IL-2 during culture helps maintain cell function and reduce the risk of exhaustion, ensuring that T cells remain effective when reinfused into the patient [59].

Figure 4 schematically shows *ex vivo* expansion strategies relying on using hydrogel scaffolds or aAPCs. Hydrogel scaffolds provide structural support for hosting immune cells while embedded stimulating factors and activating antibodies provide functional support for enhancing cell replication and functionality. In contrast, aAPCs can be used to activate immune cells while delivering stimulating factors needed to support cell expansion *in vitro*.

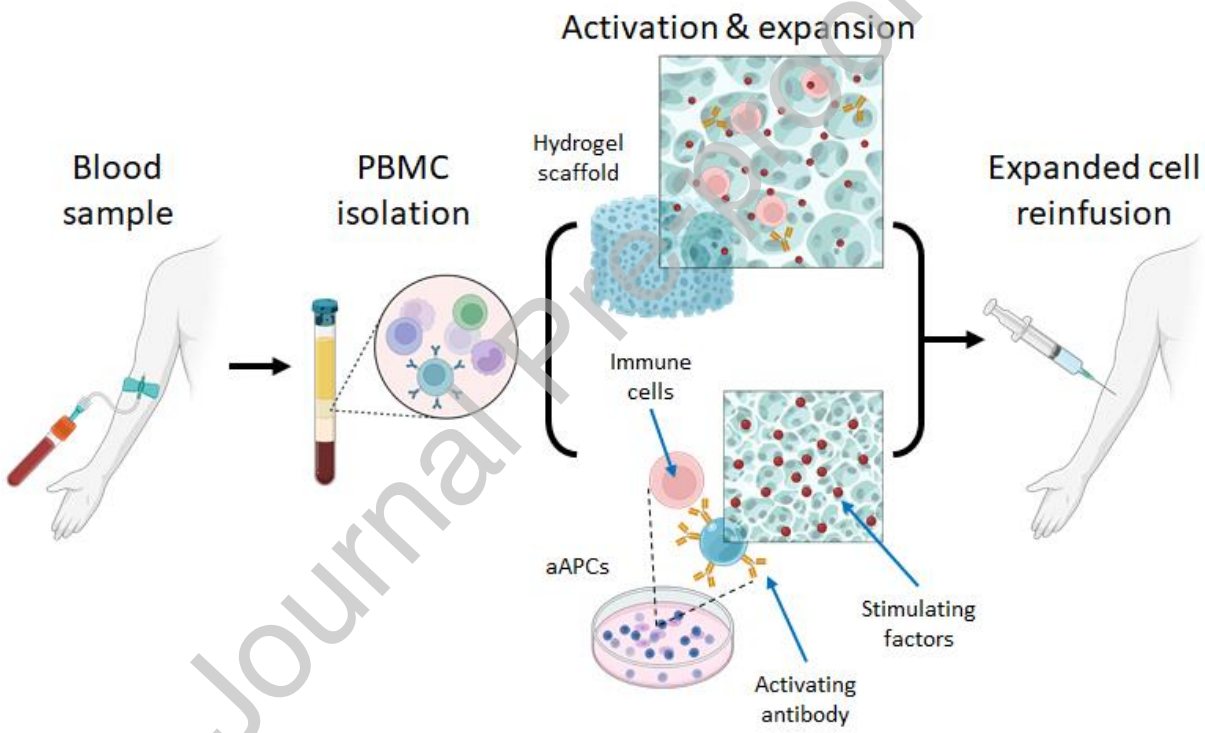

Figure 4. Scheme of *ex vivo* expansion strategies relying on using functionalized hydrogel scaffolds or artificial antigen presenting cells (aAPCs) capable of activating immune cells and supporting their expansion and function. Created with BioRender.com.

Biocompatible hydrogels represent ideal candidates for hosting and expanding T cells *ex vivo* thanks to their highly tunable physical and biochemical properties [60]. Unlike traditional two-dimensional (2D) cultures, 3D scaffolds facilitate a more physiologically relevant cell-cell interaction and signaling, potentially enhancing immune activation and expansion [61–63]. However, while these systems offer greater control over ECM-like features, their ability to fully replicate native lymphoid environments remains an area of active investigation. Key hydrogel characteristics such as stiffness, porosity, and degradation rate can be adjusted to mimic ECM properties [64,65]. For instance, Pérez del Rio *et al*. [66] designed polyethylene glycol (PEG)-based scaffolds with low molecular weight heparin (PEG-Hep), aiming to replicate LN stiffness (1-5 kPa) and ECM composition (**Figure 5a,b**). These scaffolds provided both structural support and a cytokine-anchoring mechanism, with

Hep facilitating cytokine CCL21 retention to enhance cell migration and proliferation. T cell expansion was measured by flow cytometry through (carboxyfluorescein succinimidyl ester) CFSE staining 6 days after seeding primary human $CD4^+$ T cells on PEG-Hep hydrogels, revealing a strong overexpression of memory T cells (≈90%) over the naïve phenotype (**Figure 5c**). Despite these promising results, the study did not assess the long-term stability of PEG-Hep scaffolds or their capacity to support antigen-specific T cell priming, leaving open questions regarding their suitability for sustained immunotherapy applications.

To further enhance immune cell motility and adhesion, alternative ECM-mimicking hydrogels have been explored [67]. One critical factor influencing T cell behavior is the interaction with hyaluronic acid (HA), a key ECM component in lymphoid tissues. This interaction has been demonstrated to positively impact motility, adhesion, differentiation, gene expression, and proliferation [68]. In this regard, Hickey *et al*. [69] engineered an artificial T-cell stimulating matrix (aTM), integrating thiolated HA within a polyethylene glycol diacrylate (PEGDA)-crosslinked hydrogel to mimic LN-like mechanical cues. The resulting matrix exhibited a stiffness range of 0.2-3 kPa, chosen to support mechano-transduction of T cell receptor (TCR) signaling (**Figure 5d**). Interestingly, PEGDA alone—used as a control—lacked the HA component and failed to induce comparable T cell activation. Monitoring glycoprotein CD44 expression confirmed that HA modulated T cell proliferation and phenotyping, with softer HA hydrogels (0.5-1 kPa) enhancing TCR signaling more effectively than stiffer counterparts.. Upregulation of cytokines IL-7Ra and IL-15a (**Figure 5e**), along with a significantly larger portion of $CD8^+$ T cells co-expressing IFN-γ, TNF-α, IL-2, and CD107a, indicated a strong preference toward memory T cell differentiation. Notably, T cells expanded *ex vivo* on aTM scaffolds, harvested after 8 days and transferred intravenously exhibited superior antitumor efficacy in an adoptive cell therapy (ACT) model, reducing melanoma tumor growth by ≈75% compared to untreated tumors and by ≈60% and ≈30% compared to tumors treated transferring T cells stimulated with aAPCs on tissue culture plate and HA, respectively (**Figure 5f**). While these findings highlight HA potential as a key modulator of T cell fate, further studies are needed to evaluate whether these scaffolds can consistently support diverse T cell subsets and maintain their functional properties long term.

Beyond HA-based approaches, gelatin methacryloyl (GelMA) hydrogels have also been investigated as platform for T cell expansion. For example, Joseph *et al.* [70] realized GelMA scaffolds with tunable mechanical properties, aiming to match pathophysiological stiffness of LNs. Mechanical testing revealed that low GelMA concentration (7.5% w/v) resulted in a weak crosslinked network, compromising hydrogel integrity. In contrast, hydrogels with higher GelMA concentrations (> 15% w/v) exceeded physiologically relevant stiffness values (4-24 kPa in healthy conditions [71] and 40-50 kPa during infection [72]). Only formulations within the intermediate range (10-15% w/v) achieved a balance between structural stability and biomechanical relevance, making them suitable for LN-mimetic constructs (**Figure 5g**). Bioactivity assessments using EL4 cells and primary mouse T cells—both activated using phorbol myristate acetate (PMA) and ionomycin—showed enhanced viability and IL-2 release when loaded into GelMA hydrogels, with a preference for softer GelMA hydrogels over stiffer scaffolds or 2D cell cultures (**Figure 5h**). Despite these promising outcomes, the study did not explore whether GelMA scaffolds can support antigen-specific T cell priming or maintain functionality over prolonged culture periods, raising questions about their translational potential.

An additional advantage of hydrogels-based cultures is their ability to shield cells from shear stress, a common issue in dynamic bioreactors and stirred suspension systems [73,74]. In this regard, Lin *et al*. [75] developed an innovative core-shell hydrogel system using HA-alginate fibers (diameter ≤400 μm) to host T cells and their activators (anti-CD3/CD28/CD2), thereby minimizing hydrodynamic stress (**Figure 5i**). The coaxial extrusion of the cell-laden hydrogel into a $CaCl_2$ solution enabled rapid alginate crosslinking, forming stable fibers that could be suspended in culture media. Importantly, this spatial confinement not only reduced shear-induced stress but also enhanced mass transport, creating a localized microenvironment conducive to cell expansion. To retrieve cells for downstream applications, hydrogel fibers were dissolved in EDTA acid and T cells were

collected by centrifugation. Compared to both static—in which cells were suspended in culture medium without agitation—and dynamic—in which cells were suspended in culture medium with rocking, hydrogel-encapsulated T cells exhibited significantly higher viability, reduced DNA damage, and a remarkable 320-fold expansion over 14 days in the latter case? (**Figure 5j**). However, the need for EDTA for hydrogel dissolution introduces an additional processing step that may not be optimal for large-scale clinical applications.

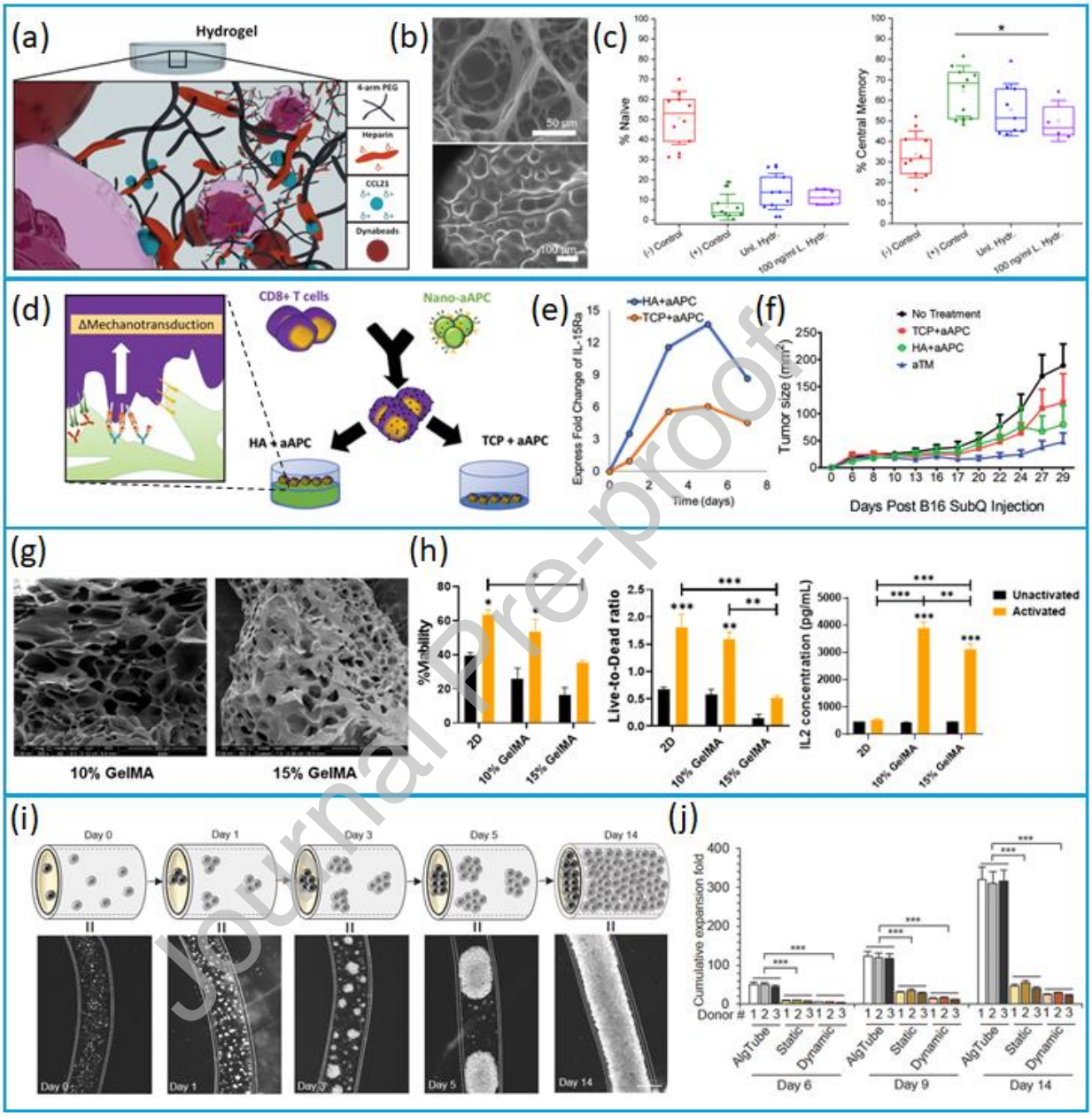

Figure 5. (a) Schematic representation of T cells cultured in the CCL21-loaded PEG-Hep hydrogels; (b) SEM images of PEG-Hep hydrogel with 4% wt PEG composition; (c) Percentage of naïve and central memory $CD4^+$ T cells on day 5. Adapted from Ref. [66], this article is distributed under a Creative Commons Attribution (CC-BY) license. (d) Scheme of antigen-specific $CD8^+$ T cell activation with nanoparticle artificial antigens presenting cells (aAPCs) on HA hydrogel versus a tissue culture plate (TCP); (e) Release of cytokine IL15Ra over one week; (f) Tumor size over four weeks indicating that adoptive T cells from aTM stimulation significantly delayed tumor growth. Adapted from Ref. [69], © 2019 with permission from Wiley-VCH. (g) SEM images of scaffolds with 10% w/v and 15% w/v of GelMA concentrations; (h) Viability, live-to-dead ratio and IL-2 secretion after 48 h of both unactivated and activated primary T cells in 2D culture and 3D-bioprinted scaffolds. Adapted from Ref. [70], © 2023 with permission from American Chemical Society. (i) Scheme and

optical images of core-shell HA-alginate fibers capable of promoting T cells growth over two weeks; (j) Cumulative T cell expansion over two weeks in HA-alginate fibers, static and dynamic suspension culturing. Adapted from Ref. [75], © 2018 with permission from Wiley-VCH.

Additionally, hydrogels can be functionalized with antibodies—such as anti-CD3 and anti-CD28 to enable localized T cells activation—and/or with bioactive molecules such as cytokines or growth factors whose release over time promotes enhanced expansion and effector functionality. As mentioned previously, T cell activation is not only affected by biochemical cues but is also highly dependent on physical parameters, including substrate stiffness [33,76]. Thus, the simultaneous modulation of both biomechanical and biochemical stimuli presents an intriguing avenue for optimizing immunotherapy strategies. However, a significant gap remains in understanding how biomechanical properties influence immune cell regulation, particularly in dynamic physiological environments. To investigate the interplay between hydrogel stiffness and biological cues in T cell regulation, Chin *et al*. [77] designed a customizable and reusable 12-well plate system integrating 2D polyacrylamide (PA) hydrogels, where PA stiffness and antibody density could be finely tuned (**Figure 6a**). By exposing Jurkat T cells to varied substrate stiffness and antibodies concentrations, the study provided insights into how these parameters affect immune activation. Specifically, T cells cultured on stiffer PA hydrogels (≈50 kPa) exhibited higher IL-2 secretion, suggesting enhanced activation, whereas softer PA hydrogels (≈7 kPa) favored greater cell proliferation (**Figure 6b**). Interestingly increasing antibody coating density further amplified IL-2 secretion, particularly in softer PA hydrogels, indicating a synergistic effect between biochemical and biomechanical cues. These findings reinforce the importance of tailoring both mechanical and molecular signals to fine-tune T cell responses, although further validation in primary T cell models is necessary to establish clinical relevance.

Beyond antibodies, hydrogels functionalization with glycoproteins such as integrins has also been explored to facilitate cell-cell and cell-ECM adhesion. For example, Guasch *et al*. [78] explored an integrin-mediated approach to enhance T cell expansion, leveraging fibronectin-derived peptides to modulate cellular adhesion and activation. Specifically, two fibronectin-mimetic peptides (i.e., cRGD and cLDV) were crosslinked within PEG hydrogels, and their impact on T cell proliferation following cell seeding was assessed. To initiate T cell activation, hydrogel slabs were decorated with hexagonally arranged gold nanoparticles (AuNPs) functionalized with anti-CD3 (**Figure 6c**). Both cRGD- and cLDV-functionalized hydrogels showed increased T cell activation, as evidenced by upregulated CD69 expression and IL-2 secretion compared to non-functionalized hydrogels (**Figure 6d**). However, only cRGD-functionalized hydrogels supported a robust T cell proliferation, also promoting a higher proportion of memory T cells over naïve phenotype (**Figure 6e**) These results suggest that integrin signaling via cRGD sequences may provide an additional regulatory mechanism for enhancing T cell expansion, potentially complementing traditional TCR-based activation strategies.

Further advancements in T cell expansion have leveraged aACPs. For example, Shou *et al*. [79] engineered aAPCs made of hybrid colloidal crystal clusters, designed to optimize long-term T cell expansion and antitumor activity (**Figure 6f**). Unlike commercial Dynabeads, the nanoporous network of these aAPCs provided a sustained release of prosurvival cytokines (e.g., IL-2) while simultaneously presenting activating antibodies anti-CD3 and anti-CD28. This dual mechanism resulted in a significantly more efficient and prolonged T cell proliferation response. *In vitro* experiments demonstrated a ≈80-fold enhancement in T cell expansion after 14 days of co-culturing, confirming the strong translational potential of this approach for large-scale manufacturing of immune cells and cancer immunotherapy (**Figure 6g**). However, while aAPCs show significant promise, their comparative performance in antigen-specific T cell priming and long-term functional maintenance remains an area requiring further study.

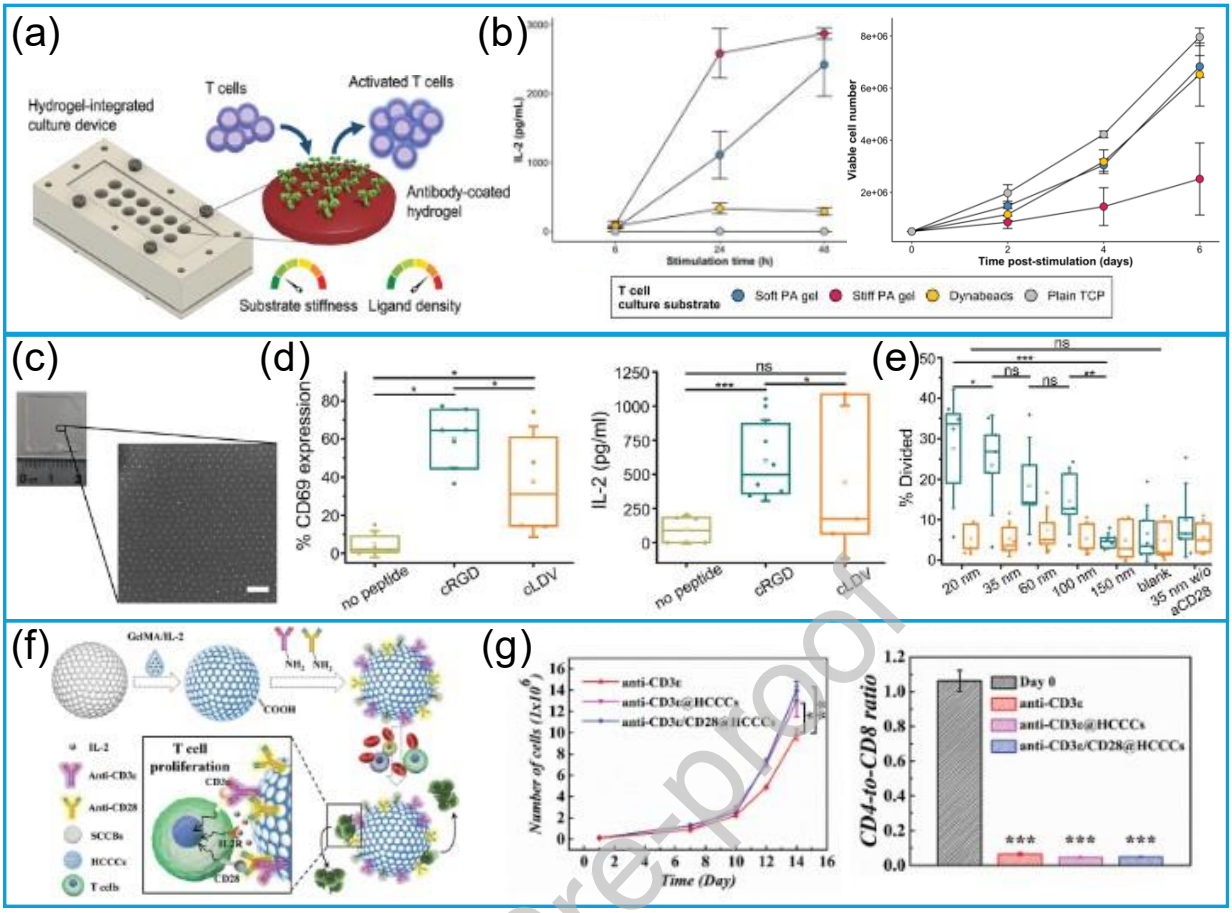

Figure 6. (a) Schematic representation of hydrogel-integrated multiwell culture chamber for T cell stimulation; (b) Secretion of cytokine IL-2 over 48 h and number of viable T cells over six days stimulated by soft gels (7.1 kPa) and stiff gels (50.6 kPa). Adapted from Ref. [77], this article is distributed under a Creative Commons Attribution (CC-BY) license. (c) Optical image of PEG hydrogel and SEM micrograph of gold nanoparticle array decorating the hydrogel surface; (d) CD69 expression and IL-2 secretion of primary $CD4^+$ T cells activated by functionalized PEG hydrogels; (e) Percentage of divided $CD4^+$ T cells obtained on cRGD- (green lines) and cLDV- (orange lines) containing PEG hydrogels decorated with anti-CD3-functionalized gold nanoparticle separated by various distances and co-stimulated with soluble anti-CD28 after one week. Adapted from Ref. [78], © 2017 with permission from American Chemical Society. (f) Scheme of T cell expansion supported by antibodies-modified hybrid colloidal crystal clusters; (g) Number of T cells and CD4/CD8 ratio evaluated after two weeks. Adapted from Ref. [79], © 2021 with permission from Wiley-VCH.

Lastly, due to their exceptional biocompatibility, hydrogels represent also promising candidates for reducing the risk of immune rejection following implants or injection into patients [80]. In particular, their ability to encapsulate and sustain immune-regulatory cells presents a valuable strategy for modulating immune responses in transplantation settings. In this regard, Kim *et al*. [81] explored this potential by encapsulating human natural and induced regulatory T cells (nTregs and iTregs, respectively) within hydrogels to enhance their immune-regulatory function. Systemic adoptive transfer of *ex vivo*-expanded Tregs has been largely studied as a strategy to locally suppress immune responses during allogeneic transplantation. However, the short lifespan and limited persistence of *ex vivo*-expanded Tregs pose significant challenges, necessitating additional strategies to achieve long-term immune tolerance. Taking inspiration from tumor cells, which express chemokine ligand 1 (CCL1) to recruit Tregs and establish an immunosuppressive environment against effector $CD4^+$ and $CD8^+$ T cells, engineered hydrogels supplemented with CCL1 and IL-2 potentially offer an

approach to locally suppress immune responses. Specifically, CCL1 enhances Tregs recruitment and stability, while IL-2 supports Tregs encapsulation, viability and functionality. In this key study [81], Tregs encapsulated in an alginate-GelMA-gelatin hydrogel and coaxially bioprinted with murine pancreatic islets and human PBMCs demonstrated remarkable immunoprotective effects. The hydrogel constructs successfully protected murine islets from xenorejection and and prevented leukocytes infiltration, highlighting its potential for advancing transplant immunotherapy. While these findings are promising, further studies are needed to assess the long-term stability and functional persistence of encapsulated Tregs in clinically relevant models.

In conclusion, hydrogel-based T cell expansion systems offer a cost-effective alternative to traditional cell expansion methods, which typically rely on expensive cytokine cocktails, prolonged culture periods, and specialized bioreactors that require constant monitoring and maintenance [36]. By providing a controlled microenvironment that supports immune cell proliferation and function, hydrogels reduce the need for high cytokine concentrations and feeder cells, thereby lowering reagent costs and minimizing labor-intensive culture processes [13]. Additionally, their scalability and ease of integration into existing clinical workflows make them an attractive option for generating large numbers of immune cells for applications such as ACT, without compromising cell quality or function [82–84]. These systems provide a biomimetic environment that closely resembles the natural ECM, promoting optimal cell growth and differentiation. By supporting efficient nutrient and oxygen diffusion, hydrogels ensure high cell viability and proliferation rates. The adaptability of hydrogels allows for precise control over mechanical properties and biochemical cues, tailoring the microenvironment to the specific needs of different immune cell types. Furthermore, their versatility in functionalization with antibodies, cytokines, and other bioactive molecules enhances the activation and expansion of T cells. This makes hydrogel-based platforms a powerful tool in advancing personalized medicine, providing tailored and effective immune cell therapies with reduced risk of adverse reactions and improved patient outcomes. A comprehensive list of hydrogel-based strategies for *ex vivo* immune cell expansion is reported in **Table 1**.

Table 1. Overview of hydrogels designed to host immune cells and enhance their expansion *ex vivo*.

HA: hyaluronic acid; Alg: alginate; Gel: gelatin; MC: methylcellulose; GelMA: gelatin methacryloyl; DLP: digital light processing; PMA: phorbol myristate acetate; FBS: fetal bovine serum; PEGDA: polyethylene glycol diacrylate; nTregs: natural regulatory T cells; iTregs: induced regulatory T cells; cRGD: cyclic Arg-Gly-Asp; cLDV: cyclic Leu-Asp-Val; PEG: polyethylene glycol; AuNPs: gold nanoparticles; Mal-LMWH: maleimide-functionalized low molecular weight heparin; CCL21: chemokine ligand 21; T_N: naïve T cells; T_{CM}: central memory T cells; T_{EM}: effector memory T cells; PBMC: peripheral blood mononuclear cells; mTregs: memory Tregs; mTconvs: memory conventional T cells; PEG-Hep: PEG-low molecular weight heparin. *Data not explicitly given in the reference; value was retrieved from graphs shown in the reference.

Biomaterials	Fabrication method	Stiffness	Mesh size	Hosted cells	Activators	Loaded cells	Growth rate	Cytokines	Phenotype	Ref.
PEG-Hep	Molding and PEG-SH/Mal-LMWH crosslinking	4.8 kPa (6% wt PEG-Hep) 3.1 kPa (4% wt PEG-Hep) 1.1 kPa (3% wt PEG-Hep)	5-50 µm (6% wt PEG-Hep) 20-75 µm (4% wt PEG-Hep) 25-105 µm (3% wt PEG-Hep)	Primary human $CD4^+$ T cells	Activation with Dynabeads® (4.5 µm superparamagnetic spheres decorated with anti-CD3/CD28) and co-stimulation with CCL21	1×10^6 cells/mL	Increase of 1.29-fold replication index and 1.06-fold expansion index after 6 days	n/a	11% of T_N ($CD45RO^-/CD62L^+$) 47% of T_{CM} ($CD45RO^+/CD62L^+$) 33% of T_{EM} ($CD45RO^+/CD62L^-$)	[66]
HA	Molding and PEGDA crosslinking	0.2-3.3 kPa	n/a	Wild-type B6 murine $CD8^+$ T cells Human $CD8^+$ T cells	Anti-CD3/CD28	0.1×10^6 cells/mL	≈25-fold expansion after 7 days	Upregulation of IL-15Rα (≈14-fold after 5 days)* Upregulation of IL-7Rα (≈2.5-fold after 7 days)* Copositive for IFN-γ, TNF-α, IL-2, CD107a	<1% T_N, ≈10% T_{CM}, ≈90% T_{EM} (0.5 kPa)* ≈1% T_N, ≈51% T_{CM}, ≈48% T_{EM} (1 kPa)* ≈5% T_N, ≈45% T_{CM}, ≈50% T_{EM} (1.7 kPa)* ≈8% T_N, ≈28% T_{CM}, ≈60% T_{EM} (2.6 kPa)* ≈25% T_N, ≈18% T_{CM}, ≈50% T_{EM} (3.3 kPa)*	[69]
GelMa	DLP with photocuring at 405 nm	19.83 kPa (10% w/v GelMA) 52.95 kPa (15% w/v GelMA)	55.67 µm (10% w/v GelMA) 20.22 µm (15% w/v GelMA)	EL4 cell line Primary mouse T cells	PMA and ionomycin	1.0×10^6 cells per 100 µL	n/a	IL-1β, IL-4, IL-6 not detected IL-5 detected in 2D culture TNFα and IL-17A detected in 3D culture	n/a	[70]

								regardless GelMA concentration IFNγ detected only in 3D culture with 15% GelMA 7.4-fold and 5.9-fold enhancement of IL-2 release in 10% and 15% GelMA, respectively, compared to 2D culture		
HA 2% (core) Alg 1.5% (shell)	Coaxial core–shell extrusion and $CaCl_2$-mediated crosslinking	n/a	Shell thickness ≤ 60 µm Outer diameter ≤ 400 µm	$CD3^+$ T cells (≈98-99% purity)	Tetrameric anti-CD3/CD28/CD2 antibodies	1.0×10^6 cells/mL	320-fold expansion after 14 days	IL-2, IL-4, IFN-γ, TNF-α release comparable with control experiment	≈60-75% of $CD4^+$ T cells ≈16-29% of $CD8^+$ T cells	[75]
PA	Molding and free-radical crosslinking	7.1 kPa (0.05% w/v bis-acrylamide) 50.6 kPa (0.4% w/v bis-acrylamide)	n/a	Jurkat $CD4^+$ T cells	Anti-CD3/CD28	1×10^5-1×10^6 cells/mL	≈2.1-fold (stiff PA) and ≈6.8-fold (soft PA) expansion after 6 days	≈3.5-fold and ≈8.5-fold enhancement of IL-2 release in soft and stiff PA after 24 h, respectively, compared to Dynabeads* ≈7.5-fold and ≈9.5-fold enhancement of IL-2 release in soft and stiff PA after 48 h, respectively, compared to Dynabeads*	n/a	[77]
cRGD-PEG-AuNPs cLDV-PEG-AuNPs	Molding with photocuring at 365 nm	n/a	n/a	Primary human $CD4^+$ T cells	Anti-CD3 (functionalized to AuNPs) Anti-CD28 (added to solution for co-stimulation)	1×10^6 cells/mL	Cells divided 3-4 times after 3 days (12% of seeded cells for cRGD and 4% for cLDV)* and 7-8 times after 7 days (34% of seeded cells for cRGD and 5% for cLDV)*	Upregulation of IL-2 (≈1.25 ng/mL for cRGD and ≈0.45 ng/mL for cLDV hydrogels after 16-20 hours)*	≈90% of $CD4^+CD45RO^+$ (memory phenotype) and ≈10% of $CD4^+CD45RA^+$ (naïve phenotype) after 6 days for cRGD hydrogel	[78]

Hybrid colloidal crystal clusters encapsulated with GelMA	Mechanical and thermal treatments to form SCCB droplets, followed by GelMA hydrogel infiltration and UV curing	n/a	≈400 µm SCCB droplet* ≈150 nm silica beads* <100 nm nanovoids*	Human PBMC	Activation with anti-CD3ε/CD28 and co-stimulation with IL-2	1×10^5 cells per mL	≈80-fold expansion *in vitro* after 14 days	n/a	Smaller amount of $CD3^+/CD56^+$ T cells compared to control experiment No significant difference in CD69 expression and $CD4^+/CD8^+$ T cells compared to control experiment	[79]
Alg-GelMA	Molding and photo-chemical crosslinking (400 nm, $CaCl_2$)	n/a	n/a	Human $CD4^+$ nTregs Human $CD4^+$ iTregs Human PBMCs	Dynabeads to activate nTregs $CD4^+$ T_N supplemented with human TGF-β, all-trans retinoic acid, rapamycin to generate iTregs	2×10^6 cells/mL	n/a	Supplemented with IL-2 and CCL1	Depletion of CD25 and FOXP3 in encapsulated (+IL2) $CD4^+$ nTregs and iTregs Enrichment of mTregs, Th2, Th22 and depletion of mTconvs, Th1, Th1/17, Th17, Th9 in $CCR8^+$ memory $CD4^+$ T cells from PBMCs	[81]
Alg-GelMA-Gel	Coaxial extrusion and photo-chemical crosslinking (400 nm, $CaCl_2$)	≈4 kPa at 5°C ≈134 Pa at shear rate of 1 Hz								

4. Injectable hydrogels

In cancer treatment, , cell-based immunotherapies have faced significant challenges in treating solid tumors effectively. One major limitation is the immunosuppressive tumor microenvironment (TME), which is characterized by physical barriers, suppressive cytokines, and regulatory immune cells that collectively hinder immune cell infiltration and persistence within the tumor[85,86]. Additionally, solid tumors exhibit heterogeneity in antigen expression, making it difficult for engineered immune cells to uniformly recognize and eliminate cancer cells[87]. Another key challenge is the limited trafficking and retention of infused immune cells at the tumor site, as systemically administered cells often struggle to extravasate and migrate efficiently within the dense ECM of solid tumors[88]. Furthermore, the metabolic competition within the TME, including hypoxia and nutrient deprivation, can impair immune cell functionality and persistence, further reducing the efficacy of cell-based immunotherapies[89].

In this context, injectable hydrogels may help overcome some of the aforementioned challenges by providing a localized, immunostimulatory niche that enhances immune cell retention, sustains their activity, and modulates the TME to be more permissive to effective immune responses. Specifically, injectable hydrogels emerged as an effective vehicle for *in vivo* delivering immune cells, stimulatory factors or drugs [90], offering a versatile platform for precise localization and controlled release within tissues and tumors (**Figure 7**) [91,92]. In contrast to hydrogels conceived to resemble LNs for *ex vivo* expansion, injectable hydrogels are specifically designed to remain liquid prior to and during the injection and then undergo a transition into a gel state once administered. This transition can be triggered by various mechanisms such as temperature-mediated [93], chemically fueled [94], or spontaneous sol-gel processes [95]. Additionally, in-situ crosslinking systems allow for the gelation of the hydrogel at the target site through chemical or enzymatic reactions [96,97]. This crosslinking method generally ensures that the gelation process is confined to the targeted area, minimizing the risk of undesired gel formation during administration. Furthermore, chemical or enzymatic crosslinking can be tailored to respond to specific environmental cues, such as pH or the presence of specific biomolecules, providing an additional layer of versatility in drug delivery and tissue engineering. Another class of injectable hydrogels is represented by shear-thinning systems [98], which exist as gels prior to injection, become flowable under shear stress during the injection, and then self-heal into a gel state once the stress is removed. Thermogelling systems, which undergo gelation upon reaching physiological temperatures, and shear-thinning hydrogels, which gelled only after injection, both serve as effective delivery platforms [98,99]. This diversity of injectable hydrogels provides flexibility depending on the specific application and desired properties. The integration of *in situ* crosslinkable hydrogels with immune cells offers the opportunity to establish an intratumoral or peritumoral niche for immune cell activation and expansion, where fresh immune cells can be regularly and effectively delivered to the tumor microenvironment [100].

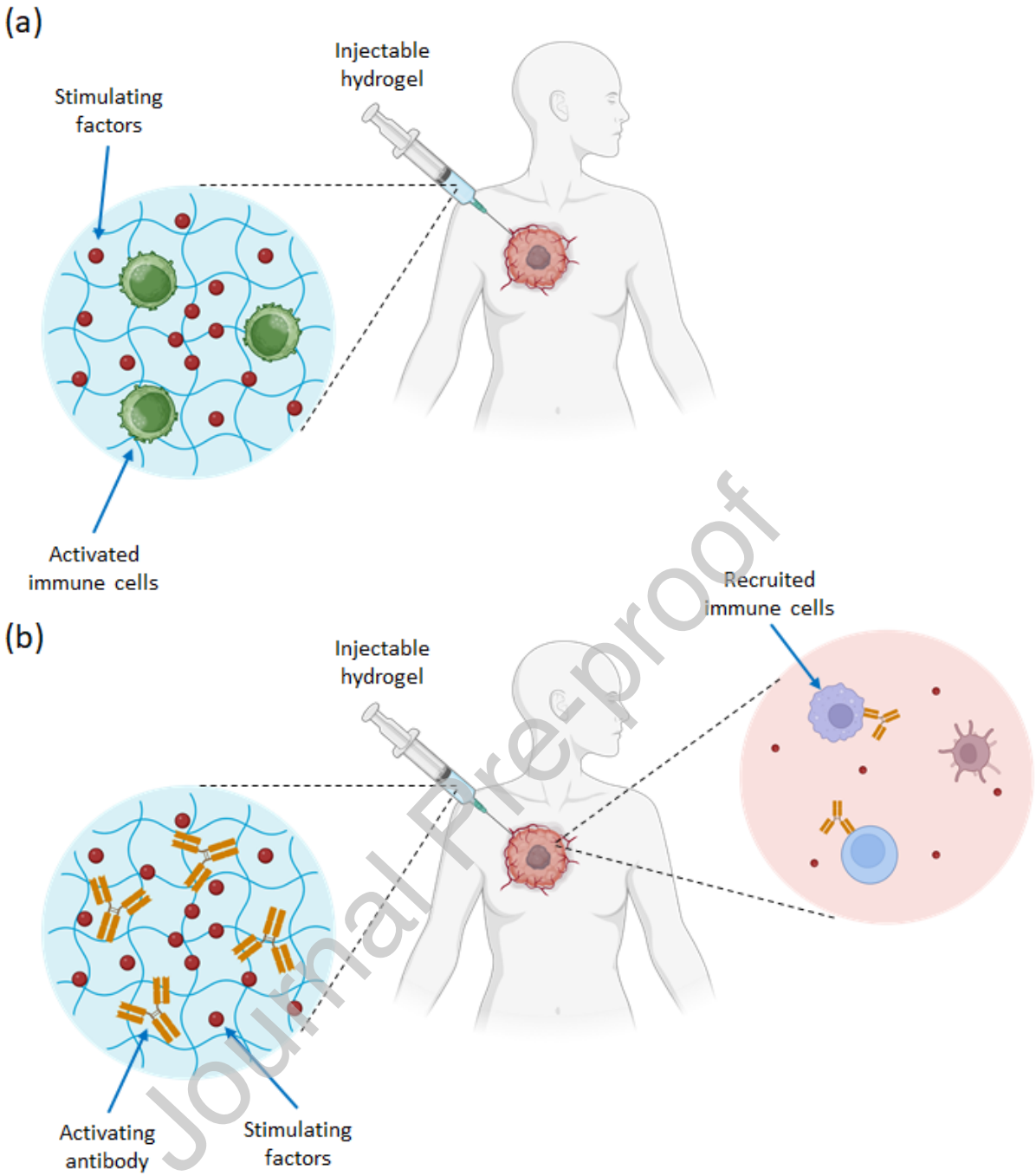

Figure 7. Example of *in vivo* strategy based on injectable hydrogels embedding (a) immune cells and stimulating factors, or (b) activators and stimulation factors for locally enhancing the immune response against tumor cells. Created in BioRender.com.

In the context of temperature-sensitive hydrogels, Weiden *et al*. [101] benefited from the reversible thermally-induced gelation (above 16 °C) of polyisocyanopeptide (PIC) to fabricate hydrogel scaffolds that allowed minimally invasive injection in liquid form and gelation at the target site thereby eliminating the need for external crosslinking agents and reducing potential toxicity (**Figure 8a**). In addition, azide-terminated monomers integrated in polymer matrix provided functional handles for incorporating immune cell-modulating cues. The hydrogel promoted *in vivo* retention of functional pre-activated T cells in mice up to 4 weeks (**Figure 8b**) without causing inflammation, indicating non-immunogenicity. Cell migration in draining

and non-draining LNs, spleen and blood of the recipient mice was detected up to 4 weeks after subcutaneous injection simultaneously with a reduction in their percentage in PIC gel (**Figure 8c**). Release of cells from the hydrogel was likely a consequence of gel degradation over time. However, despite these advantages, thermoresponsive hydrogels often lack long-term mechanical integrity, which can lead to premature degradation before the immune modulation process is fully completed. Additionally, patient variability in temperature response must be considered, as slight body temperature fluctuations (e.g., fever or hypothermia) may affect gelation kinetics, potentially resulting in inconsistent therapeutic outcomes. Moreover, while the study reported no apparent inflammatory responses, the rapid gelation process and degradation kinetics may still pose risks of localized inflammation in certain patient populations.

An innovative and promising approach to locally deliver encapsulated immune cells makes use of self-assembling peptides whose mechanical and chemical properties can be easily tailor on demand [102]. For instance, Jie *et al*. [103] engineered a self-assembling nanofiber hydrogel scaffold capable of accelerating CAR-T cell proliferation while extending their retention *in vivo* (**Figure 8d**). The scaffolds combined mechanotransduction and chemical signal modulation, offering optimal stiffness (0.5-1.0 kPa) and adhesive ligand density. Additionally, intrinsic PD-1 blocking secreted from engineered CAR-T cells suppressed the tumor-inhibitory microenvironment. Indeed, the PD-1 blocking mechanism involves inhibiting the interaction between the PD-1 receptor on T cells and its ligands (PD-L1/PD-L2), preventing the immune suppression that tumors exploit, thereby allowing T cells to attack and destroy cancer cells more effectively. This scaffold achieved a rapid 12-fold amplification of CAR-T cells in just 3 days (**Figure 8e**) and ensured long-term retention through local injection, addressing two major challenges in CAR-T immunotherapy for solid tumors. *In vivo* experiments in mice confirmed significant improvements in tumor suppression with tumor growth delayed over 70% in size after two weeks and survival rates of ≈80% (**Figure 8f**). Despite these promising results, a major limitation of this approach is the challenge of balancing gel stiffness with degradability—while softer scaffolds may enhance retention, they can also be more prone to degradation, potentially reducing their ability to sustain long-term CAR-T cell presence at the tumor site. Additionally, while PD-1 blockade offers strong tumor inhibition, excessive immune activation could raise concerns about immune-related adverse effects, including autoimmunity [104].

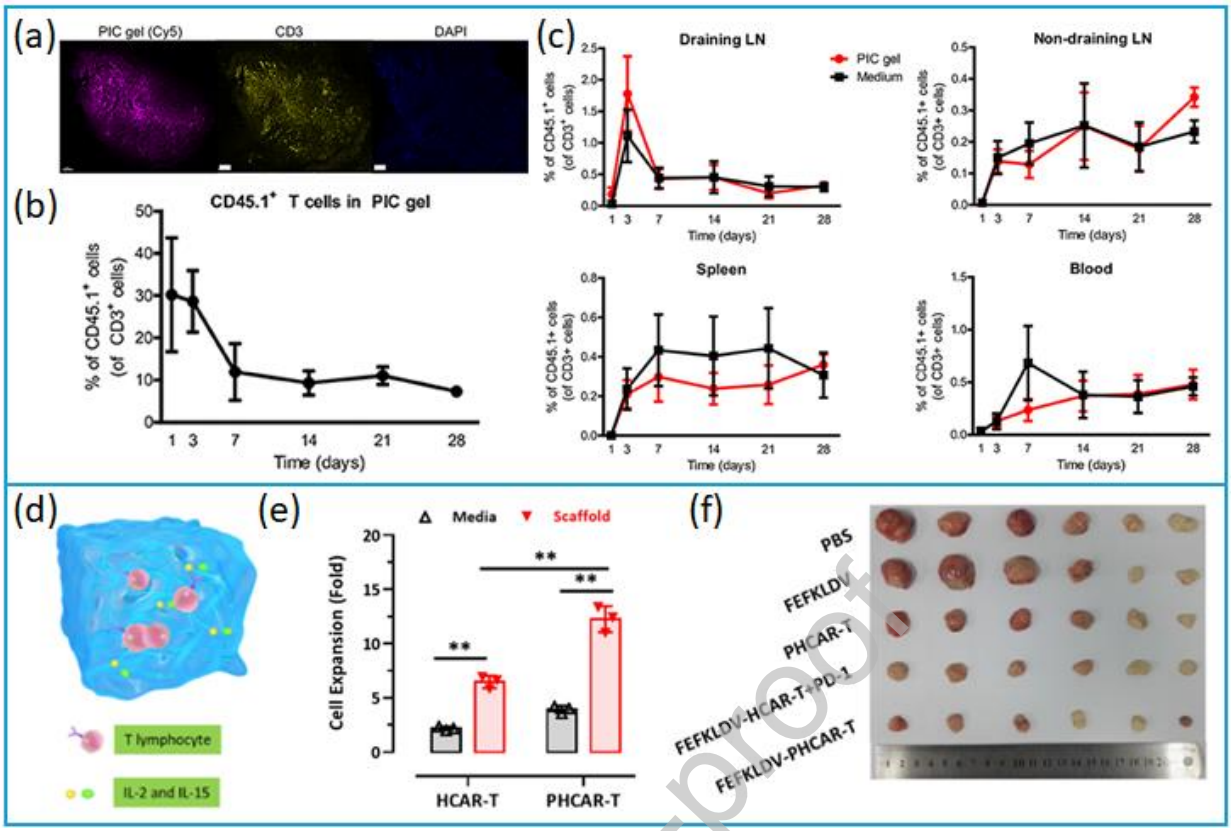

Figure 8. (a) Representative image of a 10-µm section of formalin-fixed, paraffin-embedded (FFPE)-treated PIC gel in the skin 1 day after the injection; (b, c) Percentage of $CD45.1^+$ T cells retrieved from PIC gels and surrounding skin, and from draining LN, non-draining LN, spleen and blood, respectively, over four weeks. Adapted from Ref. [101], this article is distributed under a Creative Commons Attribution (CC-BY) license. (d) Rendering of the FEFK hydrogel embedding HCAR-T cells and cytokines IL-2 and IL-15; (e) PD-1-promoted HCAR-T cell expansion in scaffold and medium evaluated after 3 days; (f) Tumor size monitored over 32 days as a result of different treatments. Adapted from Ref. [103], © 2022 with permission from American Chemical Society.

On the other hand, injection of macroporous hydrogels accommodating functionalized microparticles may further reduce invasiveness and improve method robustness due to the lack of variabilities and stresses introduced by in-vivo crosslinking. In this regard, Bhatta *et al*. [105] developed an injectable hydrogel designed to activate and expand T cells directly within the patient. The hydrogel consisted of a bulk phase of PEG crosslinked to azido-functionalized alginate via disulfide bonds, while a macroporous phase introduced via a controlled cryogenation process (**Figure 9a**). The size of macropores was approximately 150 µm, ideal for accommodating T cells. Additionally, microparticles loaded with T cell activation cues (anti-CD3 and anti-CD28) were embedded within the nanoporous gel network, and their release was minimal under normal conditions. However, in presence of T cells with abundant surface thiol groups, the disulfide bonds were disrupted, allowing the microparticles to be released into the macropores and activating the T cells. This injectable hydrogel offered a method for controlled T cell expansion *in vivo* (≈25-fold expansion after 3 days, **Figure 9b**), enhancing cytotoxic T cells responses and antitumor activity (**Figure 9c**). While this approach presents a promising step forward, disulfide-based crosslinking may introduce unintended degradation pathways in the complex *in vivo* environment, potentially impacting T cell activation. Furthermore, the reliance on endogenous thiol groups for controlled release may lead to patient-to-patient variability in efficacy, particularly in immunocompromised individuals.

Biocompatible polymer nanoparticles are also selected due to their many advantages such as facile injectability, mesh size tunability, possible RGD incorporation, and biodegradability over time [106–109]. This is the case of Grosskopf *et al*. [110], who developed a RGD-PEG-PLA-based injectable hydrogel incorporating polymer nanoparticle (PNP-1-5) for controlled delivery of encapsulated CAR-T cells and stimulatory cytokines, enhancing solid tumor treatment and reducing risk of graft-versus-host disease, a common complication in CAR-T therapy (**Figure 9d**). The hydrogel exhibits a shear-thinning behavior enabling injection through small-diameter needles or catheters and protecting encapsulated cells from harsh mechanical stresses during administration. After injection, the formulation rapidly forms robust depots with substantial resilient behavior, critical for persisting in the subcutaneous layers [98]. These PNP-1-5-loaded hydrogels enabled a dose-sparing effect, achieving superior therapeutic outcomes with fewer CAR-T cells compared to traditional subcutaneous or intravenous administration. Notably, even a 4-fold higher CAR-T cell dose delivered subcutaneously or intravenously failed to achieve complete tumor eradication as resulted in a slower regression rate than PNP-1-5 hydrogel delivery. This suggests that the hydrogel delivery system can reduce the number of CAR-T cells needed for effective treatment, potentially lowering treatment costs. It was demonstrated that 70% of solid tumors were effectively treated in mice using PNP-1-5 hydrogel, significantly higher than 10% and 40% for subcutaneous and intravenous methods, respectively (**Figure 9e**). However, the clinical translation of PNP hydrogels remains a significant challenge due to potential batch-to-batch variability and the need for precise control over nanoparticle degradation kinetics. Moreover, although the study highlights improved therapeutic outcomes, the long-term persistence and potential off-target effects of nanoparticles remain largely unexplored. Additionally, PNP-1-5 hydrogel demonstrated a strong abscopal effect when administered distally, suggesting its potential use in treating metastatic or inaccessible tumors. The results showed that even distal treatment with CAR-T cells and IL-15 within the hydrogel cured all treated animals, though at a slower rate than peritumoral administration (**Figure 9f**).

In addition to their more widespread use as training court for *in vivo* T cell stimulation and expansion, injectable hydrogels accommodating DCs represent a promising candidate in developing intratumoral vaccine nodules [111–113]. However, current limitations of this technique include low DC viability, short lifespan at the vaccination site, and inadequate immune cell recruitment, which hinder its widespread clinical adoption. In this regard, Yang *et al*. [114] benefited from the high cell retention of peptide nanofibrous hydrogels to develop a vaccine nodule containing anti-PD-1 antibodies, DCs, and tumor antigens (**Figure 9g**). Upon subcutaneous injection, the nodule sustained DCs viability and function, enhanced host DC recruitment, and facilitated the drainage of activated DCs to LNs. This resulted in enhanced antigen-specific T-cell proliferation and robust cellular immune responses. Compared to traditional methods, the vaccine nodule demonstrated superior antitumor efficiency in both preventive and therapeutic models, marked by delayed tumor growth and extended survival in mice (**Figure 9h**). This was attributed to an effective stimulation of antitumor T-cell immunity and increased infiltration of activated $CD8^+$ T-cells in tumors. While promising, the long-term immune response induced by such vaccine nodules remains uncertain, particularly concerning the potential for immune exhaustion or tolerogenic responses over time [115,116].

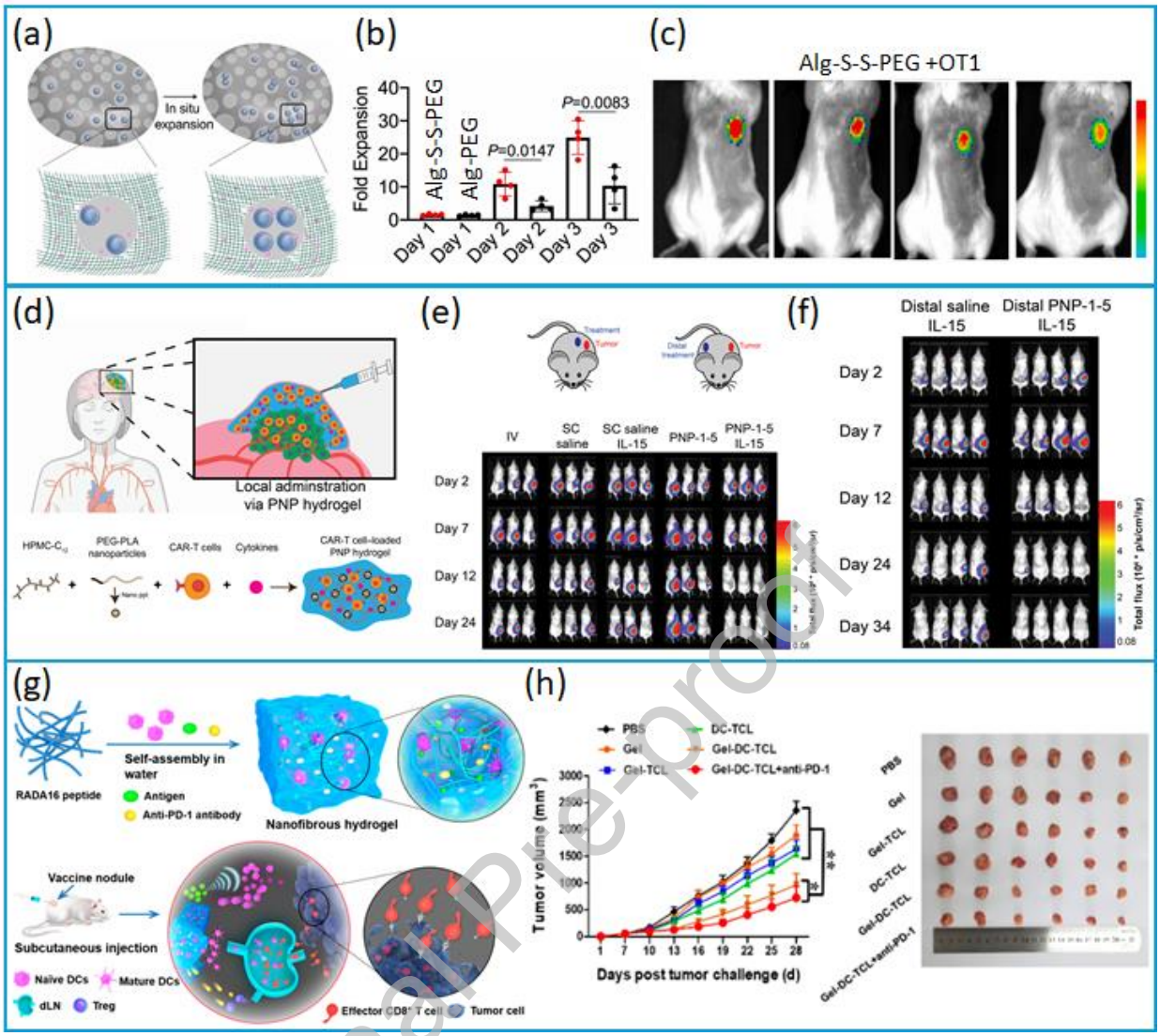

Figure 9. (a) Schematic illustration of PEG injectable hydrogels that enable controlled release of activator-bounded microparticles and *in situ* expansion of T cells; (b) Expansion of $CD8^+$ T cells in Alg-S-S-PEG and Alg-PEG gels three days; (c) IVIS images exhibiting the *in vivo* release of OT-1 cells from sky blue fluoroparticle-embedded in Alg-S-S-PEG hydrogel over ten days after injection. Adapted from Ref. [105], © 2023 with permission from Elsevier Ltd. (d) Scheme of CAR-T cell delivery to solid tumor using PNP hydrogels encapsulating CAR-T cells and stimulatory cytokines; (e) Luminescent images of tumor monitored over 24 days after hydrogel injection administered close to the tumor region with different delivery methods; (f) Luminescent images of tumor monitored over 34 days after hydrogel injection administered distant from the tumor region with different delivery methods. Adapted from Ref. [110], this article is distributed under a Creative Commons Attribution (CC-BY) license. (g) Working principle of the dendritic cell-based vaccine nodule engineered in the RADA16 peptide nanofibrous hydrogel; (h) Tumor size monitored over 28 days after cell inoculation. Adapted from Ref. [114], © 2018 with permission from American Chemical Society.

Beyond directly delivering immune cells, injectable hydrogels can also be designed as delivery platforms for immunomodulatory cues and cytokines to stimulate endogenous immune cells or to condition the tumor microenvironment for subsequent immune cell therapies. For example, He *et al*. [117] developed an injectable hydrogel microsphere-integrated training court that locally activates and amplifies tumor-infiltrating T lymphocytes (**Figure 10a**). GelMA microspheres were shaped through microfluidic and crosslinked with UV. Porosity was introduced via freeze-dried process thereby endowing microparticles with

increased surface area (**Figure 10b**), ideal for loading high densities of activators (anti-CD3 and anti-CD28 antibodies) and co-stimulatory cues (cytokines IL-7 and IL-15). When injected into osteosarcoma in mice, decorated microspheres significantly suppressed tumor growth by over 95% (**Figure 10c**) and increased $CD8^+$ and memory T cells phenotyping. However, while these results are promising, a potential limitation of this approach is the uniformity and reproducibility of microsphere fabrication, particularly regarding batch-to-batch variations in porosity and loading efficiency. This could lead to inconsistent therapeutic effects when scaled to clinical applications. Additionally, the long-term fate of the microspheres remains unclear, as uncontrolled degradation could alter the release profile of immunostimulatory factors, potentially reducing efficacy or causing unwanted immune activation.

Moreover, preconditioning the tumor site with these biomaterials can create a more favorable environment for the recruitment, activation and sustenance of adoptively transferred immune cells, such as NK cells or engineered T cells, boosting their persistence and therapeutic efficacy. In particular, NK cells play a crucial role in adaptive immunotherapy due to their ability to effectively target and eliminate tumor cells. However, their therapeutic efficacy is often limited by poor persistence, reduced infiltration into tumor sites, and an immunosuppressive tumor microenvironment. Injectable hydrogels functionalized with bioactive molecules—such as cytokines, chemokines, or nanoparticles—can enhance NK cell survival, activation, and retention at tumor sites, thereby drastically improving therapeutic outcomes. In this regard, Cheng *et al*. [118] developed an innovative injectable hemostatic gel, known as GODM-gel (**Figure 10d**), designed to prevent the recurrence of hepatocellular carcinoma after surgical resection by enhancing the effectiveness of adoptive NK cell therapy. Post-surgical recurrence remains a significant challenge in the treatment of hepatocellular carcinoma, largely due to the characteristics of the TME, which include high acidity and the presence of neutrophil extracellular traps. These factors suppress the activity of NK cells and create physical barriers that hinder their ability to eliminate residual cancer cells. The GODM-gel incorporates mesoporous bioactive glass nanoparticles (MBGNs) and deoxyribonuclease I (DNase I), and undergoes chemically triggered gelation upon administration through Schiff base formation. The MBGNs function as neutralizers of tumor acidity, reducing the infiltration of immunosuppressive cells such as regulatory T cells, M2 macrophages associated with tumors, and myeloid-derived suppressor cells. DNase I is incorporated into the gel to degrade neutrophil extracellular traps, breaking the barriers that surround cancer cells and enhancing the ability of NK cells to destroy them. Gel sensitivity to pH levels allows for the controlled release of DNase I in response to acidic conditions (**Figure 10e**), ensuring targeted and sustained therapeutic action. The gel significantly enhances the function and persistence of NK cells, increases the presence of tumor-infiltrating $CD8^+$ T cells, and improves survival rates in treated animals (**Figure 10f-h**). Despite these benefits, a critical concern is the potential off-target effects of DNase I, as systemic degradation of extracellular DNA structures could lead to unintended immune suppression or tissue damage [119]. Additionally, the tumor microenvironment is highly heterogeneous, and the ability of GODM-gel to uniformly modulate immune activity across different tumor sites and patient conditions remains uncertain.

A similar approach was adopted by Gong *et al*. [120] who developed a gelatin-based injectable hydrogel (GOSAM) designed to improve the efficacy of NK cell-based therapy using cells derived from umbilical cord blood to treat triple-negative breast cancer. Cancer stem cells in these tumors contribute to immune evasion by reducing the expression of ligands that activate natural killer cells and by degrading key immune proteins through elevated levels of autophagy, a process that enables tumor cells to survive immune attacks and resist conventional treatments [121]. GOSAM-gel incorporates two therapeutic agents: suberoylanilide hydroxamic acid, which epigenetically restores the expression of ligands that activate NK cells, and 3-methyladenine, which inhibits autophagy to preserve the activity of NK cells. These agents are loaded into MBGNs and encapsulated in the injectable matrix that forms a gel at the tumor resection site through Schiff base formation. The hydrogel is engineered to release these agents in response to the acidic environment of the tumor, ensuring localized, sustained delivery. Experimental results in mouse models demonstrated that the hydrogel significantly enhanced the therapeutic efficacy of NK cells by increasing cancer cell susceptibility to

immune attack and reducing tumor recurrence after surgery. Additionally, the hydrogel showed excellent biocompatibility, biodegradability, and hemostatic properties, aiding in surgical wound healing and minimizing bleeding. Its multifunctional design positions it as a valuable tool for post-surgical cancer therapy, providing both immune enhancement and recovery support. However, while the dual-function strategy is innovative, one potential limitation is the reliance on tumor acidity for drug release, which may vary among patients and tumor types, leading to inconsistent drug diffusion and immune stimulation. Furthermore, epigenetic modulation via suberoylanilide hydroxamic acid raises concerns about long-term genetic stability, as persistent alterations in gene expression could have unpredictable consequences beyond enhancing NK cell activity.

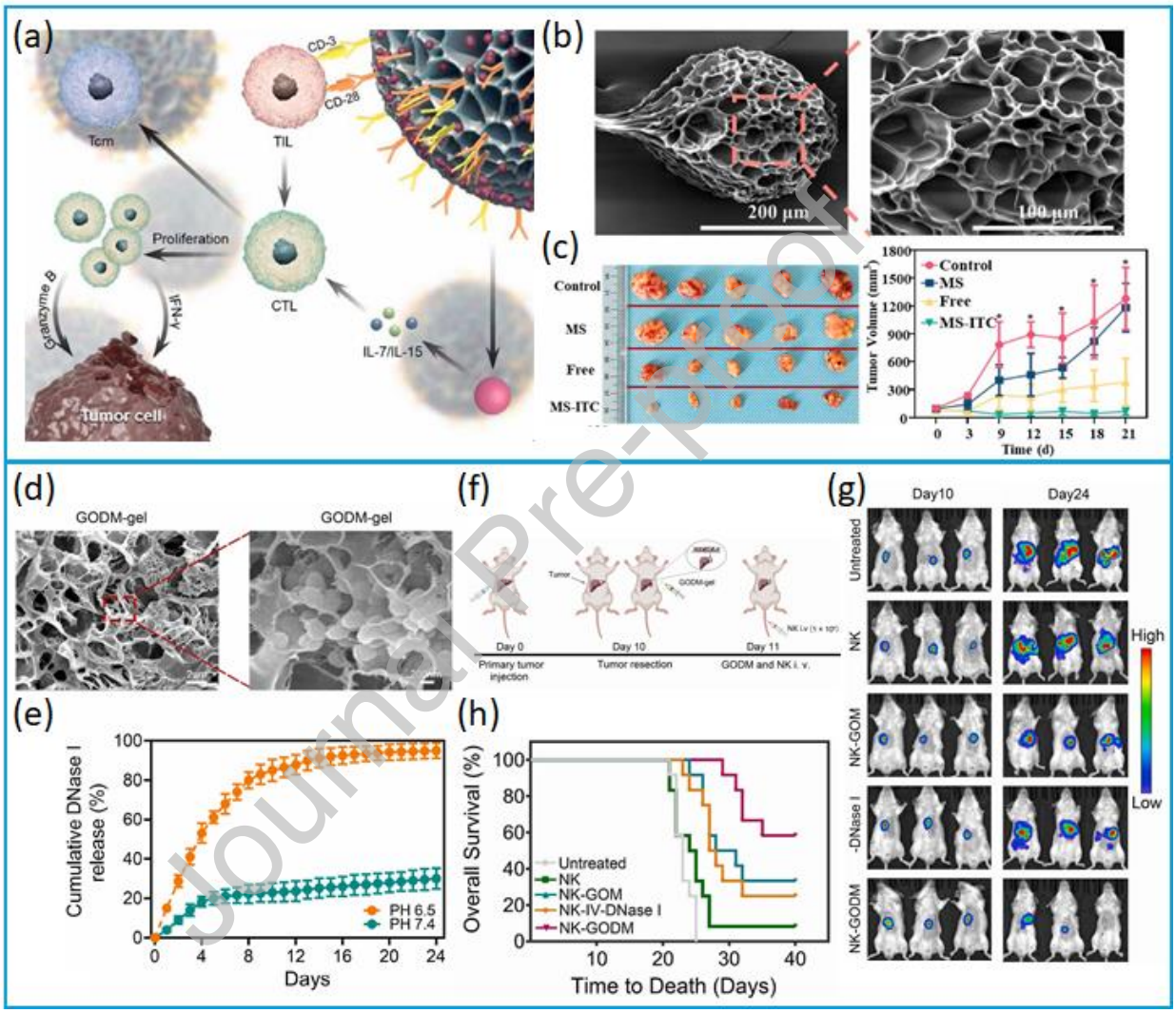

Figure 10. (a) Working principle of the injectable hydrogel microsphere-integrated training court; (b) SEM images of the GelMA microspheres; (c) Tumor size monitored over 21 days as a result of different treatments. Adapted from Ref. [117], © 2024 with permission from Elsevier Ltd. (d) SEM images of GODM-gel; (e) Release of FITC-labelled DNase I from GODM-gel in pH 7.4 and pH 6.5 PBS; (f) Scheme of the tumor treatment combing GODM-gel and adoptive NK cell transfer; (g) *In vivo* bioluminescence imaging of the recurrent tumors after liver resection; (h) Survival rate of mice treated with different treatments. Adapted from Ref. [118], © 2022 with permission from Elsevier Ltd.

In summary, injectable hydrogels can potentially represent a breakthrough in immunotherapy for cancer treatments thanks to the opportunity to minimally invasively deliver at the target site drugs and co-

stimulatory factors to enhance anti-tumor response. These hydrogels can be engineered to provide a supportive and localized matrix that not only sustains the immune cells but also facilitates their activation and proliferation within the body. The in-situ gelation of these hydrogels ensures precise conformation to tissue contours, optimizing the local immune environment. By incorporating bioactive molecules such as cytokines and antibodies, injectable hydrogels can potentiate the immune response at tumor sites, thereby improving the efficacy of cancer immunotherapies. Furthermore, the reduced need for surgical interventions enhances patient comfort and recovery, making this approach more patient-friendly. A comprehensive list of hydrogel-based strategies for *in vivo* immune cell expansion is reported in **Table 2**.

Table 2. Overview of injectable hydrogels designed to host immune cells and enhance their expansion *in vivo*.

Alg: alginate; PEG: polyethylene glycol; SCCB: silica colloidal crystal bead; GelMA: methacrylate gelatin; PBMC: peripheral blood mononuclear cells; CAR: chimeric antigen receptor; LDV: Leu-Asp-Val; RGD: Arg-Gly-Asp; PIC: polyisocyanopeptide; BMDC: bone-marrow-derived dendritic cell; OVA: ovalbumin; HPMC-C_{12}: dodecyl-modified hydroxypropyl methylcellulose; T_{EM}: effector memory T cells; T_{CM}: central memory T cells; T_{SCM}: T stem cell memory; MED8A: subcutaneous human medulloblastoma; GODM: gelatin-oxidized starch/DNase I @MBGN; MBGN: mesoporous bioactive glass nanoparticles; NK: natural killer; NET: neutrophil extracellular trap; GOSAM: gelatin-oxidized starch/SAHA@3MA @MBGN; SAHA: suberoylanilide hydroxamic acid; 3MA: 3-methyl adenine; UCB: umbilical cord blood; GZMB: granzyme B. *Data not explicitly given in the reference; value was retrieved from graphs shown in the reference.

Biomaterials	Fabrication method	Stiffness	Mesh size	Hosted cells	Activators	Loaded cells	Growth rate	Cytokines	Phenotype	Ref.
RGD-PIC	Thermal gelation at 37 °C	0.03-0.4 kPa	2-6 µm	Peripheral blood leukocytes Human pan T cells Pre-activated mouse $CD451^+$ T cells	Activation with anti-CD3/CD28 Dynabeads or, alternatively, with PHA/IL-6/IL-2	0.5-5×10^6 cells per mL	2-fold enhancement in cell proliferation and 4-fold enhancement in cell viability compared to 2D culture after 3 days*	Secretion of IFN-γ comparable among RGD-PIC, collagen, and 2D culture in vitro	No significant difference in cell differentiation compared to cells injected w/o gel	[101]
LDV-FEFK peptide	Self-assembly	0.5-3 kPa	n/a	PHCAR-T cells isolated from human PBMC and transfected with PD-1 and Her2 CAR lentivirus	Activation with anti-CD3/CD28 Dynabeads and co-stimulation with IL-2/IL-15	1×10^5 cells per mL	12-fold expansion after 3 days	Higher secretion of PD-1-blocking scFv, INF-γ and granzyme B in PHCAR T cells compared to HCAR T cells	Higher expression of $CD8^+IFN\text{-}\gamma^+$ T cells *in vivo* using PHCAR T cells-laden LDV-FEFK compared to HCAR T cells and PHCAR T cells w/o gel No significant difference in the percentage of CD4+Foxp3+ T cell using PHCAR T cells-laden LDV-FEFK compared to control experiments	[103]
Alg-S-S-PEG	Molding at -20 °C to cryogenically produce micropores	G' ≈ 3.6 kPa G" ≈ 0.2 kPa	≈50-190 µm	$CD8^+$ T cells and OT-1 cells sorted from spleens of mice	Anti-CD3/CD28 Dynabeads	5-30×10^4 cells per gel	≈25-fold expansion *in vitro* after 3 days* ≈3-fold expansion *in vivo* after 4 days*	Higher release of ICAM-1, IL-16, TNF-α, CXCL10, CCL2, IFN-γ *in vitro* for OT-1 cells loaded in gel	No significant difference in CD69 and CD40L expression *in vitro* compared to control experiment	[105]
PEG-PLA nanoparticle	HPMC-C_{12}-mediated	G' ≈ 10-	n/a	CAR-T cells isolated	Activation with anti-CD3/CD28	1×10^6 cells in	1.5-5.5-fold enhancement of cell	No significant difference	Higher ratio of $CD8^+/CD4^+$ T	[110]

	crosslinking	1000 Pa* G″ ≈ 3-200 Pa*		from human buffy coat and transfected with Antares-P2A-mNG and B7H3 CAR-P2A-Nluc	Dynabeads and co-stimulation with IL-2/IL-15	100 µL of gel	expansion *in vitro* after 8 days* 100-fold enhancement of cell expansion *in vivo* after 30 days	in TNFα, IL-6, IL-1β, GM-CSF, INFγ, IL-10 secretion *in vivo* after 3 days compared to untreated mice	cells *in vivo* after 10 days Smaller (higher) percentage of T_{EM} (T_{SCM}) *in vivo* after 10 days Higher (lower) expression of 41BB$^+$, CD39$^+$, (PD1$^+$) 10 days after treatment in MED8A tumor model	
RADA16 peptide	Self-assembly	G′ ≈ 1-100 kPa* G″ ≈ 0.01 kPa*	n/a	BMDC	Activation mediated by tumor antigens and anti-PD-1	2×10^6 cells in 200 µL of gel	Higher percentage of CD3$^+$CD8$^+$ T cells after 28 days	2-3-fold enhancement of IFN-γ secretion and 1-2-fold enhancement of OVA-specific splenocytes secretion	No significant difference in CD86 expression *in vitro* compared to 2D culture Higher expression of CD86$^+$CD11c$^+$ (≈5-fold)* and MHCII$^+$CD11c$^+$ (≈2-fold)* *in vivo* after 25 days	[114]
GelMA	Gel droplets shaped via shear forces, followed by UV curing and cryogenic formation of micropores	9.76 kPa	24.4 µm pores 214.5 µm spheres	CD3$^+$ T cells purified from mice PBMC	Activation with anti-CD3/CD28 and co-stimulation with IL-7/IL-15	1×10^6 cells per mL	13.75-fold expansion after 14 days	Higher release of TNF-α and IFN-γ compared to control experiment	Higher expression of CD69 and CD25, while lower expression of CD95, Tim3 and PD-1 both *in vitro* and *in vivo* when supplemented with IL-7/IL-15 Higher ratio of CD8$^+$/CD4$^+$ T cells *in vivo* while no significant difference *in vitro* Higher expression of both CD8$^+$CD44$^+$CD62L$^+$ and CD4$^+$CD44$^+$CD62L$^+$ T cells *in vivo*	[117]
GODM-gel	Molding at 37 °C	G′ ≈ 1-150 Pa* G″ ≈ 2-15 Pa*	1-5 µm* pores 100-200 nm spheres	Cell-free	MBGNs functionalized with DNase I	1×10^6 adoptive murine NK cells injected into	n/a	≈10-fold, ≈5-fold, and ≈4-fold enhancement of TNF-α, IFN-γ, and granzyme secretion, respectively	Enhanced CD8+ T cell-meditated antitumor immunity compared untreated control	[118]

						the mice		, compared to control*		
GOSAM-gel	Molding at 37 °C	G′ ≈ 2-60 Pa* G″ ≈ 10-20 Pa*	1-5 µm* pores 100-200 nm spheres	Cell-free	MBGNs functionalized with SAHA@3MA	5×10^6 human UCB-NK cells injected into the mice	n/a	No significant difference of TNF-α, IFN-γ, and GZMB secretion compared to untreated control	n/a	[120]

5. Implantable scaffolds

Compared to injectable hydrogels designed for systemic and localized tumor treatment, implantable hydrogels primarily serve as a postoperative strategy to prevent tumor relapse following surgical resection. Surgery remains a cornerstone in solid tumor treatment, but incomplete resection often leaves behind residual cancer cells that can lead to recurrence and metastasis. Implantable hydrogels address this challenge by providing a localized and sustained therapeutic effect directly at the resected site. These hydrogels can be engineered to encapsulate immune cells, such as CAR-T cells or DCs, to enhance the local immune response against residual tumor cells (**Figure 11**). Additionally, they can be loaded with chemotherapeutic drugs, immune checkpoint inhibitors, or cytokines to create a hostile microenvironment for cancer cell survival. Their tunable degradation profiles allow for controlled, long-term drug release, minimizing systemic toxicity while maximizing efficacy. By integrating both immunotherapy and chemotherapy, implantable hydrogels offer a promising approach to improving post-surgical outcomes and reducing the risk of recurrence in cancer patients.

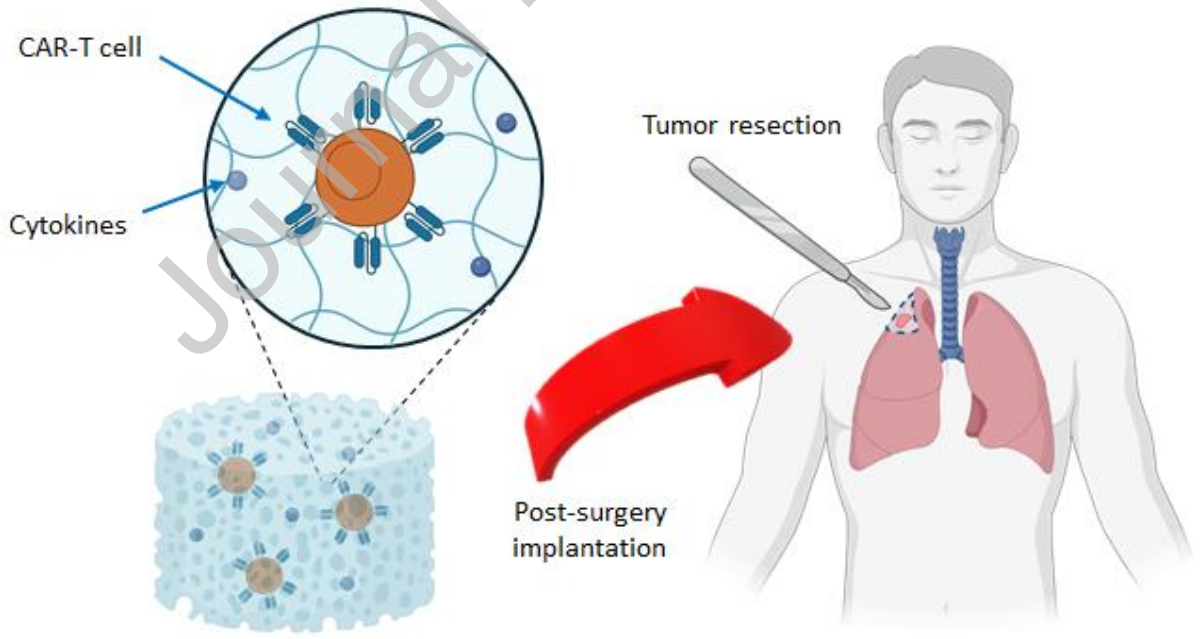

Figure 11. Example of implantable hydrogels loaded with CAR-T cells and cytokines to target remaining cancer cells and prevent relapse of surgically-resected tumors. Created with BioRender.com.

This approach has been further validated by recent studies demonstrating the effectiveness of implantable hydrogels in preventing tumor recurrence and enhancing systemic anti-tumor responses. For instance, Hu *et al*. [122] developed a HA-based hydrogel loaded with CAR-T cells and cytokines, which, when implanted into the tumor cavity after surgical resection of subcutaneous melanoma in mice, not only inhibited local tumor relapse but also reduced the growth of distant metastases. The HA hydrogel was engineered to deliver multiple therapeutic agents, including (i) CAR-T cells targeting melanoma-associated proteoglycans, (ii) PLGA nanoparticles carrying IL-15, and (iii) human platelets conjugated with anti-PDL1 blocking antibodies (**Figure 12a**). This multifunctional scaffold acts synergistically by targeting residual cancer cells with CAR-T cells and recruiting anti-PDL1-conjugated platelets via inflammatory response to suppress immune checkpoint signaling. Furthermore, the in-situ degradation of HA by hyaluronidase within 8 days ensures a controlled release profile, which highlights the potential of implantable hydrogels as a powerful post-surgical immunotherapeutic platform. CAR-T cells and aPDL1 antibody—covalently conjugated to human platelets—co-encapsulated in the hydrogel showed superior efficacy in suppressing melanoma growth *in vivo* (**Figure 12b**) and in promoting abscopal antitumor effects (**Figure 12c**).

The potential of implantable hydrogels in post-surgical cancer treatment is further reinforced by studies exploring different biomaterial scaffolds for immune cell delivery. Beyond HA-based hydrogels, fibrin gels have also been investigated as an effective delivery system for CAR-T cells in glioblastoma treatment. Fibrin gels, formed by combining fibrinogen, thrombin, and calcium, are naturally degradable, making them suitable for *in vivo* applications. For example, Ogunnaike *et al.* [123] demonstrated that macroporous fibrin gels (**Figure 12d**) embedding B7-H3–specific CAR-T cells successfully targeted glioblastoma resected from mouse brains (**Figure 12e**). Notably, 64% of mice treated with the fibrin-B7H3-CAR-T combination remained cancer-free after 3 months, a significant improvement compared to the 20% survival rate observed with direct intracavity administration (**Figure 12e**). However, the failure of fibrin gels with CD19-CAR-T cells to prevent tumor recurrence illustrates the necessity for precise biomaterial selection and tumor-specific targeting in achieving effective anti-tumor responses. Expanding beyond glioblastoma, fibrin gels have also been employed in the treatment of unresected triple-negative breast cancer. For instance, Uslu *et al*. [124] utilized fibrin gels to deliver mesothelin-specific CAR-T cells (CARM5) and found that their encapsulation within the gel enhanced tumor elimination and mice survival compared to direct administration in cell medium (**Figure 12g,h**). Although the survival benefit was significant, the occurrence of therapy-related deaths in some animals underlines the unresolved challenges of on-target, off-tumor toxicity, suggesting that improvements in the specificity of CAR-T cells or gel composition are needed for safer clinical applications.

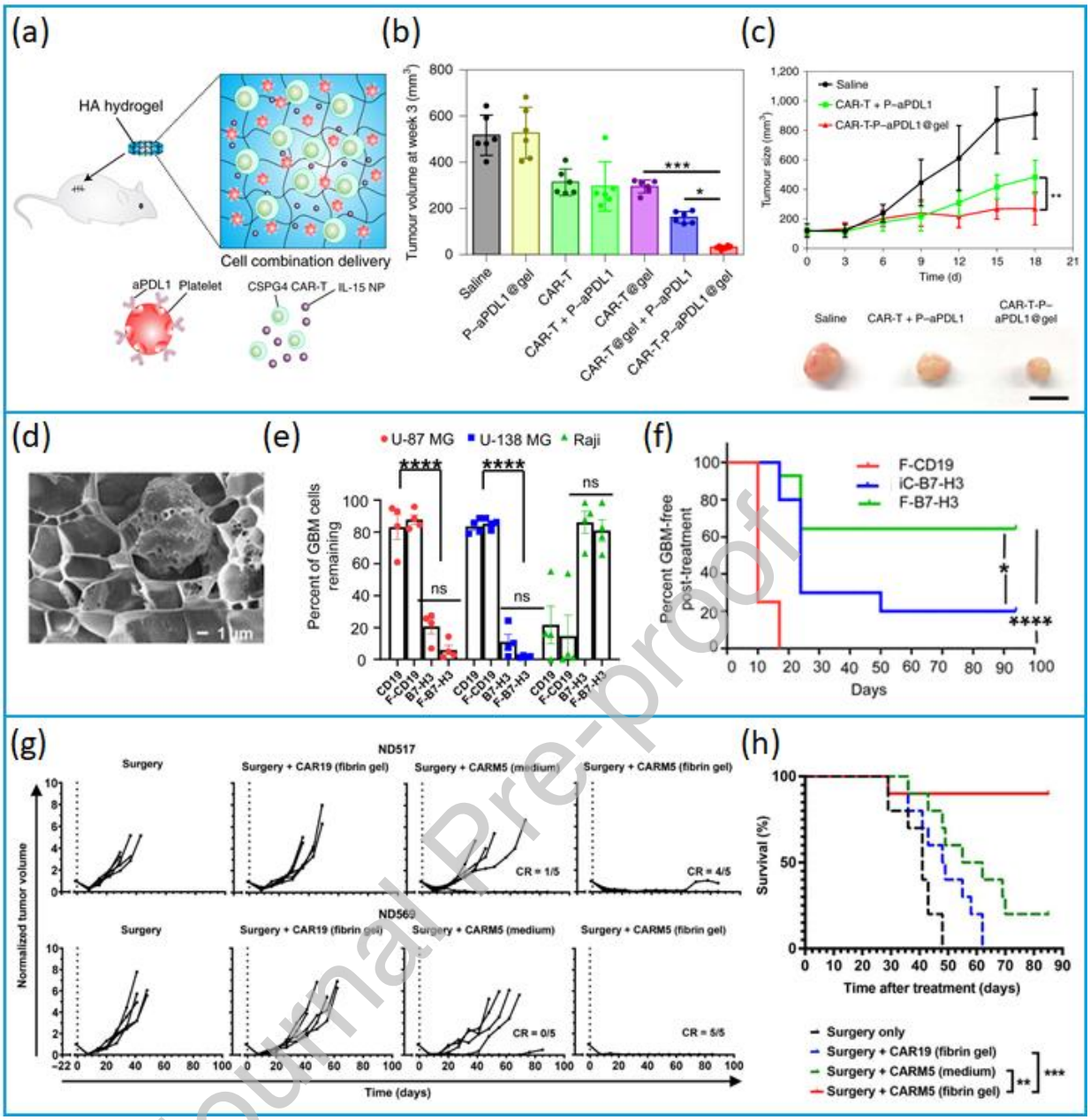

Figure 12. (a) Implantation scheme of the engineered HA hydrogel; (b) Tumor volume at week 3 after different treatments; (c) Tumor growth measured after treatment of the primary tumor (top panel) and representative pictures at day 18 (bottom panel, scale bar 1 cm). Adapted from Ref. [122], © The Authors, under exclusive license to Springer Nature Limited 2021. (d) SEM image of the fibrin gel (scale bars, 1 μm); (e) Percentage of remaining tumor cells after 5 days following tumor treatment with CAR-T cells and B7-H3 CAR-T cells growing in medium and F-CD19 CAR-T cells and F-B7-H3 CAR-T cells released from the fibrin gel; (f) survival curve of the treated mice. Adapted from Ref. [123], this article is distributed under a Creative Commons Attribution (CC-BY) license. (g) Changes in tumor volume over time after different treatment for both T cell donors (ND517 and ND569) (CR, complete remission); (h) Survival curve of treated mice. Adapted from Ref. [124], this article is distributed under a Creative Commons Attribution NonCommercial (CC-BY-NC) license.

Despite the promising preclinical outcomes of various hydrogel-based systems, the transition to clinical application remains challenging. Many hydrogels developed for *in vitro* studies have yet to be translated into human trials due to differences between *in vitro* and *in vivo* conditions, safety concerns, and potential side

effects. To address these limitations, research is increasingly focused on developing biomaterials that better mimic native tissue environments and enhance immune cell functionality. Stephan *et al*. [125] tackled this challenge by engineering an alginate scaffold containing collagen-modified peptide (CMP) to promote T cell proliferation and migration following implantation (**Figure 13a**). In-vitro studies demonstrated that CMP-coated scaffolds embedded in collagen hydrogels significantly improved T cell migration, increasing cell transit by 6.3 times compared to uncoated controls. *In vivo*, these alginate scaffolds facilitated robust T cell infiltration into peritumoral tissues and tumor-draining LNs following peritumoral implantation after tumor resection in mice (**Figure 13b,c**), underscoring their potential in post-surgical immune modulation. Building on this concept, Agarwalla *et al*. [126] introduced the so-called Multifunctional Alginate Scaffolds for T cell Engineering and Release (MASTER) system, designed for *in vivo* CAR-T cell generation and release (**Figure 13d,e**). By enabling the direct expansion and deployment of CAR-T cells within the body, MASTER significantly reduces the time and cost associated with traditional CAR-T cell therapy. In lymphoma-bearing mouse models, this system demonstrated superior CAR-T cell persistence and therapeutic efficacy compared to conventional approaches (**Figure 13f**), highlighting its potential for clinical translation. Yet, challenges related to the scalability and reproducibility of this system for human applications require further examination.

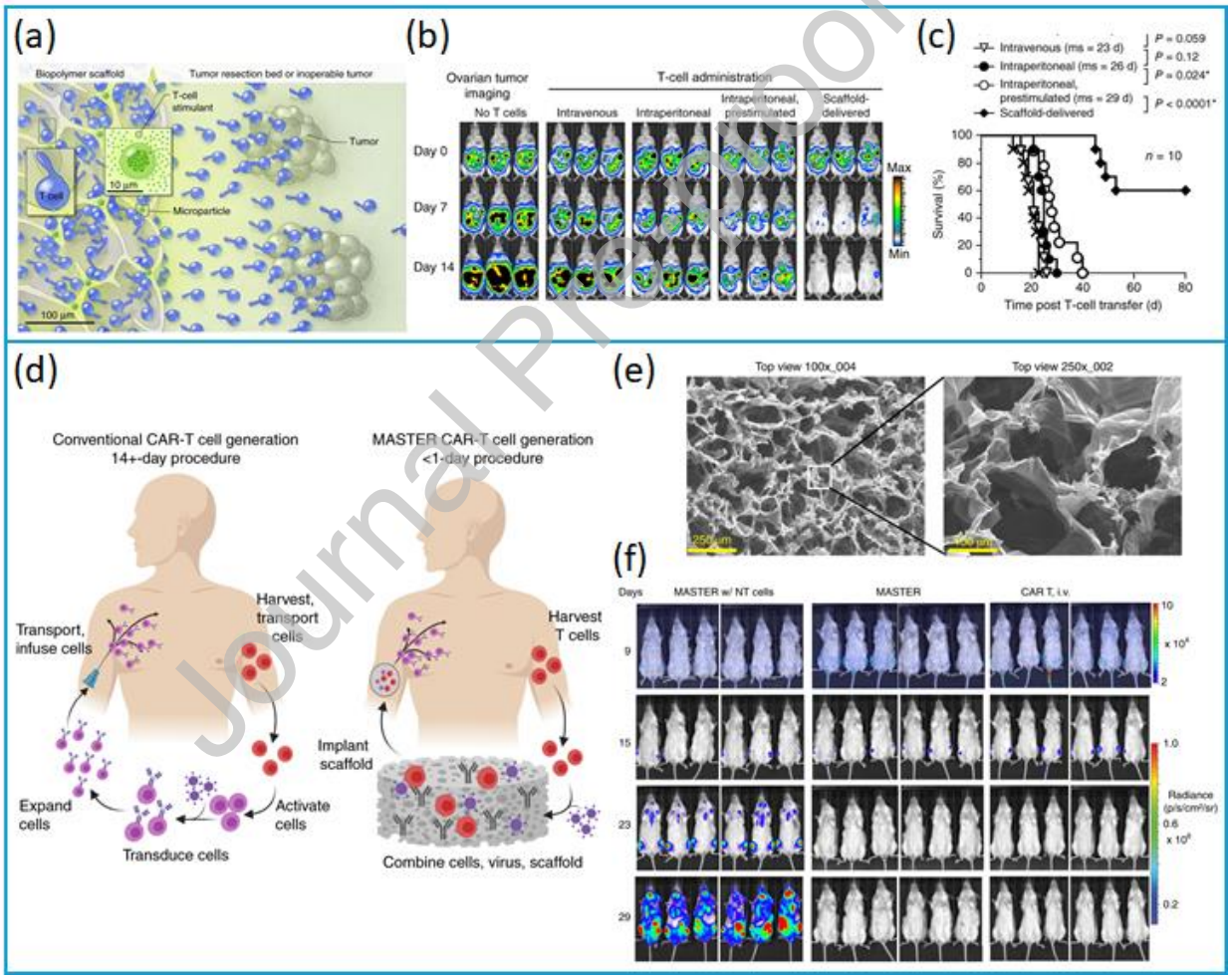

Figure 13. (a) Scheme of scaffold embedding T cells and stimulants surgically implanted at a tumor site. Stimulatory microspheres trigger cell expansion and promote their egress into surrounding tissue; (b) *In vivo* bioluminescence imaging of tumors over 14 days following different T cell administration modalities; (c) Survival curve of treated mice. Adapted from Ref. [125], © 2015 Nature America, Inc. All rights reserved. (d) Schematic comparison between conventional CAR-T cell therapy and MASTER-mediated CAR-T cell therapy; (e) SEM image of MASTER scaffold; (f) *In vivo* bioluminescence imaging of tumor treated with MASTER loaded

with non-transduced cells, MASTER loaded with CAR-T cells, and conventional CAR-T cells. Adapted from Ref. [126], © The Author(s), under exclusive license to Springer Nature America, Inc. 2022.

Beyond T cells, implantable hydrogels are also being explored for the expansion and activation of NK cells. In this regard, Kim *et al*. [127] encapsulated NK cells within a hydrogel matrix featuring interconnected micro- and macropores, optimizing the local microenvironment for immune cell survival and function. The hydrogel, composed of alginate and thermosensitive gelatin, allowed for controlled pore formation, enhancing NK cell clustering, nutrient exchange, and cytokine diffusion (**Figure 14a**). Functional assays revealed that NK cells within this hydrogel exhibited increased cytokine secretion and cytotoxic activity (**Figure 14b**), with enhanced tumor-killing capabilities (**Figure 14c**). Additionally, when applied to genetically modified CAR-NK cells, the system effectively targeted solid tumors, such as breast cancer, further demonstrating its adaptability across different immunotherapeutic strategies. However, while NK cells hold great promise, their clinical efficacy in hydrogel-based platforms is still uncertain due to variability in NK cell activity across different individuals. In contrast, Ahn *et al*. [128] employed a macroporous, biodegradable HA-based scaffold called 3D-ENHANCE, which fosters NK cell aggregation, proliferation, and activation without requiring external cytokine supplementation (**Figure 14d**). This scaffold offers a more straightforward solution that enhances NK cell viability and function through a simplified approach—facilitating the interaction between NK cells and the scaffold. Experimental results showed that NK cells expanded within this system exhibited superior viability, increased expression of cytotoxicity-related genes, and enhanced tumor-lytic activity compared to NK cells expanded in traditional 2D cultures (**Figure 14e,f**). Furthermore, the tunable degradability of the scaffold allows for controlled NK cell release, making it an ideal candidate for implantable cell reservoirs at surgical sites. However, the potential for off-target effects and tissue-specific immune responses remains an important consideration for clinical translation.

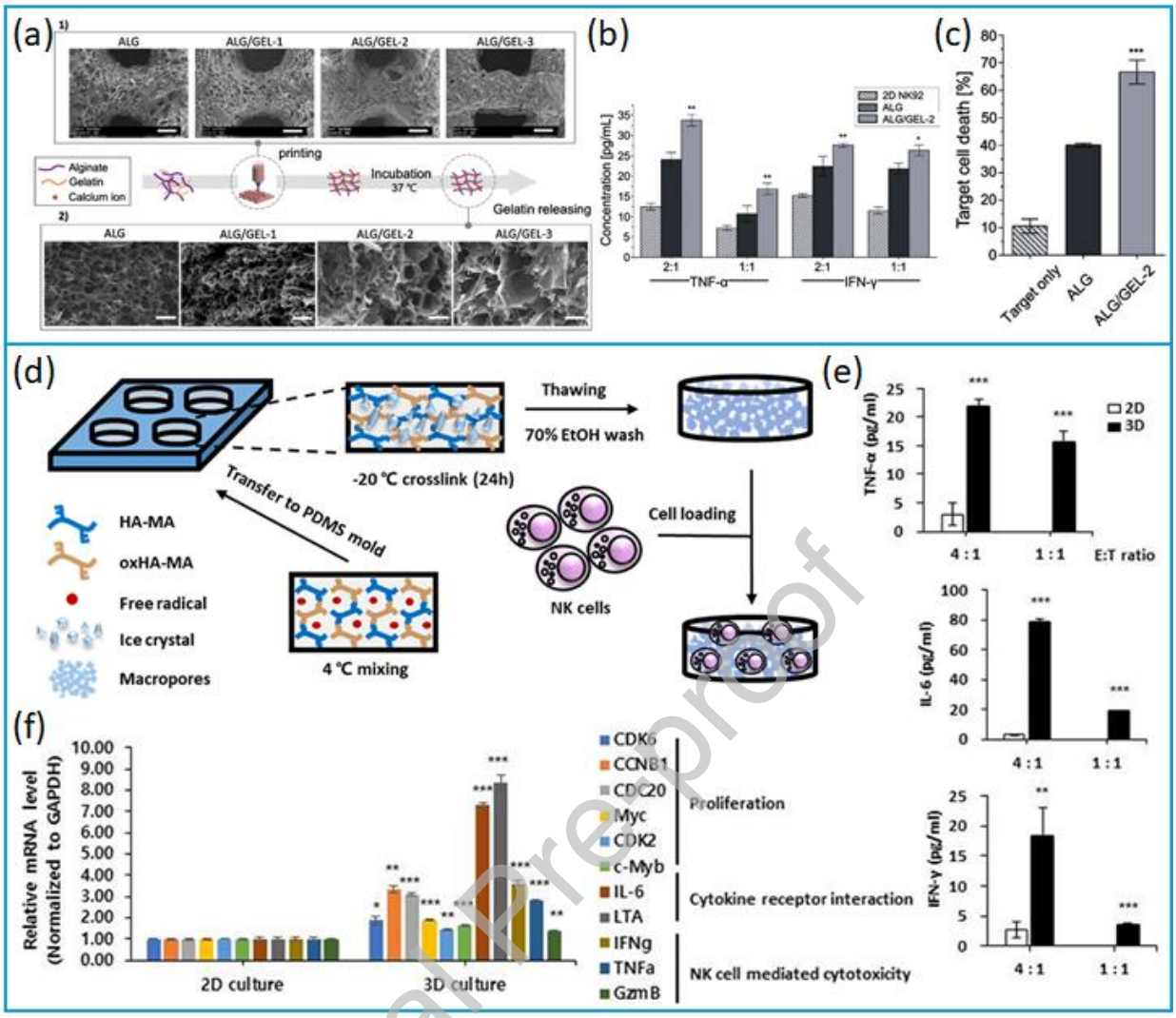

Figure 14. (a) SEM images of alginate and alginate-gelatin hydrogels immediately after printing (top panels, scale bar 500 µm) and after gelatin releasing (bottom panels, scale bar 100 µm); (b) Cytokine levels of TNF-α and IFN-γ from 2D and 3D cultured NK92 cells after 5 days at an effector-to-target ratios of 2:1 and 1:1. (c) Target cell death induced co-culturing with alginate and alginate-gelatin scaffolds loaded with CAR-NK cells. Adapted from Ref. [127], this article is distributed under a Creative Commons Attribution (CC-BY) license. (d) Scheme of 3D-ENHANCE fabrication and loading with NK cells; (e) Cytokine levels of TNF-α, IL-6 and IFN-γ from 2D and 3D cultured NK cells after 5 days at an effector-to-target ratios of 4:1 and 1:1; (f) mRNA levels measured by qRT-PCR after 5 days. Adapted from Ref. [128], © 2020 Elsevier Ltd. All rights reserved.

Together, these studies underscore the versatility of implantable hydrogels as platforms for immune cell expansion and localized therapy. By enabling the controlled release of CAR-T cells, NK cells, or immune-modulating factors, these biomaterials offer significant potential to improve post-surgical outcomes, minimize tumor recurrence, and enhance systemic anti-tumor responses. However, while their promise is clear, several challenges remain before their widespread clinical adoption. One key advantage of implantable hydrogels is their ability to create a localized immune-stimulatory niche, which not only supports immune cell survival and function but also reduces the systemic toxicity often associated with conventional immunotherapies. This targeted approach could enhance efficacy while mitigating adverse effects such as cytokine release syndrome or off-target immune activation.

Despite these benefits, the long-term fate of implantable hydrogels *in vivo* remains a critical concern. Issues such as degradation kinetics, potential fibrotic encapsulation, and variability in the immune response across different patients could impact their therapeutic consistency and reliability. Furthermore, while many hydrogels demonstrate biocompatibility in preclinical models, their interaction with the human immune system is complex and not yet fully understood. An overly aggressive immune response could lead to premature degradation or local inflammation, potentially compromising their intended function. Additionally, scalability and manufacturing consistency present practical hurdles, as translating these systems from small-scale research settings to clinical-grade production requires rigorous standardization and regulatory approval. Another significant limitation is the challenge of achieving precise control over immune cell activation and release. While current formulations offer passive release mechanisms—often dictated by hydrogel degradation or local enzymatic activity—more sophisticated, dynamically tunable systems are needed to respond to patient-specific conditions. Advances in bio-responsive or stimuli-responsive hydrogels could help bridge this gap, but their complexity may also increase regulatory hurdles. Continued interdisciplinary research combining materials science, immunology, and biomedical engineering will be crucial in transforming these promising platforms into viable therapeutic solutions.

A comprehensive list of implantable hydrogels for *in vivo* immune cell expansion and delivering of immune-modulating factors is reported in **Table 3**.

Table 3. Overview of implantable hydrogels designed to host immune cells and stimulating factor release? to reduce tumor relapse *in vivo*.

CAR: chimeric antigen receptor; MA: methacrylic anhydride; HA: hyaluronic acid; CSPG4: chondroitin sulfate proteoglycan 4; TNF: tumor necrosis factor; PBMC: peripheral blood mononuclear cells; Alg: alginate; Gel: gelatin; CSPG4: chondroitin sulfate proteoglycan 4; IL: interleukin; HA-MA: methacrylate-modified hyaluronic acid; GZMB: granzyme B. *Data not explicitly given in the reference; value was retrieved from graphs shown in the reference.

Biomaterials	Fabrication method	Stiffness	Mesh size	Hosted cells	Activators	Loaded cells	Scaffold degradation	Cytokines	Cell release	Ref.
MA-modified HA	UV curing	n/a	≈20-100 µm*	CSPG4-specific human CAR-T cells	IL-15	1-10×10^6 cells per mL	8 weeks	≈5-fold and ≈3-fold enhancement of IL-2 and IFN-γ secretion, respectively, compared CAR-T control*	5×10^4 cells per hour	[122]
Fibrin	Enzymatic reaction with thrombin	1.1-2.5 kPa	1-10 µm*	B7-H3-specific human CAR-T cells	*In vitro* activation with anti-CD3/CD28 and co-stimulation with IL-7/IL-15 prior to cell encapsulation into gel	1×10^6 cells per mL	2 weeks	*In vitro* release of IL-2 and IFN-γ in response to U-87 MG and U-138 MG tumor cells	1-4×10^5 cells per day*	[123]
Fibrin sealant (Tisseel)	Enzymatic reaction with thrombin	n/a	n/a	mesothelin-specific human CAR-T cells	*In vitro* activation with anti-CD3/CD28 Dynabeads and co-stimulation with IL-7/IL-15 prior to cell encapsulation into gel	1.5×10^6 cells per mL	n/a	≈2.5-fold, ≈10-fold and ≈100-fold enhancement of IL-2, TNF and IFN-γ, respectively, after 36 days compared to control*	5-30% of cells released after 24 hours*	[124]
Alg	Molding and $CaCl_2$-mediated crosslinking	n/a	100 µm*	mouse $CD8^+$ T and NKG2D-CAR-T cells	Activation with anti-CD3/CD28/CD137 and co-stimulation with Il-15/Il-15Rα	1.4×10^7 cells per mL	2 weeks*	No significant difference in IL-2, IFN-γ, TNF-α secretion compared to control experiment	1.4×10^7 cells per day*	[125]
Azide-modified alg	Molding and lyophilization	n/a	100-200 µm	Human PBMC with CD19.CAR-encoding gamma retrovirus	Activation with anti-CD3/CD28 and co-stimulation with IL-2	5×10^6 cells per mL	n/a	≈0.95-fold reduction and ≈1.2-fold enhancement of IL-2 and IFN-γ secretion, respectively, after 24 hours compared to spinoculation-generated CAR-T cells*	≈4×10^5 cells per day*	[126]
Alg-Gel	Molding, followed by $CaCl_2$-mediated crosslinking and thermal fusion of gelatin at 37 °C	G' ≈ 0.7-3 kPa* G" ≈ 0.1-0.4 kPa*	30.54-124.03 µm	Human NK92 cell line and human EGFR-specific CAR-NK cells	Pre-activated	2×10^6 cells per mL	5 days	≈1.81-fold and ≈2.28-fold enhancement of IFN-γ and TNF-α secretion, respectively ≈2.3-fold, ≈2.4-fold, ≈3.8-fold, ≈2.1-fold, ≈2.5-fold, ≈3.9-fold, ≈1.6-fold enhancement of CCNB1, CDC20, IL-6, LTA, IFN-γ, TNF-α, GZMB secretion, respectively, and ≈0.65-fold reduction of CDK6 secretion*	Cells released out by hydrogel degradation on day 5	[127]

HA-MA	Molding at -20 °C and thawing to cryogenically produce micropores	n/a	252.36 µm	Human NK92 cell line and human EGFR-specific CAR-NK cells	Pre-activated	0.1-1×10^6 cells per mL	4 weeks	Upregulation of CDK6, CCNB1, CDC20, Myc, CDK2, c-Myb, IL-6, LTA, IFN-γ, TNF-α, GZMB compared to 2D culture control	3-5×10^3 cells per day*	[128]

6. Conclusions and future perspectives

Integration of hydrogel-based strategies into immunotherapy represents a significant advancement in the field, offering smart solutions for both *in vivo* and *ex vivo* immune cell expansion. The inherent properties of hydrogels, such as their biomechanical tunability, biocompatibility, and ability to mimic the extracellular matrix, make them ideal for supporting growth, activation, and proliferation of immune cells. In particular, hydrogel-based strategy for *ex vivo* expansion provides a scalable and cost-effective method for generating large quantities of high-quality immune cells, essential for clinical applications such as adoptive cell therapy. By creating a biomimetic environment, hydrogels enhance cell viability and proliferation, ensuring the production of potent immune cell populations. Additionally, the versatility of hydrogels in functionalization allows for the precise tailoring of the microenvironment to meet the specific needs of different immune cell types, further optimizing therapeutic outcomes.

At the same time, hydrogels can be specifically designed for *in vivo* hardening the immune system and promoting immune cell expansion. For instance, injectable hydrogels offer a minimally invasive approach for delivering immune cells and/or bioactive molecules directly to the target site, enhancing the local anti-tumor response. This method not only reduces the need for surgical interventions but also improves patient comfort and recovery. The ability of injectable hydrogels to undergo in-situ gelation ensures precise adaptation to tissue conformation, creating an optimal environment for immune cell activation and expansion.

Despite these advantages, there are several limitations that need to be addressed to fully realize the potential of hydrogel-based immunotherapies. In the context of *ex vivo* expansion, challenges include variability in hydrogel formulations, batch-to-batch inconsistencies, and limited standardization of protocols, which may impact reproducibility and scalability for clinical applications. Additionally, some hydrogel matrices may not fully recapitulate the complex biochemical and mechanical cues of the natural immune niche, potentially affecting immune cell differentiation and function. Moreover, the cost and technical expertise required for optimizing hydrogel-based cell cultures remain significant barriers to widespread adoption.

For *in vivo* applications, one of the major hurdles is the immunogenicity and biodegradability of certain hydrogel formulations. While many hydrogels are designed to be biocompatible, some may still induce unintended immune responses or degrade unpredictably, leading to variations in therapeutic outcomes. Furthermore, the controlled release of cytokines, immune cells, or other bioactive factors remains a challenge, as diffusion rates and degradation kinetics can impact the sustained efficacy of the treatment. Another concern is the potential for hydrogel-based therapies to integrate with surrounding tissues in a way that complicates their removal or long-term monitoring. Addressing these issues will be critical to ensuring the safety, efficacy, and regulatory approval of hydrogel-based immunotherapies.

The constant technological progress and fine refinement of hydrogel-based immunotherapies hold great promise for advancing personalized medicine. Future research should focus on optimizing the design and functionality of hydrogels to further enhance their efficacy, reproducibility and safety. This includes exploring new materials and fabrication techniques to improve the mechanical properties, degradation rates, and bioactivity of hydrogels. Moreover, combining hydrogel-based approaches with emerging technologies such as gene editing, advanced imaging, and machine learning could lead to the creation of highly targeted therapies. For instance, integrating hydrogel systems with CRISPR/Cas9 technology could allow for precise genetic modifications of immune cells, enhancing their specificity and efficacy against tumor cells or pathogens. Additionally, the integration of phototherapies such as photodynamic and photothermal therapy could further boost the immune response. Indeed, recent studies have showed that phototherapy elicit a tumor-specific immune response and can be significantly enhanced using photosensitizing agent such as graphene oxide [129,130] or nanoparticles delivering photoreactive agents and immune modulators [131].

Ultimately, rigorous preclinical trials and clinical validation will be crucial to overcoming current limitations and translating hydrogel-based immunotherapies into widespread clinical practice. Future trials should aim

to refine treatment protocols, assess long-term safety, and optimize hydrogel compositions for different patient populations. By addressing these challenges, hydrogel-based strategies have the potential to revolutionize immune cell therapies, bringing more effective and accessible treatments to patients worldwide.

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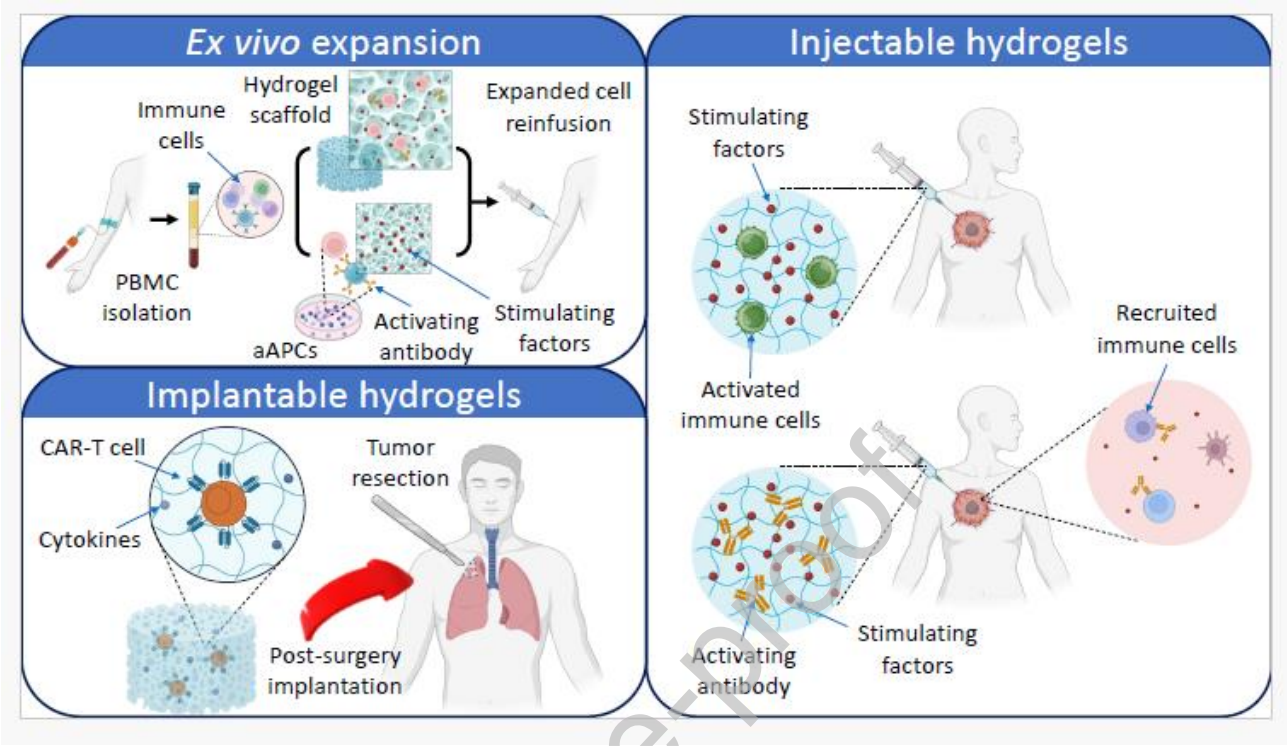
Statement of Significance

This review highlights the transformative potential of hydrogel-based 3D scaffolds in advancing personalized immunotherapy. By integrating in vivo and ex vivo strategies, hydrogels provide an innovative platform to enhance immune cell expansion, addressing critical challenges in immunotherapy. The discussion emphasizes the unique biomechanical and biochemical tunability of hydrogels, enabling precise mimicry of the extracellular matrix to support T cell proliferation, activation, and memory formation. These advances offer scalable, cost-effective solutions for producing high-quality immune cells, contributing to more effective cancer treatments, autoimmune disease management, and infectious disease control. By bridging materials science and immunology, this work underscores the pivotal role of hydrogels in shaping the future of immune-based therapies.

Graphical Abstract



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