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Review article

# Adaptive immunity of materials: Implications for tissue healing and regeneration

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ABSTRACT

Recent cumulative findings signify the adaptive immunity of materials as a key agenda in tissue healing that can improve regenerative events and outcomes. Modulating immune responses, mainly the recruitment and functions of T and B cells and their further interplay with innate immune cells (e.g., dendritic cells, macrophages) can be orchestrated by materials. For instance, decellularized matrices have been shown to promote muscle healing by inducing T helper 2 (Th2) cell immunity, while synthetic biopolymers exhibit differential effects on B cell responses and fibrosis compared decellularized matrices. We discuss the recent findings on how implantable materials instruct the adaptive immune events and the subsequent tissue healing process. In particular, we dissect the materials' physicochemical properties (shape, size, topology, degradation, rigidity, and matrix dynamic mechanics) to demonstrate the relations of these parameters with the adaptive immune responses *in vitro* and the underlying biological mechanisms. Furthermore, we present evidence of recent *in vivo* phenomena, including tissue healing, cancer progression, and fibrosis, wherein biomaterials potentially shape adaptive immune cell functions and *in vivo* outcomes. Our discussion will help understand the materials-regulated immunology events more deeply, and offer the design rationale of materials with tunable matrix properties for accelerated tissue repair and regeneration.

## 1. Introduction

The immune system plays a pivotal role in maintaining tissue homeostasis, combating diseases, and facilitating tissue repair and regeneration [1–3]. In addition to its primary function of defense against external pathogens and safeguarding the body, the immune system has the ability to sense, react to, and remodel biomaterials implanted within the body to enable the healing process [4–6]. Biomaterials have been designed to engage with intricate biological systems, ultimately to enable favorable outcomes in tissue functions. Therefore, the precise modulation of the immune system by implanted biomaterials is recognized as a pivotal determinant of the efficacy of treatments for a wide range of defects and diseases.

Both innate and adaptive immunity comprising the immune system interacts closely with each other. Innate immunity constitutes the immediate, non-specific defense mechanisms that are activated upon detection of antigens, including biomaterials, within the body. Conversely, adaptive immunity involves an antigen-specific immune response that mobilizes a specialized cohort of immune cells tailored to target the recognized antigen [7,8]. Each immune system consists of

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distinct cell types. Innate immune system comprises various cell types, such as polymorphonuclear cells (granulocytes, eosinophils, basophils), mast cell, phagocytic cells (neutrophils, dendritic cells (DCs), monocytes, and macrophages), and lymphocytes (natural killer cells, gamma-delta T cells and innate lymphoid cells) whereas the adaptive immunity includes antigen-specific T and B lymphocytes [9]. Especially, T and B lymphocytes are found throughout our body, including in the tonsils, spleen, lymph nodes, blood, and tissues containing biomaterials, and their distribution depends on the interactions with innate immune cells and direct activation, which can impact whole-body systems over a long-term period [10].

The immune system has an immense influence on the development and performance of biomaterials. Initially designed to be bioinert to minimize immune responses and avoid rejection [11], biomaterials have since evolved as the complexity of immune system became apparent. Modulating immune responses has thus been recognized to significantly influence the integration and performance of biomaterials. While immunological responses have traditionally focused on innate immune cells such as macrophages and occasionally neutrophils, there is an increasing recognition of the importance of adaptive immunity. Indeed, various physicochemical properties of biomaterials, including topography, surface chemistry, degradability, and stiffness, play crucial roles in directing macrophage polarization towards anti- or pro-inflammatory phenotypes, which in turn impact tissue repair and regeneration [12–16]. However, understanding the role of adaptive immunity is increasingly recognized as essential for predicting long-term compatibility and efficacy of biomaterials, particularly in individuals with specific immune diseases. Moreover, developing robust *in vitro* platforms capable of modeling the intricate interactions of the immune system *in vivo* is becoming increasingly imperative.

In this review, we discuss the implications of adaptive immunity in the context of biomaterials for tissue repair and regeneration following injury, trauma, or cancer. We commence with an overview of adaptive immunity and its interplay with innate immunity in tissue healing and regeneration process. We further outline how various subsets of adaptive immune cells (T cells ($CD4^+$, $CD8^+$, Th17, or $Foxp3^+$ Treg (T regulatory cells)), and B cells) actively respond under the influence of biomaterials. Subsequently, we illuminate the potential impact of physicochemical properties of biomaterials (size, shape, topography, stiffness, degradation, and dynamic mechanics) on adaptive immunity, based on the current exciting *in vitro* and *in vivo* findings. This discussion highlights the intricate dynamics of materials-regulated immunology events, shedding light on the design rationale behind biomaterials endowed with tunable matrix properties for tissue healing and regeneration.

## 2. Overview of adaptive immunity and its interplay with innate immunity in tissue regeneration process involved with biomaterials

In this section, we focus on the fundamental immune response in the tissue healing process that occurs upon implantation of biomaterial (as depicted in Figs. 1 and 2). We consider two theoretical scenarios: the use of i) non-degradable, sterile biomaterials (Fig. 1) and ii) degradable biomaterials (Fig. 2). This distinction is important because the sources initiating immune responses and the predominant type of immunity (innate vs. adaptive) may vary depending on the degradability of the biomaterials. Through this analysis, we aim to gain comprehensive insights into the adaptive immune responses triggered by biomaterials and their implications for effective tissue healing and regeneration.

Upon the implantation of biomaterials in the body, the immune response is initiated by the first line of defense composed of immune cells, such as neutrophils, macrophages, and dendritic cells. These cells detect the presence of biomaterials and attempt to engulf them. Depending on the degradability of the biomaterials, two types of immune responses are postulated: i) an innate immune response or ii) a complex immune response involving both innate and adaptive immunity.

The innate immune response is a non-specific reaction to foreign substances, where immune cells mount a generalized defense

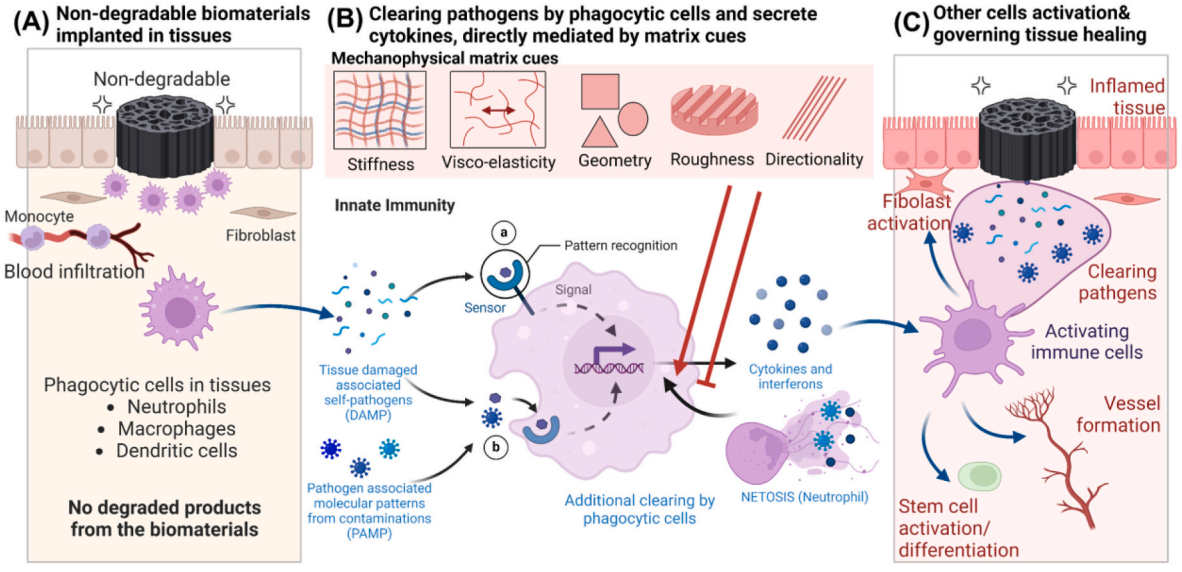


**Fig. 1.** Impact of non-degradable biomaterials on immunity for tissue healing. (A) Following the implantation of biomaterials in tissues, phagocytic cells, including neutrophils, macrophages, and resident dendritic cells, encounter pathogens and initiate the innate immune response. The focus here is on external sources from non-degradable biomaterials as initiators of innate immunity because innate immunity is dominant than adaptive immunity in this scenario. **(B)** Phagocytic cells efficiently clear pathogens and secrete cytokines, which can be directly influenced by the chemo-mechanical cues provided by the biomaterials. Factors such as surface chemistry, geometry, topography, and matrix mechanics play crucial roles in modulating the cytokine response. Additionally, specific receptors on cell membranes or endosomes recognize DAMPs or PAMPs resulting from surgical contamination or biomaterial synthesis. Neutrophil NETosis, characterized by the formation of net-like structures comprising decondensed chromatin and proteases, aids in pathogen clearance. **(C)** The secretion of cytokines by phagocytic cells can activate other cells in the vicinity of the biomaterials, influencing tissue healing. These include fibroblasts, stem cells, and blood-forming cells, which play crucial roles in the regenerative processes.

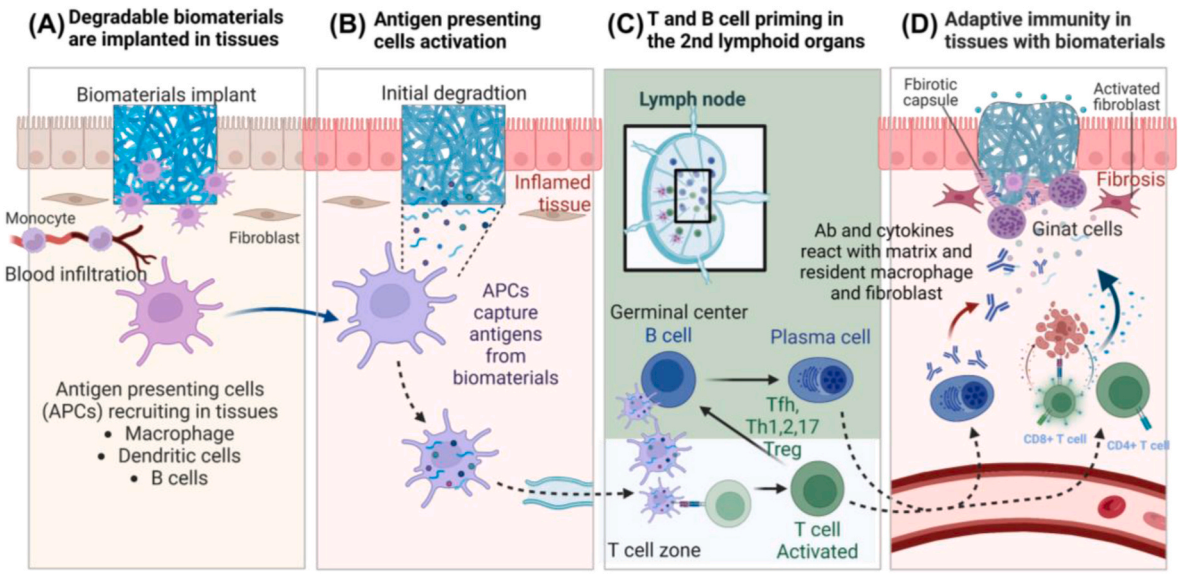


**Fig. 2. Impact of Implanted Degradable Biomaterials on Adaptive Immunity for Tissue Healing. (A)** Upon implantation of biomaterials into tissues, resident dendritic cells and tissue phagocytic cells such as neutrophils and macrophages are recruited to the site. **(B)** When these cells encounter pathogens or degraded components from the biomaterials, they engulf them, and some of these components are presented on their surface through the Major Histocompatibility Complex (MHC) structure. These antigen-presenting cells (APCs) then migrate to the nearby lymph nodes. **(C)** In the lymph nodes, T cells and B cells are activated by the antigens presented by the APCs. The activated T cells differentiate into various subtypes including Th1, Th2, Th17, Treg, and Tfh cells, while the activated B cells differentiate into plasma cells that produce antibodies. **(D)** The activated B cells, plasma cells, and T cells (including $CD4^+$ helper T cells such as Th1, Th2, Th17, and Treg, and $CD8^+$ cytotoxic T cells) begin to migrate towards the implanted biomaterials and initiate adaptive immune responses. The antibodies and cytokines released by these cells interact with the extracellular matrix, resident macrophages, and fibroblasts, leading to the formation of a fibrotic capsule and the presence of multinucleated giant cells, characteristic features of the immune response to implanted biomaterials.

mechanism against the biomaterials [17,18]. On the other hand, the complex immune response involves a coordinated interplay between innate and adaptive immunity, resulting in a more specific and targeted reaction to the components of each biomaterial [19,20]. In the complex immune response, innate immune cells recognize the biomaterials as foreign entities and initiate an immediate response. Concurrently, they communicate with adaptive immune cells, such as T cells and B cells, which possess the ability to recognize specific antigens presented by the biomaterials. This interplay between innate and adaptive immunity results in a precisely orchestrated immune response against the various components of biomaterials.

The nature of the immune response is influenced by multiple factors of biomaterials, including the composition, structure, degradability, and mechanics. Understanding these immune processes is crucial for designing biomaterials that can effectively interact with the immune system and ultimately induce desired tissue healing outcomes.

### 2.1. *Immune responses related with non-degradable biomaterials*

Theoretically, non-degradable biomaterials that remain intact without generating nano- or micro-sized debris in body fluid, encounter various immune cells, including dendritic cells, macrophages resident in the tissue, and neutrophils derived from the bloodstream, within a few hours. Recruited macrophages join within days. However, these cells do not function as antigen-presenting cells (APCs) to deliver biomaterial component antigen signals to T cells. Some examples of clinically available non-degradable biomaterials with excellent biocompatibility include titanium (titanium oxide), zirconia, alumina, gold or gold alloys, silicone (polydimethylsiloxane), polyacrylamide, and polyether ether ketone [21,22]. In this scenario, the predominant immune response is governed by neutrophils and macrophages as part of the innate immune system. This innate immune response triggers both pro-inflammatory (peaking at ~3 days) and anti-inflammatory (occurring after ~3 days) reactions, characterized by the release of specific cytokines (IL-1β, TNFα, and IL-6 for pro-inflammatory, and IL-4, IL-10, and IL-13 for anti-inflammatory) and growth factors (such as TGF-β, VEGF, PDGF, among others). The specific immune response is influenced by the chemo-mechanical cues of the biomaterials, including surface chemistry, topography, and stiffness.

In contrast, the role of T and B cells in mediating adaptive immunity related with biomaterials is yet to be deeply investigated. While the biomaterial components may not directly contribute to the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), surgical conditions can trigger the presence of unavoidable DAMPs and PAMPs due to NETosis (neutrophil extracellular trap release), cell death in tissues, or floating bacteria in the air. Furthermore, unintentional remnants of external DNA/RNA or pathogen invasion during manufacturing or handling, even under surgically aseptic conditions, cannot be entirely ruled out. These conditions can induce adaptive immunity to some extent following the innate immune response, but do not typically induce severe adaptive immunity, including antigen presentation. Hence, this section considers a theoretically aseptic immune response primarily induced by innate immunity when non-degradable biomaterials are implanted without the presence of DAMPs and PAMPs.

In the early stages after biomaterial implantation, neutrophils rapidly and non-specifically respond within hours. They play a crucial role in eliminating potential pathogens and debris associated with the biomaterials through processes like phagocytosis, release of enzymatic contents (myeloperoxidase, neutrophil elastase, cathepsin G), and the formation of neutrophil extracellular traps (NETs). Neutrophils are the most abundant myeloid leukocytes, constituting 50–70 % of all white blood cells, and are integral to the body's innate immune response against infection and inflammation. NETosis is particularly effective in trapping and neutralizing non-self-pathogens, including bacteria, fungi, protozoa, viruses, and their components such as lipopolysaccharides (LPS). Moreover, NETosis can be triggered in the absence of pathogens during tissue healing and regeneration, facilitated by activated platelets, cytokines, chemokines (such as IL-8 and TNF), precipitated microcrystals, sudden increases in intracellular calcium, and various physiological

stimuli.

Recent reviews have detailed the relationships between NETosis and chemo-mechanical factors in sterile biomaterials [23,24]. For instance, neutrophils can directly sense the stiffness of polydimethylsiloxane (PDMS) substrates in the range of 0.2–32 kPa. Stiffer substrates induced increased NETosis, dependent on focal adhesion kinase (FAK) activity, and higher secretion of pro-inflammatory cytokines and chemokines [25]. Hydrophilicity also influences NETosis, with hydrophilic micro-structured titanium surface (~2 GPa) preventing NET formation compared to hydrophobic counterpart or smooth surface [26].

However, the studies on interactions between neutrophils and biomaterials are still in their infancy mainly regarding the biomaterial parameters, such as nano/micro-scale topography, concave/convex geometry, 3-dimensionality, and confined area. Exploring these aspects is necessary to gain a comprehensive understanding of how neutrophils interact with implanted biomaterials, and ultimately to provide design strategy for biomaterials in tissue repair [27]. In addition to neutrophils, resident dendritic cells (DCs) present in tissues contribute to the recruitment of circulating monocytes to the site of injury by secreting specific chemokines, leading to a second wave of innate immune cell response. However, the initiation of an antigen-specific immune response mediated by antigen-presenting neutrophils and DCs, which can bridge the innate and adaptive immunity, has had limited studies related with non-degradable biomaterials.

Following neutrophil recruitment, infiltrating monocytes from the blood accumulate in the vicinity of the biomaterial implantation site. These monocytes differentiate into highly phagocytic macrophages that play a crucial role in engulfing dying neutrophils and biomaterial debris to facilitate clearance. Moreover, macrophages secrete soluble factors that contribute to the progression of the innate immune response and tissue healing in conjunction with biomaterials [28]. During the early stages of the inflammatory cascade (~3 days), macrophages release pro-inflammatory factors that coordinate and support the clearing response. When the foreign body or dead tissue is resolved, pro-inflammatory macrophages undergo a phenotypic transition towards an anti-inflammatory state that promotes tissue repair and ultimately leads to the resolution of inflammation (~14 days). When biomaterials are non-degradable, they persist within the tissue and continuously impact the inflammatory and resolution phage [29]. Consequently, the direct interaction between macrophages and the chemo-mechanical cues of the biomaterials governs the overall status of inflammation, tissue repair, and/or resolution.

Adaptive immune cells also modulate innate immune responses by directly sensing the biomaterials. B cells, typically recognized as key APCs in adaptive immunity, can sense biomaterials and secrete cytokines without requiring T cell activation. However, the magnitude and nature of the cytokine response differ from the functional B cells activated by $CD4^+$ T cells [24]. In fact, experimental findings on the precise role of B cells in response to non-degradable biomaterials are largely limited. Yet, a recent study has demonstrated the direct response of synthetic biopolymer (polycaprolactone (PCL)) to naïve B cells (fresh B cells before being activated by T cells or biomaterials) [30]. Interestingly, PCL implantation was found to modulate the functional response of B cells, including their activation and duration, in a manner distinct from biodegradable decellularized extracellular matrix (dECM) counterpart [31]. This suggests the possibility of non-degradable biomaterials directly influencing the function of B cells without T cell activation. One potential mechanism for activating B cells involves the activation of Toll-like receptors (TLRs) or B cell receptors (BCRs) in conjunction with T cell activation, triggered by the biomaterial cues [32]. Further research is needed to elucidate the specific roles of adaptive immune cells in the interaction with non-degradable biomaterials to gain a comprehensive understanding of the biomaterial-host interactions *in vivo*.

Collectively, neutrophils, macrophages, and DCs within tissues play crucial roles as first-line innate responders, governing the immune response to non-degradable biomaterials. However, unlike adaptive immunity, these cells lack the ability to identify specific pathogens or develop memory for defending against subsequent invasions. Instead, they rely on the recognition of molecular patterns (DAMPs or PAMPs), through receptors. These receptors can be potentially activated by chemo-mechanical cues from the biomaterials themselves, as well as by self-damaged cells. As a result, when faced with a similar invasion in different locations or at different times, these innate immune cells respond in a consistent manner, lacking the adaptability seen in adaptive immunity. However, recent endeavors start to unravel the functional response of adaptive immune cells (e.g., B cell) to non-degradable biomaterials, highlighting the potential for B cell-mediated memory-dependent innate immune responses. This aspect will be explored in more detail in the following sections.

### 2.2. Degradable biomaterials and the actions of antigen presenting cells

The majority of biomaterials exhibit a certain degree of degradability over time, prompting initial interactions with innate immune cells that engulf biomaterial components and trigger an inflammatory response similar to the aforementioned innate immunity. However, a notable distinction arises when immune cells function as antigen-presenting cells (APCs). These APCs recognize and internalize danger signals originating from biomaterials, such as debris or enzymatic byproducts, then subsequently present them to the adaptive immune system. The process of adaptive immunity can be broadly divided into three stages: i) recognition of antigens derived from biomaterials by APCs, ii) delivery of antigen signals to T cells and B cells, and iii) activation and proliferation of T and B cells to facilitate adaptive immune responses (as illustrated in Fig. 2A–D).

#### 2.2.1. Recognition of biomaterials and immune process by antigen presenting cells

The recognition of biomaterial components by APCs has recently been evidenced, where FITC fluorescent-labeled degradable hydrogels were implanted in a subcutaneous tissue and the related cell-biomaterial interactions were investigated [33]. Indeed, infiltrating immune cells were found to progressively degrade and engulf the hydrogels over a 21-day period, and the internalization peaked at between days 7 and 14, indicating an increasing uptake of the hydrogel by the infiltrating cells at the implantation site. Of note, the predominant cell populations infiltrating the degradable hydrogel scaffolds were macrophages and DCs, particularly Langerhans cells, accounting for approximately 75–90 % of the infiltrating cells. These cells acted as APCs, responsible for initiating and directing the adaptive immune response. Following the uptake of debris or byproducts from the biomaterials, the APCs migrate to secondary lymphoid organs, including the draining lymph nodes and the spleen. Within these organs, they present peptide/molecule-major histocompatibility class II (MHC II) complexes directly to antigen-specific T cells, thereby activating the adaptive immune response. Experimental evidence confirmed an increase in the population of $CD11c^+$ APCs that internalized FITC-labeled hydrogels in the draining lymph nodes and spleen.

In degradable biomaterials, specific degraded products can be categorized as either DAMPs or PAMPs, depending on the inherent composition of the raw materials. For instance, DNA/RNA can function as DAMPs, while LPS/peptidoglycan can serve as PAMPs [34]. Furthermore, even under sterile conditions, certain biomaterials such as hyaluronan and fibrinogen, albeit lacking microbial or nucleic acid components, are known to activate DAMP signaling. Additionally, molecules like heat shock proteins, chromatin-associated protein high-mobility group box 1, DNA/RNA, biglycan, or heparan sulfate, when released by stressed or damaged cells during biomaterial implantation, can induce DAMP or PAMP-associated inflammation [5]. It remains an area of interest to explore whether the physical structure of these molecules directly engages specific pattern recognition receptors

(e.g., TLR1-10) on APCs, as DAMPs or PAMPs. Once tissue-resident or recruited immune cells capture and recognize DAMP and PAMP-associated molecules, they trigger the secretion of inflammatory factors, thereby recruiting additional immune cells to the site of action. These immune cells deliver potential biomaterial-derived antigen signals to T and B cells, consequently compounding adaptive immune responses.

#### 2.2.2. Delivery of biomaterial-derived antigen signals to T and B cells for innate immunity activation

Following the migration of APCs to secondary lymphoid organs, the presentation of biomaterial-derived antigen signals to T and B cells is crucial for the initiation of adaptive immunity. This process begins with the delivery of biomaterial-derived antigen signals. It is well-established that both macrophages and DCs are highly effective APCs due to their robust expression of MHC II. This provides antigen signals to $CD4^+$ T cells (helper T cells), activating them to mount an immune response. Along with this, macrophages and DCs express other co-stimulatory molecules, such as CD80 and CD86, which are essential for efficient T cell activation. On the other hand, B cells typically exhibit lower levels of MHC II expression on their surface, limiting their ability to effectively present antigens to T cells. To upregulate MHC II expression, B cells rely on the assistance of other immune cells, such as DCs or activated T cells, unlike macrophages and DCs [35]. Therefore, due to their robust phagocytic and superior antigen-presenting ability, macrophages and DCs are considered the primary APCs for delivering biomaterial-derived antigen signals to adaptive immune cells.

T cells are central in adaptive immunity, and various subsets of T helper cells engage in tissue healing and regeneration processes. There are five primary subtypes of $CD4^+$ T helper (Th) cells: Th1, Th2, Th17, Treg, and T follicular helper (Tfh). Each subtype exhibits distinct responses to biomaterials [36,37]. Th1 cells produce inflammatory cytokines, such as IFN-γ, IL-2, and TNF-α, and are primarily involved in defending against intracellular viruses and bacteria. Consequently, their interaction with biomaterials elicits a pro-inflammatory response. In contrast, Th2 cells secrete regenerative cytokines, such as IL-4, IL-5, and IL-13, and play a crucial role in defending against extracellular parasites and certain bacteria. Their interaction with biomaterials promotes tissue repair and regenerative process. On the other hand, Th17 cells produce cytokines like IL-17, IL-21, and IL-22, which contribute to inflammation and tissue repair in a context-dependent manner. Treg cells regulate the overall adaptive immune response. They suppress excessive immune reactions, prevent autoimmunity by inhibiting tissue-damaging Th1 and Th17 cells, and produce cytokines such as IL-10 and TGF-β to enhance Th2 cell function. Consequently, modulating Treg cells has recently been considered a key to determining the tissue-regenerative outcomes associated with biomaterials [38,39]. T follicular helper (Tfh) cells play a pivotal role in orchestrating humoral immune responses by migrating to B cell follicles, secreting IL-21 and IL-4, and facilitating B cell maturation within the germinal center reaction.

The differentiation and function of various T helper cells are regulated by a complex interplay of cytokines and other signals within the immune system, and can be influenced by a variety of physical and biochemical factors presented by biomaterials. Assessing T cell immune responses over extended *in vivo* periods (approximately 21 days) serves as a crucial indicator for successful biomaterials. A typical type 1 (Th1) foreign body granuloma, characterized by a central core of macrophages and foreign material, surrounded by fibroblasts, myofibroblasts, and lymphocytes, is associated with a robust Th1/M1 immune response, often accompanied by the presence of multinucleated giant cells in inflammatory biomaterials. On the other hand, a type II (Th2) foreign body reaction, observed mostly in bio-inert and compatible biomaterials, does not typically exhibit foreign body giant cells [32].

In terms of MHC I presentation by APCs, $CD8^+$ T cells (cytotoxic T cells) recognize the molecule-MHC I complex, which triggers their activation to target and attack molecules potentially derived from biomaterials. Upon activation and differentiation into cytotoxic T cells, they secrete pro-inflammatory cytokines, such as IFN-γ, TNF-α, and IL-2. These cytokines not only induce the specific elimination of target cells, displaying a particular antigenic complex (molecule-MHC I) recognized by their T cell receptors, but also nonspecifically lyse unrelated bystander target cells. Importantly, the secretion of these cytokines activates and stimulates other immune cells, thereby promoting inflammation. Consequently, it can be hypothesized that $CD8^+$ T cells are recruited to the site of implantation following activation by molecules presented by APCs. They contribute to the foreign body reaction through the production of pro-inflammatory cytokines, elimination of cells carrying specific molecules, and unintentional lysis of neighboring host cells. In this scenario, blocking the activation of $CD8^+$ T cells theoretically holds the potential to reduce the foreign body reaction, improve tissue integration of implanted biomaterials, and subsequently enhance regeneration [40].

B cells are activated when they recognize biomaterial molecules through their surface receptors, aided by APCs. Concurrently, helper T cells can enhance B cell activation through co-receptor engagement. Following activation, B cells undergo proliferation and differentiation into plasma cells, which secrete antigen-specific antibodies, or memory B cells that provide long-term protection against future encounters with the same molecules. In certain conditions, biomaterial molecules can directly link the B cell receptor, resulting in a T-cell independent B cell response producing IgG3. This type of response typically generates low-affinity, short-lived antibodies. In contrast, the T-cell dependent B cell immune response, involving production of IgG1 or IgG2a, occurs with the assistance of T cells. The secreted antibodies bind to specific antigens, a process known as immunorecognition. This binding facilitates opsonization by phagocytic cells like macrophages and neutrophils, or activates the complement system to form membrane attack complexes. B cell reactions can be assessed by monitoring the increase in total serum IgG concentration, as IgG is the most abundant class of antibodies produced by plasma cells, which are differentiated cells derived from activated B cells. Recent studies have reported a substantial elevation of IgG levels above baseline on days 4 and 7 after scaffold implantation, indicating an immediate systemic B cell-related response [33].

The specific subtype of IgG produced in response to biomaterials varies, and in mice, there are four distinct subtypes: IgG1, IgG2a, IgG2b, and IgG3. Each subtype possesses unique structural characteristics, functions, and distribution within the body, playing different roles in the immune response. IgG1 is the most abundant subtype in mice and is primarily involved in defense against viral pathogens, along with IgG3. IgG2a and IgG2b, on the other hand, contribute to defense against bacterial pathogens. IgG1 is typically associated with a Th2 immune response and is effective in opsonization (marking pathogens for phagocytosis) through the complement system. In contrast, IgG2a and IgG2b are associated with a Th1 immune response and are particularly involved in mediating antibody-dependent cellular cytotoxicity. While IgG3 production is generally linked to a Th2-type immune response, it can also be induced by Th1-type cytokines such as IFN-γ, displaying a complex and context-dependent response. In summary, IgG1 is typically associated with a Th2 “tissue repair” type response against extracellular biomaterials in the local microenvironment, while IgG2a is associated with a Th1 “foreign body” response against molecules taken up intracellularly [41].

Metal ions released from degradable metals and ceramics hold potential for modulating adaptive immune responses, which is beneficial for their therapeutic applications in tissue regeneration [42–45]. For instance, zinc ions have been shown to enhance the proliferation and differentiation of Treg while suppressing the development of Th17 cells by attenuating STAT3 activation, thereby promoting tolerance in uncontrolled autoimmune reactions [46,47]. Similarly, calcium ions and their receptors have been observed to facilitate the activation and maturation of T cells [48,49]. Moreover, silver ions have been demonstrated to modulate the balance between Th1 and Th2 responses

depending on the concentrations [50]. Given the influence of these metal ions on various immune cell behaviors, further research is needed to deepen our understanding of how these therapeutic ions interact with adaptive immunity.

For understanding the immune response to degradable biomaterials, the discrepancies between *in vitro* and *in vivo* immune responses cannot be ignored. While *in vitro* studies provide valuable insights with simple models, they often fail to capture the full spectrum of immune interactions observed in living systems with multiple immune cells. A striking example of this is seen in the degradation behavior of peptide-based hydrogels [51]. *In vitro*, D-amino acid-containing peptide cross-linkers (D-MAP) show minimal degradation (~5 %) when exposed to collagenase I, in contrast to their L-amino acid counterpart (L-MAP), which degrades rapidly (~100 % degradation within 30 days). However, when applied to *in vivo* wound models, D-MAP hydrogel unexpectedly exhibits enhanced degradation rates compared to L-MAP hydrogel, with minimal persistence after 21 days. This result underscores the presence of additional factors in the *in vivo* environment, such as diverse enzyme populations, cellular interactions including innate and adaptive immune cells, and complex signaling cascades, which can significantly alter biomaterial degradation kinetics and immune response [52,53]. The observation emphasizes the importance of comprehensive *in vivo* studies to fully understand and predict the immune and tissue regenerative performance of degradable biomaterials for clinical applications.

## 3. Impact of matrix chemo-mechanical properties on adaptive immune cells: insights from *in vitro* studies

The chemo-mechanical cues of biomaterials, encompassing ligand type and density, nano/microtopography, dimensionality (2D/3D), anisotropy, stiffness, and viscoelasticity, have significant impacts not only on innate immune cells but also on adaptive immune cells (Fig. 3A). Recent research has been dedicated to unraveling the mechanisms through which adaptive immune cells (mostly T cells) respond to these matrix cues, facilitated by precisely tailored biomaterial platforms. In this part, we scrutinize pertinent *in vitro* studies to gain insights into the intricate interplay between adaptive immune cells and these matrix cues. The effects of chemo-mechanical cues of matrices on adaptive immunity is briefly summarized in Table 1. The *in vitro* studies will guide design principles of biomaterials to finely modulate the adaptive immunity *in vivo*. Despite the increasing understanding of how matrix chemo-mechanical properties influence T cell behavior, the effects of these factors on B cells have been largely overlooked. Exploring the impacts of matrix properties, such as ligand type and density, nano/microtopography, dimensionality, anisotropy, stiffness, and viscoelasticity, on B cell activation, differentiation, and function through well-designed *in vitro* studies is essential in the future.

### 3.1. Importance of ligand type and density

The composition and density of extracellular matrix (ECM) ligands can profoundly influence adaptive immune cell responses including T cell activation and subsequent functions [54]. *In vivo*, the type and amount of ECM proteins vary across tissues, impacting local T cell development, migration, and activation. Pan T cells are activated by cytokines (*i.e.,* IL-2), while $CD4^+$ helper and $CD8^+$ cytotoxic T cells are activated through binding of T cell receptors (TCR) to peptide-MHC (pMHC) on APCs. During recirculation, initially activated T lymphocytes move through different microenvironments and interact with ECM proteins via integrin receptors [55]. Simultaneously, the intensity of T cell activation is governed by costimulatory signals arising from integrin binding to ECM ligands [56,57]. Different tissues display distinct ECM proteins (e.g., collagen, fibronectin, laminin) depending on the site, age, and microenvironment that interact with T cell integrins in a ligand-specific manner [58,59]. Furthermore, the density of ECM ligands regulates T cell activation [60]. Sparse ligands may be insufficient for T cell binding to ECM and enhancing intracellular tension, while excessive ligand density can overwhelm TCRs, impeding proper activation.

For example, T cells freshly isolated from the interstitial ECM of tissue express a distinct subset of functionally active integrins that

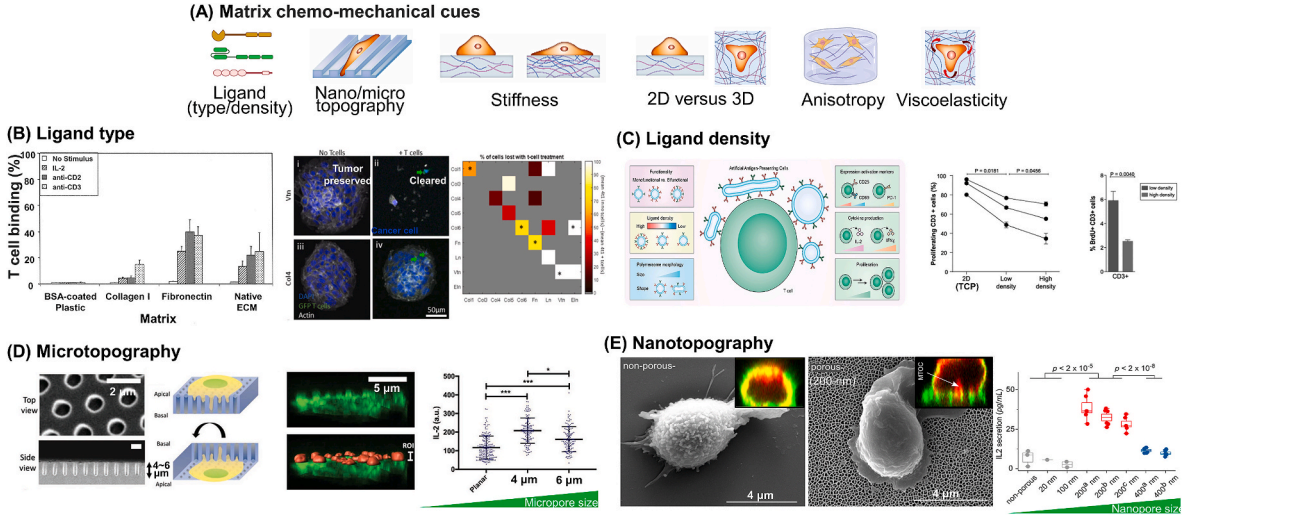


**Fig. 3.** Impact of matrix chemo-mechanical cues (particularly ligand type/density and nano/microtopography) on adaptive immune cells. (**A**) Summary of key matrix chemo-mechanical cues, including ligand type/density, nano/micro-topography, stiffness, 3D architecture, anisotropy, and viscoelasticity. (**B**) Effect of matrix ligand type on T cell binding and tumor cell clearance. T cell adhesive interactions differ depending on ligand identity and chemical stimuli (left). Reproduced with permission [58]. Copyright 2003, The American Association of Immunologists, Inc. Tumor cell clearance by T cells depends on matrix ligand (right) Reproduced with permission [60]. Copyright 2022, Elsevier Ltd. (**C**) Influence of ligand density on T cell activation. Increased ligand density generally enhances T cell activation in a topography-dependent manner (left) Reproduced with permission [76]. Copyright 2022 The Authors, Published by American Chemical Society. However, excess ECM ligands can impair T cell proliferation (right). Reproduced with permission [63]. Copyright 2019 BMJ Publishing Group Ltd & Society for Immunotherapy of Cancer. (**D**) Micro-sized substrate pits stimulate higher IL-2 secretion from T cells compared to flat surfaces, correlated with T cell microvilli projection into pits. Reproduced with permission [79]. Copyright 2020 John Wiley & Sons, Inc. (**E**) Matrix nano-topography modulates T cell activation with 200 nm features eliciting maximal IL-2 production, among different nano-pore sizes examined. Reproduced with permission [81]. Copyright 2021, National Academy of Science.

**Table 1**
Summary of the effect of biomaterials and mechanical cues on adaptive immune cell functions *in vitro*. M: *Mus musculus*, mouse; R: *Rattus norvegicus*, rat; H: Homo sapiens, human.

| Refs | Species | Immune cell | Matrix and cellular chemo-mechanical cues (chapter) | Biological functions influenced by adaptive immune cells |
|---|---|---|---|---|
| [58] | H | Primary T cell lamina propria T cell (LPT) and peripheral blood T cell (PBT) | ECM coating (collagen I, fibronectin, naïve ECM) (3.1) | LPT: binding collagen and fibronectin ↑ |
| [60] | M | Primary T cell | ECM composition (3.1) | T cell clearance: collagen 4 or 6 ↓ fibronectin or vitronectin ↑ |
| [76] | H | Primary T cell | Topology and ligand density of artificial antigen presenting cells (3.1 and 3.2) | High ligand density → T cell activation ↑ Low ligand density → shape and size more important (Large tubular polymersome) |
| [63] | H | Primary T cell | 3D collagen density ↑ (3.1) | T cell proliferation ↓ $CD4^+$ cell ratio ↑ |
| [79] | M | Primary $CD4^+$ | PDMS micropit (3.1 and 3.2) | 4 μm micropit IL-2, IFN-γ ↑ |
| [81] | H | Primary T cell | Pore size (20–400 nm) (anodic aluminum oxide, polycarbonate, polystyrene) (3.1 and 3.2) | Pore size of 200 nm IL-2 secretion ↑ T cell activation ↑ |
| [61] | H | Primary T cell | galectin-1 ↑ (3.1) | T cell adhesion ↓ TNF-α, IFN-γ ↓ |
| [70] | M + H | EL-4 Primary $CD8^+$ | Synapse topography (3.2) | Positive curvature Perforin ↑ |
| [71] | M | Primary $CD4^+$ | TCR ligand (3.2) | ICAM-1 ligand → IL-2↑ |
| [72] | M | Primary $CD4^+$ | Micropattern (3.2) | Activation site distance → asymmetric division |
| [73] | M | Primary $CD4^+$ | Micropattern (3.2) | Anti-CD3 and anti-CD28 pattern → IL-2 ↑ |
| [74] | M | Primary $CD4^+$ | Magnetic microfluidics (3.2) | Micropatterned APC and magnet → T cell activation ↑ |
| [75] | M | Primary T cell | Artificial antigen presenting platform (3.2) | Antigen presenting on PLGA polymer → Ex-vivo T cell expansion ↑ |
| [77] | M + H | B16–F10, T2, B3Z Primary T cells | APC-mimic scaffold (3.2) | Anti-CD3, anti-CD28 and IL-2 APC-mimic scaffold → 2–10 times polyclonal expansion |
| [84] | H | Primary $CD4^+$ | 3D culture (Dynabead or Matrigel) (3.3) | 3D culture → T cell proliferation ↑ |
| [82] | M | Primary $CD4^+$ | 3D, ECM stiffness ↑ APC stiffness ↑ (3.3 and 3.4) | T cell activation ↑ Proliferation ↑ Pro-inflammatory cytokines ↑ Migration ↑ |
| [85] | M + H | Primary $CD8^+$ | Hyaluronic acid-based hydrogel (3.3) | pMHC and antiCD28 signaling, stiffness ↓ → T cell expansion ↑ |
| [86] | H | Primary $CD4^+$ | CCL21-loaded 3D PEG hydrogel (3.3) | T cell proliferation ↑ |
| [87] | M | Primary T cells | DLP-based 3D bioprinting (3.3) | Stiff matrix → viability↓, IL-2↓ |
| [94] | H | Jurkat | Anisotropic Janus particle (3.3) | Anti-CD3, fibronectin bull's eye particle T cell activation↑ |
| [95] | M | Primary $CD8^+$ | Anisotropic PLGA aAPC (3.3) | T cell activation ↑ Anticancer activity ↑ |
| [96] | M | Primary $CD8^+$ | Shape of aAPC (3.3) | T cell activation ellipsoidal > spherical |
| [97] | M | Primary $CD8^+$ Primary $CD4^+$ | APC stiffness↑ (3.4) | $CD8^+$ degranulation↑ $CD4^+$ activation↑ |
| [107] | M | Primary naïve B cell | ECM stiffness ↑ APC stiffness ↑ (3.4) | Affinity discrimination ↑ |
| [106] | M + H | Primary $CD8^+$ | Tumor cell stiffness↑ (3.4) | Cytotoxicity on tumor cells↑ |
| [113] | H | Primary $CD8^+$ Primary $CD4^+$ Jurkat | Fluid shear stress↑ | T cell activation ↑ TNF-α, IL-2, and IFN-γ ↑ |
| [104] | M | Primary $CD4^+$ | ECM stiffness ↑ (3.4) | Metabolism ↑ Proliferation ↑ T cell response ↑ |
| [103] | H | Primary $CD4^+$ | ECM stiffness ↑ APC stiffness ↑ (3.4) | Glycolytic activity ↑ Pro-inflammatory cytokines ↑ Proliferation ↑ Migration ↑ |

contribute to enhanced adhesion to purified collagen, fibronectin, and cell-derived ECM compared to peripheral blood T cells, indicating ECM microenvironment instructs newly arrived T cells for further activation (Fig. 3B) [58]. Basal membrane proteins, predominantly laminin, exhibit a dampening effect on T cell activation, particularly noticeable in cytotoxic $CD8^+$ T cells, ultimately promoting tumor growth [59]. A parallel exploration of ECM components elucidated a subtype-specific cytotoxic T cell response against cancer, with collagen subtypes (collagen 1, 3, 4, 5, 6) and fibronectin, laminin, and vitronectin revealing distinctive effects. Collagen 4 and 6 induced negligible (~0 %) T cell clearance of cancer cells, while fibronectin or vitronectin achieved nearly 100 % clearance (Fig. 3B) [60].

Also, to manipulate ECM-dependent T cell attachment and activation, various binding proteins were employed. Galectin-1, a family of beta-galactoside-binding proteins, was shown to inhibit IL-2 induced T-cell adhesion to intact ECM, laminin and fibronectin, and to a lesser extent to collagen type IV, in a dose-dependent manner. Concurrently, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) production was markedly reduced with galectin-1, recapitulating T cell activation depending on the cell binding affinity to ECM [61]. Another report revealed tuning integrin ligands of α5β1 and αvβ3 could modulate the activation of human T lymphocyte. Three RGD-disintegrins (Flavoridin, Kistrin and Echistatin) derived from snake venoms interact with integrins on human T lymphocyte surface and synergistically activate T cells via FAK phosphorylation-actin polymerization-PI3K pathway, a conventional mechanotransduction pathway [62]. In summary, the composition of ECM ligands within a tissue can profoundly influence T cell adhesion, activation, and cellular function, particularly in the context of clearance against specific targets, such as antigens.

On the other hand, the ECM density-dependent T cell activation was relatively less explored, particularly when isolating from other biophysical cues, such as ECM stiffness and viscoelasticity. The availability of pMHC complexes to the TCR cluster is a critical factor in activating T cells. In parallel, the density of anti-CD3 antibodies closely correlates with T cell activation, with maximal response achieved at a density of 316 molecules/$\mu m^2$ (with a 60 nm interparticle distance) and a discernible effect observed at approximately 59 molecules/$\mu m^2$ (at a

150 nm distance) (Fig. 3C) [63,64]. Regarding ECM density, it is postulated that inadequate ECM ligand density may hinder robust T cell adhesion necessary for proper activation, while an excess of ECM ligands can impede T cell mobility and synapse formation with APCs. Achieving an optimal balance in ECM ligand type and density is crucial for preserving T cell homeostasis and favoring implantable biomaterials.

When designing biomaterials for applications like vaccines or implantable devices, careful tuning of ECM ligand composition and density is imperative to induce appropriate T cell responses without triggering uncontrolled inflammation [65,66]. *In vitro* studies utilizing tailored ligand presentations have provided insights into how matrix biochemistry modulates adaptive immune cell function, offering guidance for the design of immunomodulatory biomaterials [67]. The precise engineering of ECM ligand presentation in terms of type, density, and conformation enables the modulation of $CD4^+$ helper and $CD8^+$ cytotoxic T cell responses to align with specific application requirements [68].

### 3.2. Nano/microscale topographies and geometrical confinement

When adaptive immune cells including T cells infiltrate tissues, their behavior is intricately shaped by the nano/microtopology of the ECM, including the nano-/micro-sized collagen fibers and ligament structures, as well as geometric constraints imposed by the basement membrane and the surfaces of rigid tissues such as bone and cartilage [69]. Additionally, the tissue matrix geometry, particularly in regions where T cells and APCs interact, plays a crucial role in influencing T cell activation by spatially exposing APCs' ligands. Hence, understanding the effects of nano/microscale topographies and geometrical confinement is pivotal in adaptive immunity.

To examine the geometric impact on the immunological synapse, microscale physical patterns of activating sites were engineered. When T cells were seeded on polydimethylsiloxane (PDMS) with various micro-sized circle patterns (diameters ranging from 10 to 20 μm) coated with anti-CD45, a reduction in T cell stiffness – a marker of T cell activation by cytoskeletal force – was observed compared to a non-patterned flat surface, irrespective of anti-CD3-induced T cell activation [68,70]. Furthermore, to explore the influence of the spatial location of activating sites, full, multi-focal, or donut-shaped contacts coated with anti-CD3 were fabricated [71]. It was observed that on full contact, T cells interacted stably with activation sites, underwent proliferation, and secreted cytokines. Conversely, T cells on multi-focal or annular-shaped contacts, preventing centralized clustering of TCR ligands, failed to form stable contacts with activation sites, exhibited aberrant PKC-θ clustering, and significantly reduced production of IFN-γ. A similar study also revealed distinct incidences of asymmetric T cell division, a key process generating cell fate diversity during immune activation, based on the spatial location of activation sites [72]. The incidence of asymmetric T cell division varied by altering the diameters (4–10 μm) of the activation sites (co-coated with anti-CD3 and anti-CD28) and the distance (15–25 μm) between them.

To further understand the spatial effect of activation sites, colocalization or segregation of anti-CD3 and anti-CD28 (with diameters of 1–2 μm) was imprinted by micropatterning on a flat glass surface. This demonstrated that positioning anti-CD28 at the cell periphery (segregation), surrounding an anti-CD3 feature, enhanced IL-2 secretion compared to having these signals combined in the center with colocalization [73]. When $CD4^+$ $CD25^+$ Tregs were analyzed on the same colocalization or segregation micropatterned platform, reduced adhesion ability and actin cluster formation, but enhanced adherent area through the integrin lymphocyte function-associated antigen-1 (LFA-1) pathway (ICAM-1 background coating), were observed. This is attributed to a lack of sustained TCR signaling, facilitating different activation of the rare T cell subtype, Tregs [74]. Collectively, these findings suggest that the density and spatial patterning of activation sites on APCs or the ECM represent critical factors modulating T cell activation in a cell type-dependent manner.

Extensive research has been conducted on the impact of nano/microscale topographies on adaptive immune cells, particularly T cells, focusing on *ex vivo* expansion or activation using nano/micro particles. This has gained significance interest in the growing field of T cell therapies in clinical settings, often facilitated by commercially available micro-sized microbeads like Dynabeads. A comparative study between nano (130 nm) and micro (8 μm) poly (lactic-*co*-glycolic acid) (PLGA)-based artificial APCs revealed that micro-sized particles provided superior stimulation to T cells, likely due to increased interactive area with T cells from micro particles [75]. In another study, shape (from spherical to rod), size (nm to μm) and ligand density (high, intermediate, low) were systemically engineered to maximize T cell activation using PEG-PDLLA polymersome. Results indicated that T cell activation was more pronounced with a large rod-type scaffold (2000 × 50 nm) compared to smaller spherical scaffolds (160 nm), in a ligand density-dependent manner [76]. This underscores the crucial role played by ligand presentation through topography and density in promoting T cell responses. Furthermore, to leverage the benefits of rod-type topography, high-aspect-ratio mesoporous silica micro-rods (70 μm length, 4.5 μm diameter and 10.9 nm pores) were fabricated as artificial APCs. With lipid bilayers, anti-CD3, anti-CD28, and IL-2 incorporation, these micro-rods significantly enhanced polyclonal expansion, ranging from 2-fold to 10-fold compared to commercially available materials [77]. A parallel finding emerged using polystyrene particles of varying shapes with similar ovalbumin antigen loading. Intriguingly, spherical particles (diameter: 193 nm) induced a more robust Th1 immune response, while rod-shaped particles (major axis length: 1530 nm) elicited a predominant Th2 response [78].

To investigate the influence of substrate micro-sized topography on T cell activation, μm-scale pits with varying depths (4 or 6 μm) were created on planar substrates. *In vitro* culture of $CD4^+$ T cells on these micropits induced the formation of actin-rich protrusions and increased expression of IL-2 and IFN-γ compared to flat controls, with 4 μm pits eliciting an even greater activation response (Fig. 3D) [79]. The findings are consistent with previous research demonstrating that T cells can form actin-rich microvilli structures containing abundant TCRs in response to surface topological cues [80]. Moving to an exploration of nanotopographical features provided by the ECM, such as pore depth, pore size, and interpore distance, an engineered nanoporous surface (20–400 nm) was systematically created using anodic aluminum oxide. The study demonstrated that nanoporosity promoted the formation of microvilli on T cells, and a 200 nm nanoporous surface notably enhanced T cell signal transmission and activation even in the absence of biochemical cues (Fig. 3E) [81].

In summary, both nano- and micro-scale topography and geometry are critical physical factors regulating T cell activation and subsequent adaptive immune cell behaviors. The interactive surface area between activation sites and T cells, along with the type and density of ligands presented by the ECM, constitutes key parameters for fine-tuning immune cell activation responses.

### 3.3. Effects of three-dimensionality and anisotropy

While extensive knowledge exists regarding culturing cells in 2D conditions, inherent limitations arise when attempting to mimic the native 3D tissue structure [82]. In 3D matrices, adaptive immune cells exhibit more physiologic shapes, biological behaviors, and multicellular arrangements compared to 2D, owing to 3D contacts with the surrounding ECM and neighboring cells in all dimensions. The mechanotransduction resulting from a 3D network of fibrous, viscoelastic, or confined structures significantly differs from forces experienced on 2D substrates. Additionally, 3D architectures impose diffusion barriers, establishing physiologically relevant gradients of nutrients, oxygen, signaling molecules, and waste - a feature lacking in unrestricted 2D contexts [83].

Matrigel, a well-defined gelatinous protein mixture containing laminin, collagen IV, and other growth factors, was initially selected to culture T cells in 3D scaffolds to recapitulate the natural 3D environment of secondary lymphoid organs. This 3D culture condition has shown higher proliferation rates of cells compared to pseudo 3D microfiber (300 μm) scaffolds and 2D cultures (Fig. 4A) [84]. Furthermore, to preserve and augment rare, antigen-specific $CD8^+$ T cells, a 3D matrix consisting of hyaluronic acid (HA) with polyethylene glycol diacrylate (PEGDA) cross-linker was introduced. The biophysical properties of the HA-based gels, including stimulatory ligand density, stiffness, and ECM proteins, were found to potentiate T cell signaling and influence the phenotype of both murine and human T cells. Importantly, the combination of the 3D ECM environment and TCR signaling results in a rapid and robust expansion of antigen-specific $CD8^+$ T cells [85]. Similarly, 3D PEG hydrogels covalently combined with low molecular weight heparin for anchoring CCL21, enhancing T cell proliferation and migration, are engineered to resemble the lymph nodes [86]. The 3D hydrogel demonstrated increased primary human $CD4^+$ T cell proliferation compared to conventional 2D expansion systems with Dynabeads®. Multiple studies have confirmed the beneficial effects of T cell activation in 3D environments [87].

Anisotropy, characterized by directional cues that do not act equally in all directions (non-isotropic), is a key feature of fibrous ECM and serves as a crucial regulator of cell behavior in 3D environments. The aligned, fibrillar architecture of native tissue ECM introduces directional cues that cells actively respond to by reorienting their cytoskeletons and polarizing organelles [88]. This anisotropic feature can impact adaptive immune cell behaviors both in native tissues and in engineering scaffolds for immune therapy. Despite the growing interest in anisotropy, studies with adaptive immune cells are scarce due to limited platforms with finely tunable nano/micro structures and physical properties [89].

Some recent studies have explored fibrous hydrogels, including polymeric micro/nanofibers or particles mimicking anisotropic features of ECM in 3D, using electrospinning and 3D printing techniques (Fig. 4B) [90–93]. A fibrous hydrogel was fabricated by embedding fragmented fibers (~11 x ~0.7 μm) in hyaluronic acid (HA)-based gels. It exhibited fiber density–dependent strain-stiffening properties similar to natural ECM. When cells were encapsulated, fiber density–dependent cell spreading and proliferation, fiber remodeling, and hydrogel contractility, reminiscent of natural ECM, were observed. Furthermore, to investigate the influence of particle shape on interstitial pore characteristics in 3D to guide cell invasion, anisotropic rod-shaped particles

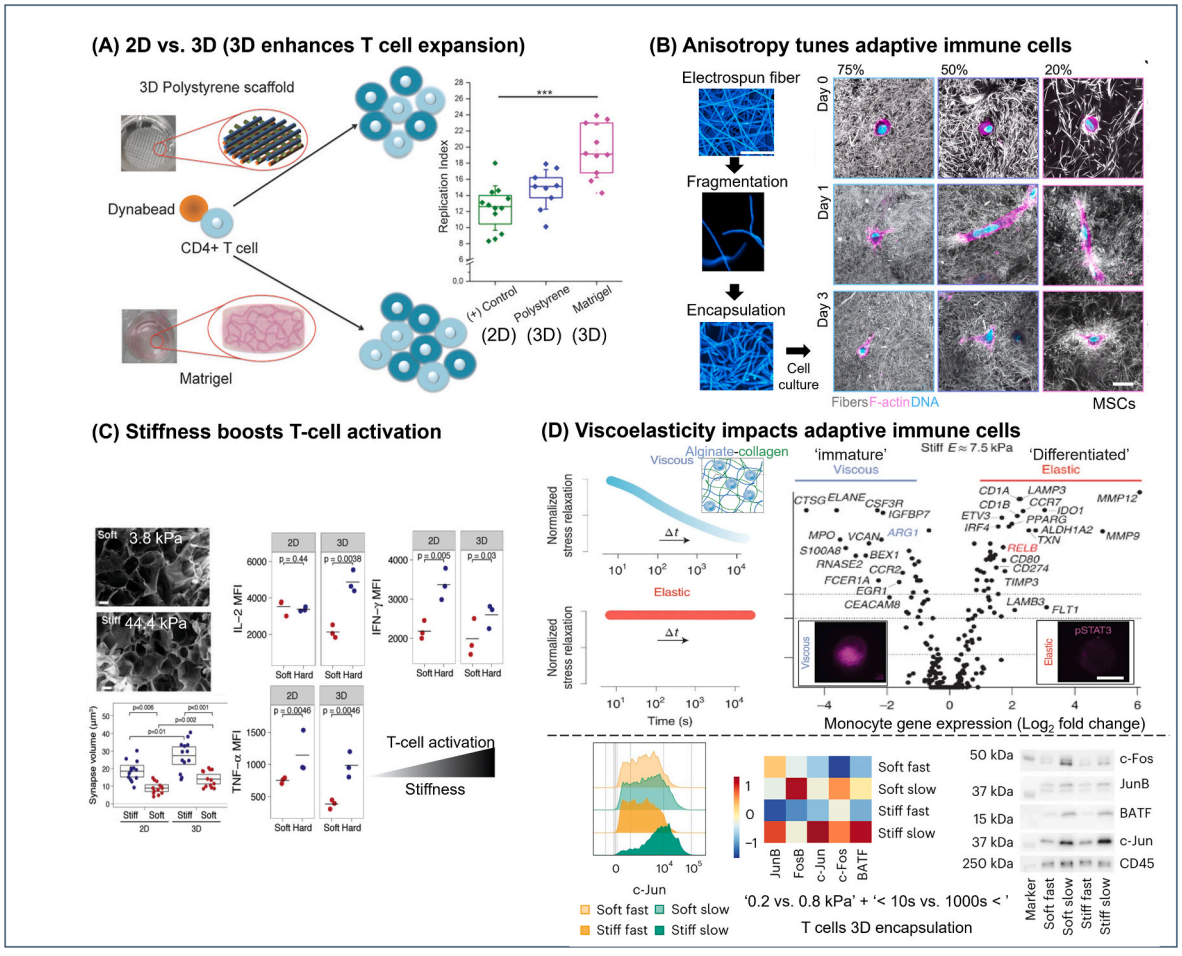


**Fig. 4.** Impact of matrix dimensionality (2D/3D), anisotropy, stiffness, and viscoelasticity on adaptive immune cells. (**A**) 3D scaffolds like polystyrene sponges or Matrigel enhance proliferation of $CD4^+$ T cells compared to suspension culture (+control). Reproduced with permission [84]. Copyright 2018 American Chemical Society. (**B**) Anisotropic fibrous architecture in 3D gels directs MSC morphology in a fiber density-dependent manner. Reproduced with permission [90]. Copyright 2021 American Association for the Advancement of Science. (**C**) T cells cultured in 2D and 3D alginate gels of varied stiffness (3.8 and 44.4 kPa) containing anti-CD3/CD28-coated microparticles. Cytokine secretion (IL-2, IFN-γ, TNFα) increases with matrix stiffness, correlating with enlarged immune synapse size. Reproduced with permission [82]. Copyright 2020, Elsevier B.V. (**D**) Stress relaxation profiles differ between viscous and elastic alginate/collagen gels. Monocytes in viscous gels display enhanced 'immature' gene expression while those in elastic gels exhibit 'dendritic cell-like differentiation' gene expression, validated by STAT3, a marker of immature monocytes, immunostaining. T-cell populations generated from 3D ECM (collagen type I based) with different viscoelasticities have different patterns of c-Jun and AP-1 protein expression. Reproduced with permission [109,112]. Copyright 2022 and 2023, Nature publishing group.

were embedded in HA gels. It was shown that there was robust *in vitro* sprouting (density and length) of endothelial cell spheroids in hydrogels with rod-shaped particles (100 × 220 μm) embedded compared to hydrogels with spherical particles (100 or 150 μm diameter) of the same diameter or volume. This enhanced sprouting was due to the anisotropy feature of the rod-shaped particles.

Combined together, anisotropic features in 3D environments may provide cells with appropriate cues to recapitulate their native morphology and functionality *in vivo*. Further studies exploring how local matrix alignment impacts key behaviors like migration and activation of adaptive immune cells are warranted. A multitude of findings demonstrate that T cell activation and proliferation are enhanced by ligand or topological anisotropy in 3D systems, further supporting the key role of matrix asymmetry in modulating adaptive immunity [94–96]. As previously discussed in Section 3.2, cues like aligned micropatterning and high aspect ratio particles promote greater expansion and effector functions of T cells compared to traditional isotropic surfaces or particles. These examples underscore how asymmetry is vital for recreating complex cell-matrix interplay that tunes responses of adaptive immunity [97,98].

### 3.4. Influence of static ("stiffness") and dynamic ("viscoelasticity") matrix mechanics

Native tissues exhibit complex mechanical properties, including stiffness (or elastic modulus) as well as time-dependent viscoelastic behaviors, such as stress-relaxation, creep, and plastic deformation. The interplay of these static and dynamic matrix mechanical factors critically impacts resident cell function in development, homeostasis, and disease [83,99]. As the ECM remodels during regeneration, fibrosis, inflammation, or cancer progression, marked changes in tissue mechanics regulate immune cell recruitment and activation that mediate the repair events and pathological progression [100]. Adaptive immune cells actively probe tissue stiffness through contractile forces at integrin adhesions as they migrate during immune surveillance and response [9]. Especially T cells and B cells identify cognate antigens either as peptides bound to the MHC molecules on the surface of APCs or as immobilized whole proteins, respectively. From this contact, intra-/inter-cellular forces are generated, modulating their interactions, making the physical properties as one of the key influences to adaptive immune cell responses [101].

There have been a relatively large number of studies related to static mechanics (stiffness)-dependent T and B cell activation that have been conducted, as summarized by some of the review papers, which can be referred to for an in-depth discussion [9,32,99]. For instance, it was revealed that dendritic cells increase their cortical stiffness during maturation to prime T cells more effectively. This allows stiff dendritic cells to activate T cells at much lower antigen levels compared to soft dendritic cells [97], providing clues to investigate the effect of ECM stiffness. Furthermore, T cells sense the ECM stiffness of matrices (Fig. 4C), altering their spreading [102,103], migration, gene expression, cytokine secretion [87,98,103], proliferation [85], metabolism [104], and cytotoxic function [105,106]. B cells also use mechanical forces to extract high-affinity antigens preferentially from follicular dendritic cells in lymph node germinal centers, likely sensing dendritic cell mechanics to promote specific antigen uptake [107]. A recent publication reporting that different biomaterial implants with distinct mechanical properties modulate *in vivo* B cell responses during muscle injury repair [31], pointing to the possibility of stiffness-dependent B cell activation in a 3D microenvironment.

More than the static stiffness, dynamic mechanics of tissues, known as viscoelasticity, is more critical in dictating immune cell response because ECM exists in a viscoelastic state rather than perfect elasticity [108]. Many pioneering studies have recently highlighted the role of viscoelasticity to tune different kinds of cells such as MSCs, monocytes, or chondrocytes (Fig. 4D) [109–111]. Amongst these, one recent study investigated adaptive immune cells (T cells) in a viscoelastic environment by adjusting stiffness and stress relaxation through click reactions and variations in collagen concentration [112]. In the study where $CD8^+$ T cells were 3D-cultured for three days in fast-relaxing (non-click crosslinked), slow-relaxing (click crosslinked), soft (2 mg/ml), or stiff (4 mg/ml) collagen matrices, variations in T cell phenotype were observed, which were found to be associated with the AP-1 pathway. The findings suggest that adaptive immune cells including B cells may sense the dynamic mechanics of tissues and biomaterials, conforming their phenotypes and functions in the repair process.

While stiffness has emerged as an important regulator of adaptive immune function, the exploration of viscoelastic tissue dynamics remains an avenue yet to be thoroughly investigated. Also, although significant strides have been made in understanding adaptive immune responses within sophisticated 2D systems, the essential next step involves validating these impacts in representative 3D microenvironments, providing a more accurate reflection of *in vivo* conditions. Advances in biomaterials now permit independently controlling stiffness and viscoelasticity (e.g., stress relaxation) to uncover these interactions. Such efforts promise to delineate critical stiffness and viscosity targets for tuning desired T and B cell behaviors in diverse contexts, such as tissue repair, infection, autoimmunity, and cancer.

## 4. Biomaterials-modulated adaptive immunity: *In vivo* findings and clinical implications

Here, we have discussed the significance of biomaterials in modulating adaptive immunity, particularly T and B cell responses, for tissue healing and regeneration from key *in vivo* studies (Table 2). The advent of exogenous T cell expansion therapies like CAR-T has spurred interest in harnessing implanted biomaterials to manipulate adaptive immune responses, aiming to enhance tissue repair and regeneration. In this part, we specifically discuss the significance of biomaterials in modulating T cell and B cell responses involved, particularly in tissue healing and regeneration, with a focus on *in vivo* findings. Activated adaptive

**Table 2**
Summary of the effect of biomaterials and matrix cues on adaptive immune cell functions *in vivo* M: *Mus musculus*, mouse.

| Refs | Species | Immune cell | Biomaterials and matrix cues (described section) | Biological functions impacted by adaptive immune cells |
|---|---|---|---|---|
| [31] | M | B cells | PCL, dcECM (composition) (4.4) | Fibrosis, antigen presentation, antibody production |
| [114] | M | $CD4^+$ T cells | Cardiac muscle-derived dECM (composition) (4.1) | Th2 immunity, IL-4 expression, muscle regeneration |
| [115] | M | T cells | Urinary bladder matrix (UBM) (size, composition) (4.1) | Th2 immunity, IL-4 expression, corneal regeneration |
| [116] | M | $CD4^+$ T cells | UBM (composition) (4.1&4.2) | Th2 immunity, tumor inhibition |
| [117] | M | $CD4^+$ T cells, Tregs | MSC-derived exosomes on fibrous scaffolds (composition) (4.1) | Th2 immunity, IL-10 and IL-4 expression, skin wound healing |
| [122] | M | $CD8^+$ T cells | Alginate-based scaffolds (stiffness, composition) (4.2) | Cytotoxic T cell activation, tumor suppression |
| [124] | M | T cells, B cells | Alginate spheres (size) (4.3) | Fibrosis, Treg-mediated immune suppression |
| [126] | M | Foxp3+ Tregs | PDMS (surface roughness) (4.3) | Fibrosis, macrophage infiltration |
| [128] | M | Th17 cells, γδ+ T cells | PCL, PDMS (composition) (4.3) | IL-17 production, fibrosis |
| [51] | M | B cells | Peptide-based hydrogels (chirality, degradation) (4.4) | Antibody production (IgG1, IgG2a), skin regeneration |

immune cells with different subsets such as $CD4^+$ Th2, $CD8^+$ cytotoxic T cells, Foxp3+ Treg, Th17 cells, and B cells, induced by specific biomaterial cues, have demonstrated critical roles in clinical implications, such as accelerating wound healing, reducing fibrous capsule formation, and augmenting anti-cancer effects. These findings underscore the transformative potential of biomaterials in steering adaptive immunity towards diverse clinical applications, ranging from regenerative therapies across multiple tissue types to biomaterial-based innovations in cancer immunotherapy. As these discoveries advance into clinical practice, biomaterials may stand ready to revolutionize the treatment landscape for a spectrum of diseases and conditions.

### 4.1. Biomaterial-modulated $CD4^+$ Th2 cell responses and the implications in tissue regeneration process

The concept of T cell-mediated tissue regeneration through biomaterials closely relates to Th2 responses. In a recent pioneering study by the Elisseeff group, experiments were conducted to elucidate the role of biomaterials in T cell regulation (Fig. 5A) [114]. The authors implanted various decellularized extracellular matrices (dECMs) in a volumetric muscle loss injury. Among the dECMs, cardiac muscle-derived matrix demonstrated the highest capacity for muscle healing in wild-type mice. This was accompanied by hypertrophy of local draining lymph nodes and a significant increase in IL-4 expression, a gene encoding a canonical Th2 cytokine. To gain further insights into the role of T cells in this regenerative process, similar experiments were performed on $Rag1^{-/-}$ mice, which lack mature T and B cells. In these mice, the enhanced muscle tissue regeneration and up-regulation of IL-4 observed in the wild-type counterparts were absent in cardiac muscle-derived dECM. However, when $Rag1^{-/-}$ mice were reconstituted with wild-type $CD4^+$ T cells (T-WT), Th2 immunity was restored. Conversely, reconstitution with $CD4^+$ T cells deficient in Th2 cytokines (T-Th2 (−)) compromised these regenerative effects. These findings underscore the crucial role of $CD4^+$ T cells and Th2 immunity in tissue regeneration facilitated by optimized dECM biomaterials.

In a subsequent study, the same group further elucidated the involvement of Th2 immune response in corneal tissue regeneration using dECMs derived from porcine urinary bladder matrix (UBM) of two different sizes: micron sized (hundreds of μm) and ultrafine (tens of μm). The results demonstrated that both UBM treatments led to faster healing and reduced vascularization of the cornea. Notably, they observed a significant increase in IL-4 expression from T cells in the cornea and draining lymph nodes, which is a characteristic feature of Th2 immunity (Fig. 5B) [115]. To investigate the specific role of Th2 immunity in this process, UBM treatment was performed on mice with suppressed Th2 immunity, achieved by depleting eosinophils or using $Gata1^{-/-}$ mice. Intriguingly, corneal regeneration was inhibited in these Th2-immunity suppressed mice, despite no changes in Th2 immunity. These findings suggest that stimulating Th2 immunity is essential for establishing a favorable regenerative environment in the wounded cornea. Since eosinophils are known to produce IL-4, the key cytokine associated with Th2 immune response, this study highlights the importance of eosinophils and Th2 immunity in promoting regenerative environments in wounded cornea.

Further studies extended this concept to cancer treatment (Fig. 5C) [116]. Implantation of UBM resulted in increased recruitment of $CD4^+$ lymphocytes compared to the injection of cancer cells alone, and it promoted Th2 immunity within the tumor microenvironment and other pathological conditions. When UBM particles were co-injected with cancer cells, tumor formation was inhibited in a $CD4^+$ T cell-dependent manner. The tumor-inhibitory effect observed in the UBM microenvironment was completely abolished in $Rag1^{-/-}$ mice (lacking mature T and B cells). However, when $CD4^+$ T cells were transferred intravenously into $Rag1^{-/-}$ mice prior to UBM implantation, tumor inhibition in the UBM microenvironment was rescued. Furthermore, impairing tumor growth through the delivery of the canonical Th2 agonist IL-4 in wild-type mice supported the notion of Th2 immunity playing a crucial role in the anti-cancer effects induced by the UBM microenvironment. While UBM-associated macrophage polarization has been identified as another underlying mechanism for the anti-cancer effects, the specific polarization phenotype of macrophages induced by UBM has not been thoroughly analyzed due to the complexity of the polarization process. Interestingly, other synthetic adjuvant biomaterials such as aluminum hydroxide and mesoporous silica particles, which are known to induce immune responses, did not inhibit tumor formation when co-injected with cancer cells in wild-type mice and were found not to induce IL-4 expression, unlike UBM. Collectively, biomaterials that accelerate Th2 immunity, including UBM, are considered to shape the immune microenvironment to combat cancer formation, highlighting their potential in cancer immunotherapy.

To potentiate Th2 immunity for tissue regeneration, exosomes derived from MSCs were also employed upon fibrous scaffolds (Fig. 5D) [117]. MSC-exosomes have been shown to possess immunomodulatory functions, promoting Th2 immune responses, M2-like macrophage polarization, and the expansion of regulatory T cells (Tregs), similar to their parent MSCs [117–121]. Based on the enhanced expression of anti-inflammatory cytokines and M2 markers observed *in vitro* with a fibrous scaffold alone, it was hypothesized that *in vivo*, a synergistic Th2 immune response could be achieved with exosomes. When exosomes were administered alone, an increased number of $IL\text{-}10^+$ Th2 cells in local tissues and $IL\text{-}4^+$ Th2 cells in lymph nodes were observed. However, in the scaffold with exosomes, no synergistic Th2 immune response was detected; it only maintained the Th2 immunity observed with exosomes alone. Nonetheless, synergistic effects were observed in terms of total $CD4^+$ T cell and Treg recruitment in the scaffold with exosomes. Also, this exosome-containing scaffold exhibited accelerated healing of large skin wounds within two weeks, demonstrating enhanced wound closure, re-epithelialization, collagen deposition, and blood vessel formation compared to scaffolds or exosomes used individually. In summary, the scaffolds functionalized with exosomes synergistically combined the effects of the scaffold as a “recruiter” and the exosomes as a “trainer” for immune cells, particularly $CD4^+$ Th2 helper cells, at both local and systemic levels. This approach facilitated enhanced tissue regeneration by harnessing the immunomodulatory properties of the exosomes and the structural properties of the scaffold, offering novel design principles of biomaterials through T cell regulation for accelerating tissue regeneration.

### 4.2. Biomaterial-activated $CD8^+$ cytotoxic T cells and the efficacy for anticancer therapies

Immunotherapies for cancer are currently under intensive investigation for clinical therapy. For example, inhibitors of the programmed cell death-1 (PD-1) pathway and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) target the inhibitory pathways in $CD8^+$ cytotoxic T cells. Cancer cells often evade the immune system by expressing PD-L1 (programmed death-ligand 1) on their surface, which binds to PD-1 receptors on $CD8^+$ cytotoxic T cells, dampening their killing activity. PD-1 inhibitors counteract this evasion strategy by allowing $CD8^+$ cytotoxic T cells to recognize and destroy cancer cells. In this section, we focus on the modulation of chemo-mechanical cues of implantable biomaterials for effective anticancer therapies.

To enhance the recruitment and activity of antitumorigenic endogenous $CD8^+$ cytotoxic T cells, biomaterials have been developed with tunable chemo-mechanical properties. One recent study on alginate-based, biodegradable, and macroporous implantable biomaterials is a notable example (Fig. 6A) [122]. The material was engineered to have specific chemo-mechanical properties. First, the material was designed to deliver IL-2 which recruits endogenous T cells. Furthermore, the surface was coated with stimulatory antibodies (anti-CD3 and anti-CD28) to activate and proliferate $CD8^+$ T cells, mimicking the function of APCs. Among other properties, the stiffness of 50 kPa (among

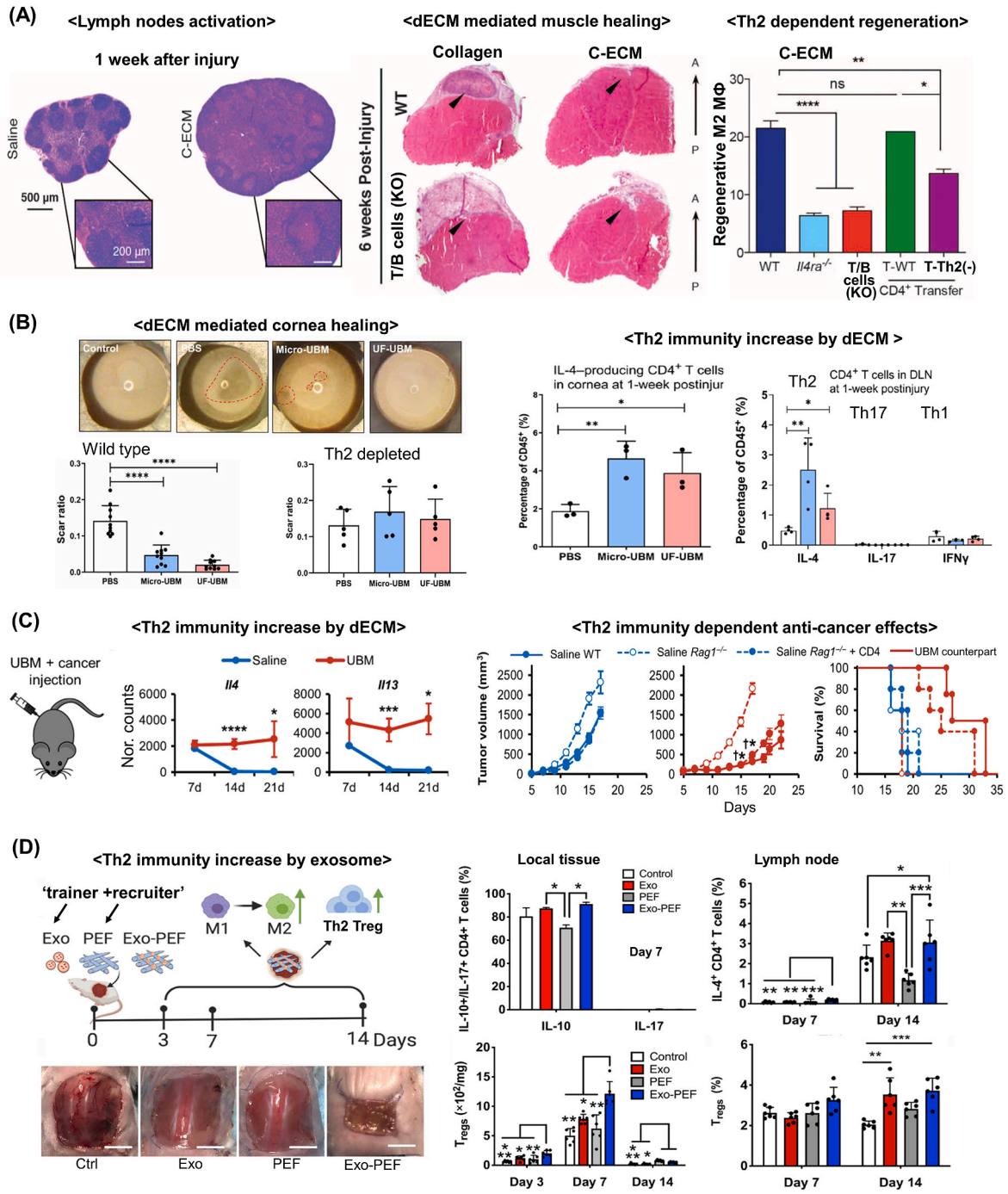


**Fig. 5.** Modulation of CD4$^+$ Th2 helper cells (Th2 immunity) *in vivo* through biomaterials for tissue regeneration. (A) Cardiac muscle-derived decellularized extracellular matrix (dECM) exhibited remarkable muscle healing capacity in wild-type mice, accompanied by hypertrophy of local draining lymph nodes. However, this enhancement was lost in Rag1$^{-/-}$ mice lacking mature T and B cells. Restoring Th2 immunity by reconstituting Rag1$^{-/-}$ mice with wild-type CD4$^+$ T cells (T-WT) rescued the regenerative effects, whereas reconstitution with Th2-deficient CD4$^+$ T cells (T-Th2 (−)) compromised tissue regeneration. This underscores the crucial role of Th2 immunity in tissue regeneration facilitated by optimized dECM biomaterials. Reproduced with permission [114]. Copyright 2016, American Association for the Advancement of Science. **(B)** Urinary bladder-derived dECM (UBM) enhanced healing in corneal tissues in a Th2-dependent manner, irrespective of particle size (ranging from microsized to few microns). Th2 immunity was observed both in the local cornea and distant lymph nodes. Reproduced with permission [115]. Copyright 2021, American Association for the Advancement of Science. **(C)** The Th2 immunity-inducing microenvironment generated by dECM was employed for cancer treatment. UBM significantly upregulated the levels of IL-4, IL-13, and Th2-related gene expression. UBM led to tumor inhibition in wild-type mice, while this effect was compromised in Rag1$^{-/-}$ mice. Additional reconstitution with CD4$^+$ T cells restored the anti-tumor effect. Treatment with a Th2 immunity booster (IL-4c) mimicked the antitumor effect of UBM. Reproduced with permission [116]. Copyright 2019, American Association for the Advancement of Science. **(D)** Synergistic Th2 immunity between two biomaterials (scaffolding materials and functional exosomes) was investigated in skin regeneration. Surprisingly, both exosomes alone and exosomes combined with scaffolds (exo-PEF) increased the number of IL-10$^+$ and IL-4$^+$ Th2 cells in local tissues and lymph nodes to a similar extent. However, exo-PEF exhibited a synergistic effect on Th2 cell recruitment. This complex scaffold synergistically healed large skin wounds within two weeks, suggesting that scaffolds and exosomes acted as the "recruiter" and "trainer" for Th2 immune cells, respectively. Reproduced with permission [117]. Copyright 2021, American Association for the Advancement of Science.

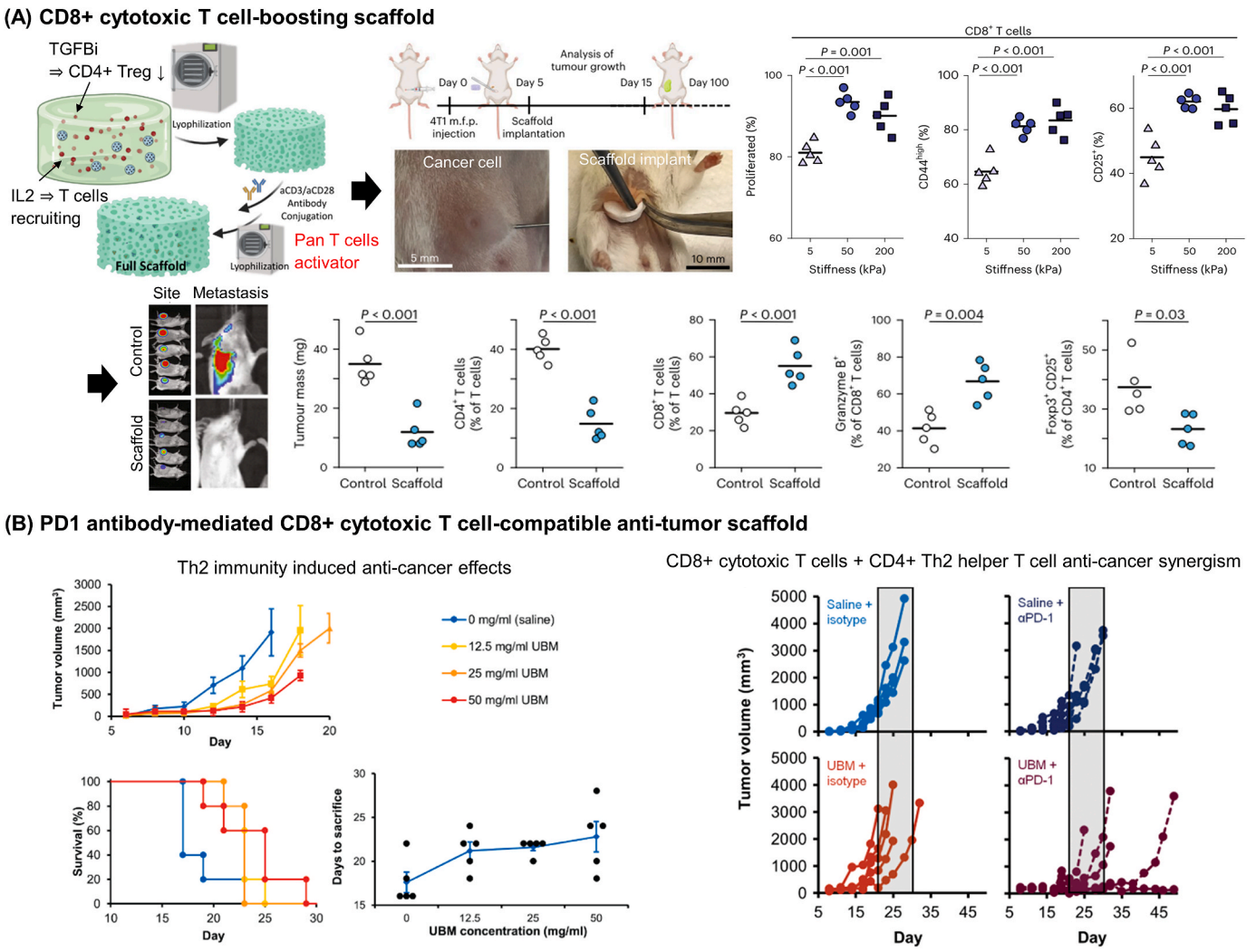


**Fig. 6.** Modulation of CD8$^+$ cytotoxic T cell activity *in vivo* through biomaterials for anti-cancer immunotherapy. (A) A scaffold was designed to recruit and activate endogenous CD8$^+$ cytotoxic T cells for tumor treatment. The scaffold was loaded with stimulatory antibodies against CD3 and CD28 (aCD3/aCD28), interleukin-2 (IL2), and a TGFβ inhibitor. This combination aimed to enhance T cell activation and recruitment while reducing the inhibitory effect of anti-tumor immune effector CD4$^+$ Tregs. Through *in vitro* studies, CD8$^+$ cytotoxic T cell activation was optimized among different scaffold stiffnesses (ranging from 5 to 200 kPa). Subsequently, biomaterials with a stiffness of 50 kPa were implanted adjacent to the tumor tissue five days after injecting cancer cells into the mammary fat pad. The results showed a significant reduction in adjacent tumor size and metastasis, accompanied by an increase in the fraction and activity of CD8$^+$ cytotoxic T cells Reproduced with permission [122]. Copyright 2022, Nature publishing group. (B) An anti-cancer scaffold was designed to facilitate PD-1 antibody-mediated CD8$^+$ cytotoxic T cell therapy. The study first confirmed the anti-tumor effect of UBM alone, which induced Th2 immunity. Subsequently, the scaffold's synergistic anti-tumor therapy was assessed when co-delivering with PD-1/PD-L1 immune checkpoint inhibition. The results indicated that Th2 immunity carving biomaterials could be clinically applicable as a synergistic adjuvant with PD-1 antibody cancer therapy, enhancing CD8$^+$ cytotoxic T cell activity. Reproduced with permission [116]. Copyright 2019, American Association for the Advancement of Science.

the range from 5 to 200 kPa) was found to be optimal for CD8$^+$ cytotoxic T cell activation. Once implanted adjacent to the tumor in the intra-mammary fat space, the engineered scaffold demonstrated significant reductions in adjacent tumor sizes, leading to improved overall survival and reduced metastasis by day 30. Moreover, the scaffold exhibited the ability to suppress both local and distant breast tumor growth, while also inducing a robust anti-cancer memory response. As discussed in the previous section, the UBM scaffold has shown promising potential in providing a microenvironment for CD4$^+$ Th2 immunity-dependent tumor inhibition. Building on this research, scientists have taken a synergistic approach to activate CD8$^+$ cytotoxic T cells, aiming to enhance cancer immunotherapy. CD4$^+$ T cells are known to play a crucial role in boosting anti-tumor immunity by supporting CD8$^+$ cytotoxic T cells through the secretion of effector cytokines like IFN-γ and TNFα [123].

Prompted by this insight, a synergistic combination was contemplated, seeking to enhance cytotoxic T cell activity through PD-1/PD-L1 blockade and simultaneously boost Th2 immunity via the UBM immune microenvironment (Fig. 6B) [116]. The results demonstrated that PD-1/PD-L1 immune checkpoint inhibition effectively synergized with the tumor-inhibitory UBM microenvironment. This finding suggests the potential clinical application of biomaterials designed to enhance Th2 immunity in combination with PD-1 antibody cancer therapy, ultimately strengthening the CD8$^+$ cytotoxic T cell activity. By harnessing the power of both immune responses, this synergistic approach holds promise for improved cancer treatment outcomes and may pave the way for innovative combination therapies in cancer immunotherapy.

Long-term modulation of the immune response is crucial for overcoming the "bench to clinic death valley" in cancer treatment, particularly in preventing recurrence and metastasis. Recent studies on biomaterial-activated CD8$^+$ cytotoxic T cells highlight the potential

for sustained anti-tumor responses through engineered scaffolds within the tumor microenvironment. Innovations in material design are now focusing on "smart" biomaterials that adapt to the evolving immune landscape of cancer progression. These include scaffolds with programmable degradation profiles for staged release of immunomodulatory factors and materials that remodel in response to tumor-associated enzymes, continually exposing new immunostimulatory epitopes. Furthermore, the synergistic combination of Th2 immunity-inducing biomaterials with checkpoint inhibitors, such as UBM with PD-1 antibodies, offers promising avenues for long-term cancer control. Future designs may also incorporate nanoparticles for sustained or time-dependent (or pH-dependent) release of immunomodulators,

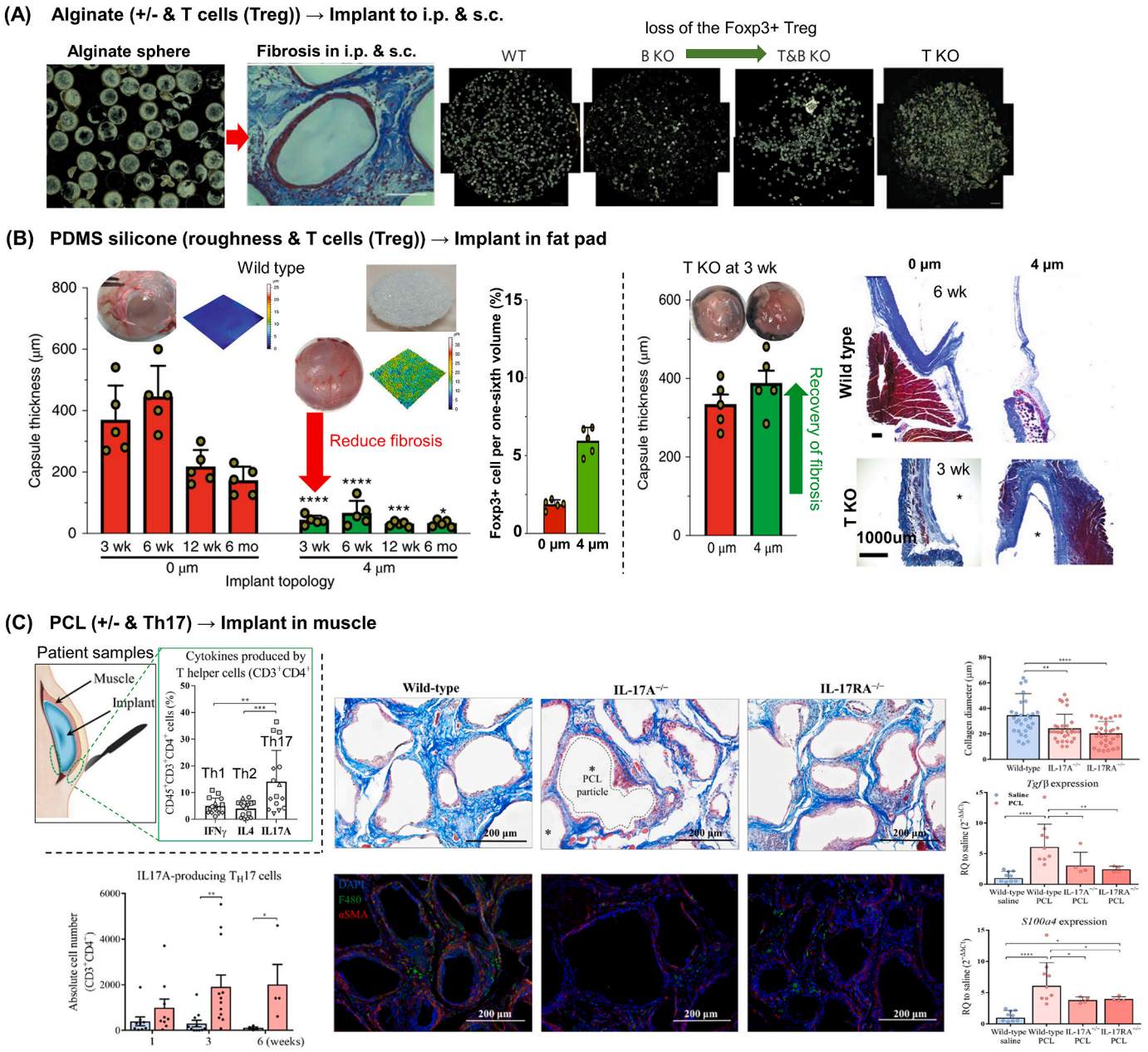


**Fig. 7.** Regulated Foxp3$^+$ Treg or Th17 cells, mitigating biomaterial-induced fibrosis *in vivo*: (A) Treg cell-dependent anti-fibrosis effect of alginate spheres. Sterilized alginate spheres with a diameter of 500 μm induced fibrosis in wild-type mice in both intraperitoneal and subcutaneous regions. In B-cell-deficient (B KO) mice, partial loss of fibrosis occurred. However, additional T-cell loss (T & B KO) led to a retrieved loss of fibrosis similar to wild-type levels, possibly due to the loss of the Treg subset, which plays a crucial role in suppressing overreaching immune reactions. Similarly, antagonistic roles of T cells against biomaterial-induced fibrosis were observed in T-cell-deficient mice (T KO), where more severe fibrosis was observed. Reproduced with permission [124]. Copyright 2017, Nature publishing group. **(B)** T-cell-dependent roughness-mediated fibrosis observed in PDMS breast implants. PDMS implants with a roughness of 4 μm provoked diminished capsule thickness compared to smooth implants (0 μm) due to an increase in the number of Foxp3$^+$ Treg cells. When 4 μm PDMS was implanted in T-cell knock-out mice, fibrous capsule formation returned to thicker features, revealing the T-cell-dependent fibrosis change influenced by surface roughness. Reproduced with permission [126]. Copyright 2021, Nature publishing group. **(C)** Th17-dependent fibrosis observed from PCL particles. Clinical tissue samples taken from areas surrounding PDMS breast implants showed the presence of CD4$^+$ Th17 cells producing IL17. Implanted synthetic PCL particles in muscle tissues revealed chronic IL17 production by Th17 cells in mice. Knock-out mice lacking IL17A or its receptor (IL17RA) showed reduced fibrotic responses to PCL, suggesting the potential for antibody or small molecule therapy against IL17 signaling to mitigate fibrosis induced by PCL particles. Reproduced with permission [128]. Copyright 2020, American Association for the Advancement of Science.

enhancing $CD8^+$ cytotoxic T cells or Th2 immunity locally. These approaches, which focus on prolonged immune modulation with fewer side effects, have the potential to significantly enhance the efficacy, safety, and durability of cancer immunotherapies.

### 4.3. Regulation of $Foxp3^+$ Treg or T17 cells and the mitigation of biomaterial-induced fibrosis

In the case of biomaterials-induced fibrosis, which significantly influences the success of tissue healing, T cells have been identified as major players in regulating this process. Clues about their involvement came from a study conducted by Anderson group using sterilized alginate spheres (diameter = 500 μm) (Fig. 7A) [124]. When alginate, known to induce fibrosis in wild-type mice, was implanted into the soft tissue (intraperitoneal or subcutaneous regions) of B cell-deficient mice (B KO), a partial reduction in fibrosis was observed. However, in mice with additional T cell loss (T & B KO), the reduction in fibrosis was reversed, bringing it closer to the wild-type level. This outcome was likely due to the loss of the $Foxp3^+$ Treg subset, which plays a crucial role in suppressing excessive immune reactions [125]. Similarly, an antagonistic role of T cells in fibrosis was detected in only T cell-deficient mice (T KO), where more severe fibrosis was observed. These findings highlight the significant impact of T cells in determining the extent of fibrosis induced by biomaterials, implying their potential as key regulators in tissue regeneration processes involving biomaterials.

In a separate study by the Langer group, the role of $Foxp3^+$ Treg cells in downregulating fibrosis surrounding biomaterial implants was investigated. They used miniature PDMS breast implants with varying surface roughness in mice and rabbits to study fibrosis effects (Fig. 7B) [126]. PDMS is commonly used in the medical field, including breast implants. The implant surface architectures are generally modified to limit unnecessary immune responses, fibrosis, and cancer risks associated with breast implants. The roughness of PDMS implants in the study ranged from smoother (0 μm) to moderately rough (4 μm) and rougher (90 μm) implants. Interestingly, the implants with a moderate roughness of 4 μm, including the commercial counterpart, exhibited reduced fibrous capsule formation and macrophage infiltration compared to the other implants (0 μm and 90 μm). This decrease in fibrosis was attributed to the presence of lower levels of inflammatory macrophages ($CD11b^+$) and a higher expression of $Foxp3^+$ Treg cells. To further investigate the role of T cells, they implanted 4 μm PDMS in T cell knockout mice (C57BL/6-Nude, T KO). In these mice, fibrous capsule formation thickened, accompanied by an increase in tissue-resident inflammatory macrophages ($CD11b^+$). Collectively, the optimal surface roughness at the single-cell scale (4 μm) demonstrated the least fibrous capsule formation in PDMS implants under the fat pad. This regulation appeared to be mediated by T cells, particularly $Foxp3^+$ Treg cells, influencing the recruitment of pro-inflammatory macrophages to the surrounding tissue. These findings open up new possibilities for controlling fibrosis around biomaterial implants through targeted immunomodulation, potentially leading to improved tissue regeneration outcomes. Further research may also be extended to utilize and deliver specific subtypes of T cells (such as Treg, Th1, Th2, Th17, cytotoxic T cells, etc.) along with biomaterials for fine-tuning fibrous capsule formation and minimizing material-associated fibrosis [127].

Subsequent research aimed to elucidate the specific role of Th17 cells, in the development of fibrosis induced by biomaterials (Fig. 7C) [128]. Clinical tissue samples taken from the areas surrounding PDMS breast implants have revealed the presence of Th17 and $\gamma\delta^+$ T cells producing IL-17, and interestingly, IL-17 expression was found to strongly correlate with fibrosis markers. Moreover, the synthetic biopolymer PCL implanted in muscle tissues of mice was shown to induce chronic IL-17 production by Th17 and $\gamma\delta^+$ T cells. On the other hand, mice lacking TCR in $CD4^+$ T cells failed to produce IL-17 in response to biomaterials, indicating that $CD4^+$ T cells are the source of antigen-specific IL-17 in response to PCL implantation. This was further supported by elevated levels of IgG1 antibodies in the serum. To target the Th17 immune response and potentially mitigate fibrosis, IL-17 signaling was investigated. Knock-out mice lacking IL-17A or its receptor (IL-17RA) showed reduced fibrotic responses to synthetic materials, suggesting the potential for therapeutic intervention through IL-17 signaling inhibition. Blocking IL-17 signaling using IL-17 antibodies or chemicals like Navitoclax, which can eliminate IL-6-producing senescent cells (since IL-6 contributes to Th17 cell differentiation) and subsequently reduce IL-17 expression, mitigated PCL-associated fibrosis. Overall, the Th17 immune response has been identified as a significant contributor to the development of fibrosis induced by biomaterials. This research highlights potential therapeutic targets, such as IL-17 or its signaling pathway, which could be explored to reduce the fibrotic response and rejection of biomaterial implants and ultimately to improve the biocompatibility and tissue regeneration.

The studies discussed above illuminate the complex and persistent nature of immune responses to implanted biomaterials, particularly in the context of fibrosis. The long-term interplay between different T cell subsets, especially Foxp3+ Tregs and Th17 cells, emerges as a critical factor in determining the extent of fibrosis and overall tissue regeneration outcomes. These immune effects can persist for extended periods, with T cell-mediated responses continuing to shape the local microenvironment weeks or even months after implantation. Recent innovations in material design have begun to leverage these insights to create "immunomodulatory biomaterials" that can actively guide the long-term immune response [32]. For instance, the development of implants with optimized surface roughness or stiffness to promote sustained Treg responses along with Th2 responses, and reduce chronic fibrosis represents a promising approach to controlling the long-term immune landscape. Strategies targeting persistent IL-17 signaling, such as the incorporation or controlled release of IL-17 antibodies or senolytic drugs like Navitoclax from biomaterials, showcase how understanding long-term immune effects can lead to novel therapeutic interventions. Another innovative approach could involve the design of biomaterials with programmable degradation profiles that release immunomodulatory factors or provide mechanical cues (e.g., changes in stiffness, such as softening or stiffening gels) in a time-controlled manner, matching the dynamics of the evolving immune response [129–132]. These advancements point towards a future where biomaterials are not just passive scaffolds but active participants in guiding the long-term immune response towards favorable tissue regeneration outcomes. By fine-tuning the balance between pro-fibrotic (e.g., Th17) and anti-fibrotic (e.g., Treg) responses over extended periods, these smart materials could potentially mitigate chronic fibrosis, enhance implant integration, and improve long-term outcomes in fields ranging from regenerative medicine to implantable medical devices.

### 4.4. Influence of biomaterials on adaptive immunity through B cells: humoral immunity and antigen uptake

As part of the adaptive immune response, B cells primarily function to produce antibodies or complement components, which play a crucial role in accelerating the clearance of antigens, including molecules from biomaterials, both locally and systemically. This process helps to modulate the overall immune response [133]. Next, activation of other immune cells by antigen presentation and physical binding, and cytokine secretion are considered secondary roles of B cells. B cells, traditionally recognized as key adaptive immune cells, could function as APCs like innate immune cells, leading to direct sensing biomaterials and secreting cytokines without requiring T cell activation. However, the magnitude and nature of the cytokine responses are more pronounced with the activation by $CD4^+$ T cells [134]. Along with the fact that antibody IgG1 secreted from activated B cells binds to wounded tissue within 6–24 h and delayed wound healing occurs in splenectomy, which decreases B cell activation with reduced IgG1 levels, understanding the intricate interactions between B cells and biomaterials is

essential for optimizing the design and development of biomaterials and not only vaccine development and immune modulation against various diseases, but also for regenerative medical applications [135].

There have been a few reports on the role of B cells in response to implantable biomaterials. In pursuit of fine-tuning antibody production within humoral immunity, the focus has shifted to targeting B cells using biomaterials as carriers for biologic antigens (vaccines), notably in the form of micro- and nano-particles. This strategy aims to address a range of diseases, such as infections, autoimmune disorders, and cancer [136–138]. When considering humoral immunity from ECM-based biomaterials, some molecules possibly considered as antigen types are released during biomaterial implantation in tissue. For example, collagen, glycoproteins, and even intracellular proteins could serve as potential antigens for B cell response (Fig. 8A) [139]. In contrast, synthetic biomaterials may lack obvious sources of antigens. However, it is hypothesized that tissue damage-related self-antigens, adsorbed proteins, and non-peptide-repeating structures could potentially act as antigens in synthetic biomaterials [140]. When the antigens are released, they are recognized by APCs, inducing expression of effector molecules to Th cells, found in secondary lymphoid tissues, including tonsils, spleen, and lymph nodes (Fig. 8B). Activated T cells then deliver antigens and activate B cells, prompting their proliferation and differentiation into resting memory cells and antibody-secreting plasma cells, called 'thymus-dependent B-cell activation' (Fig. 8C, i-1). Depending on the location of plasma cells they can live several months (in lymph node) or few days (in tissues). The secreted IgG1 and IgG2a/b from plasma cells can facilitate phagocytosis of biomaterials targeted for opsonization, an immune process whereby opsonins tag pathogens for elimination by phagocytes (Fig. 8C, i-2). Concurrently, direct antigen uptake and cytokine secretion to boost innate immunity are also essential roles of B cells recruited to injured tissues as antigen-presenting B cells, termed 'thymus-independent B-cell activation' (Fig. 8C–ii). Activated B cells directly secrete IgG3 and cytokines like TNF-α, lymphotoxin, and IL-6 to stimulate innate immune cells (Fig. 8C–iii), potentially driving foreign body reactions against implanted biomaterials and resulting in granuloma and fibrosis (Fig. 8C–iv). Therefore, inflammatory synergism may occur through B cells by the activated innate immune cells as APCs and secreted antibodies as a humoral response.

One study demonstrated that the synthetic biopolymer PCL could differently modulate the functional B cell response (activation and duration) and consequent fibrosis compared to biodegradable natural tissue-derived decellularized ECM (dcECM) counterpart, suggesting the possibility of direct intervention of biomaterials with functional B cells (Fig. 9A) [31]. One potential mechanism for activating B cells from biomaterials is through direct, chemo-mechanical activation of TLRs or B cell receptors (BCRs), in conjunction with T cell activation, which remains an unsolved question [32]. These biomaterials were implanted into a volumetric muscle loss injury in immune-competent C57BL/6 wild-type (WT) mice, as well as mutant mice devoid of mature B cells ($muMT^{-}$). The results revealed that the fibrotic response induced by PCL was compromised in the mutant mice lacking B cells, suggesting the involvement of B cells in the fibrotic process. Further analysis in the WT mice showed that B cells were recruited differently at the biomaterial implant sites and secondary lymphoid organs (draining LNs) depending on the type of biomaterial used. When natural tissue-derived dcECM was implanted, there was an increase in long-lived-differentiated plasma B cells ($CD19^{-}$ $B220^{+}$) at the implant site during the early period (3–7 days). At the same time, fewer differentiated B cells ($CD19^{+}$ $B220^{+}$) were found in the draining LNs. On the other hand, when synthetic PCL was implanted, an increase in plasma B cells ($CD19^{-}$ $B220^{+}$) was observed at a later stage (3 weeks), and the implant site exhibited a higher presence of antigen-presenting B cells (MHC $II^{+}$ of $CD19^{-}$

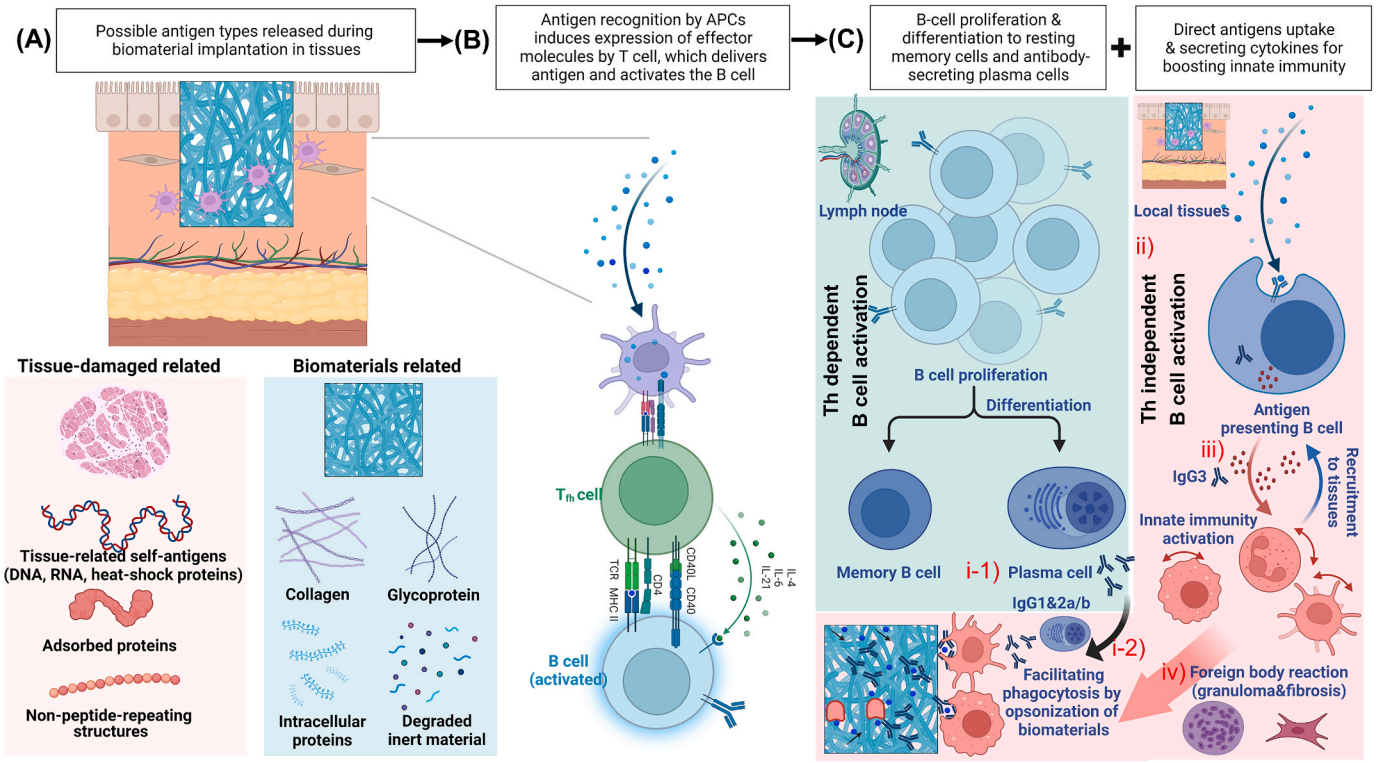


**Fig. 8.** Schematic showing the interaction cascades between antigens from biomaterials and B cells. (A) In humoral immunity, B cells can react with tissue-damaged components (e.g., damage-related self-antigens, adsorbed proteins, and non-peptide-repeating structures) and biomaterials degrading components (e.g., collagen, glycoprotein, intracellular proteins, degraded inert material). The role of biomaterials components as antigens is still under investigation. **(B)** Antigen recognition by antigen-presenting cells (APCs) induces the expression of effector molecules by T cells, which deliver antigens and activate the B cell. **(C)** B-cell proliferation and differentiation into antibody-secreting plasma cells and resting memory cells. Antibodies facilitate opsonization of antigens and subsequent phagocytosis. Additionally, biomaterials can modulate innate immunity through direct antigen uptake and cytokine release, boosting innate immunity as antigen-presenting cells.

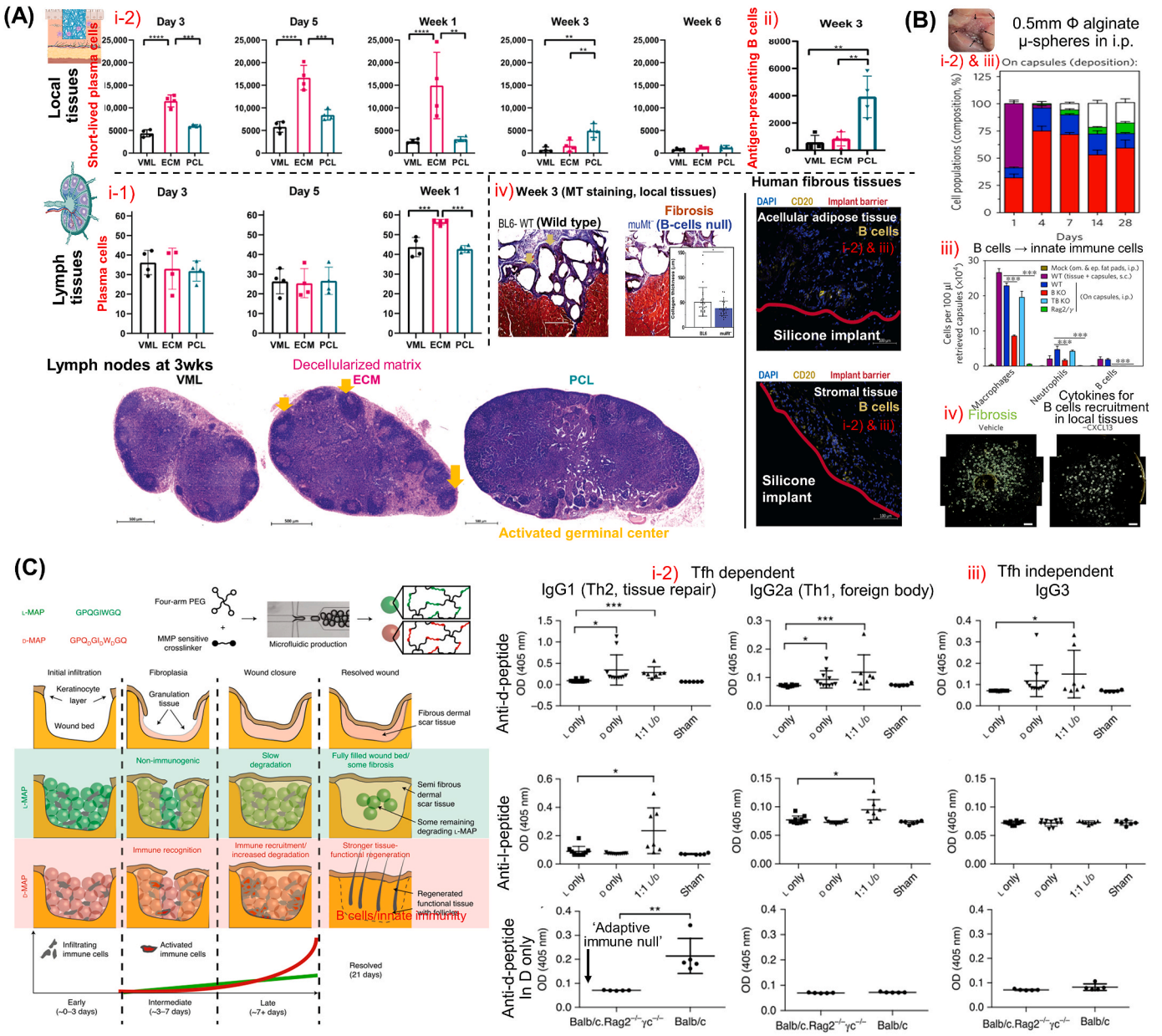


**Fig. 9.** Effects of biomaterials on B cell responses *in vivo* - humoral immunity and antigen uptake. (A) Biomaterial implants (decellularized ECM or PCL) in quadriceps muscle tissues have diverging influences on B cells in local tissues (short lived plasma cells, **i-1**) and in the lymph nodes (love lived plasma cells, **i-2**). PCL induces antigen-presenting B cells in local tissues, as shown by the presence of activated germinal centers (dense circular regions) in histology at the 3-week time point (**ii**). B-cells null mice (muMt$^-$) exhibit reduced PCL-mediated fibrosis. CD20$^+$ human B cells were detected in acellular adipose and stromal tissues surrounding silicone (human breast implant tissue expanders) which had an abnormal foreign body reaction (an example of immune response against biomaterials, **iv**). Reproduced with permission [31]. Copyright 2021, American Association for the Advancement of Science. **(B)** B cells recruitment in local tissues (**i-2** (short lived plasma cells) and **iii** (B cells acting as APCs)) is a central component of the foreign body response to biomaterial implants. When 500 μm alginate spheres are implanted in the intra-peritoneal space, B cells are recruited to the implants after 7 days. B cells knockout impairs innate immune macrophage and neutrophil recruitment in tissues, majorly representing the APCs roles of B cells (**iii**). Inhibition of B cell recruitment cytokines (CXCL13) decreased fibrosis (**iv**). Reproduced with permission [124]. Copyright 2017, Nature publishing group. **(C)** Different types of antibodies are produced depending on the chirality of peptides consisting of hydrogels for skin wound healing. "Tissue repair" antibodies (IgG1, **i-2**), "foreign body" antibodies (IgG2a, **i-2**), and Th-independent antibodies (IgG3, **iii**) are more generated in d-peptide hydrogels than l-peptide hydrogels. Reproduced with permission [51]. Copyright 2021, Nature publishing group. **i)-iv)** were noted in each case to help understanding of which kind of B cell immune response (as described in Fig. 8) being related to the biomaterials.

B220$^+$). These findings indicate that different types of biomaterials can induce distinct B-cell responses locally and systemically. The involvement of B cells in the fibrotic response to biomaterials highlights their significance in the modulation of the immune reaction and healing process, shedding light on potential strategies to fine-tune the body's response to implanted biomaterials for better biocompatibility and tissue regeneration.

The upregulation of Prdm1, a gene associated with antibody secretion and the generation of long-lived mature B cells, in sorted B cells from lymph nodes (LNs) provided further evidence for the involvement of dcECM in B cell responses. Histological analysis of LNs at 3 weeks revealed denser germinal centers in lymphoid follicles from dcECM, indicating the proliferation and maturation of B cells in the periphery of the LN. Additionally, class switching to IgG in dcECM suggested Th-

dependent antigen presentation at the germinal center, enhancing the antibody's binding affinity to its target antigen. These data suggested that dcECM biomaterials may promote high-affinity T cell–dependent B cell responses, contributing to a more favorable regenerative microenvironment. On the other hand, fibrosis-inducing PCL did not show initial activation and recruitment of plasma B cells at the injured site. Instead, at 3 weeks, PCL exhibited a high number of plasma B cells along with antigen-presenting B cells (MHC $II^+$ of $CD19^-$ $B220^+$), indicating the specific role of B cells in determining biomaterial-induced fibrosis. Antigen-presenting B cells have distinct capabilities not only in infection, pathogenesis, and autoimmunity but also in the type 2 wound healing response, possibly contributing to ECM deposition of fibroblasts and subsequent fibrosis surrounding PCL implants [141,142].

The presence of clusters of B cells in human tissue samples covering breast capsule silicone implants further confirmed the potential clinical relevance of B cells in the foreign body response to biomaterials [31]. B cells started to be recruited to 500 μm alginate spheres after 7 days of implantation in the intraperitoneal space as a central foreign body response (Fig. 9B) [124]. The B-cell-knockout further impaired the recruitment of innate immune cells (macrophages and neutrophils), and the inhibition of B cell recruitment cytokines (CXCL13) also decreased fibrosis. Overall, although B cells constitute a small percentage of cell types present locally in biomaterial tissue implants, their potential importance in biomaterials-induced regenerative medicine was highlighted. Their interactions and responses with biomaterials can significantly impact the immune reaction and healing process, suggesting that targeting B cells could be a promising approach for improving the biocompatibility and success of biomaterial implants.

The investigation of exogenous biomaterial components, such as synthetic monomers or peptides, against B cells was carried out using peptide-based degradable microporous hydrogels (Fig. 9C) [51]. The results showed that the enzymatic degradation of the hydrogels by matrix metalloproteases (MMPs) led to the production of Th dependent antibodies (IgG1 or IgG2a) against each type of peptide, depending on the chirality of the peptide crosslinker. Specifically, mice developed D-chiral-peptide specific IgG1 and IgG2a antibodies in response to D-chiral-peptide based particles or their combination, while L-chiral-peptide specific IgG1 and IgG2a were not produced in response to the L-chiral counterpart. The *in vivo* wound healing results further confirmed the role of B cells in inducing adaptive immunity. The D-chiral hydrogel induced more neogenesis of hair follicles in full-thickness skin wounds in wild-type mice compared to the L-chiral hydrogel. However, in an adaptive immunity knock-out mouse model ($B6.Rag1^{-/-}$ $\gamma c^{-/-}$), which lacks both T cells and B cells, the D-chiral hydrogel did not regenerate hairs or sebaceous glands. This suggests that the D-chiral hydrogel induces antibody responses (IgG1 and IgG2a) in the implant site and promotes type 2 immunity, involving the recruitment of myeloid cells, possibly in coordination with B cells secreting cytokines or opsonization by antibodies. This immune response leads to phagocytosis by immune cells, individual macrophage accumulation (without forming multi-nucleated giant cells), increased degradation, and functional regeneration of skin tissues. In contrast, the B-cell non-reactive L-chiral counterpart did not show similar regenerative effects. Interestingly, the study found that anti-D-peptide-specific IgG2a was induced only when the D-chiral hydrogel was used in a wound model, but not in a relatively less invasive subcutaneous implant model. This suggests that the regenerative D-chiral-peptide based hydrogel does not generate sufficient antibody for Th1 'foreign body' response (IgG2a) on its own. Instead, it primarily mediates IgG1-based Th2 'tissue repair' type response, specifically promoting skin regeneration.

Indeed, B cells, despite being relatively small in number at the local injury or implant site, can have a profound impact on the overall physiological state of an organism through their systemic response. The interaction between a local biomaterial implant and the systemic B cell response highlights how biomaterials can influence the body's overall immune response and healing processes. One promising application of this interplay is the development of efficient vaccines using biodegradable 3D porous biomaterials. Through subcutaneous implantation of scaffolding biomaterials, an augmentation in $CD4^+$ T follicular helper (Tfh) cells has been achieved, concurrently prolonging the formation of germinal centers (GC) in lymph nodes. This, in turn, boosts B cell activation and enhances antibody production. Target antigens for vaccination are encapsulated within the biomaterials for sustained release, thus amplifying the humoral immune response. This approach has shown promising results in protecting against model antigens and even lethal influenza virus, leading to improved *in vivo* survivability.

Moreover, the augmentation of biomaterial stiffness via an increased weight fraction of the scaffold polymer has been observed to yield increased antigen-specific antibody titers. In fact, the performance of these biomaterial-based vaccines surpassed that of traditional positive adjuvants like aluminum. This highlights the synergistic potential of using biomaterials as systemic adjuvants and carriers for vaccination to enhance humoral immunity [143]. Looking ahead, the investigation into various physicochemical properties of biomaterials, spanning degradation rate, components, geometry, and matrix mechanics warrants further exploration. This aims to advance the promotion or regulation of systemic humoral immunity. By fine-tuning these properties, biomaterials hold great promise in enhancing vaccine efficacy and potentially improving the body's response to injury and implant repair strategies. This research in biomaterial design and its interplay with the immune system holds immense potential for advancing regenerative medicine and immunotherapy applications.

### 4.5. Consideration of cellular crosstalks in the biomaterial-modulated immune responses between adaptive and innate immune cells

The intricate interplay among T cells, B cells, and innate immune cells such as macrophages and DCs orchestrates the immune response to biomaterials, thereby facilitating tissue healing and regeneration. Simultaneously, diverse matrix cues associated with biomaterials modulate these intercellular communications. Within this section, we illuminate recent findings at the intersection of these cellular crosstalks and biomaterials, ultimately governing the immune responses between adaptive and innate immune cells elicited by biomaterials (Table 3).

First, T cells and macrophages engage in multifaceted crosstalk, playing pivotal roles in mediating both adaptive and innate immune responses. Several instances of T cell-macrophage crosstalk are elucidated in recent literature. Upon antigen encounter, naive $CD4^+$ T cells undergo differentiation into Th1 or Th2 cells, determining their polarization. Th1 cells release cytokines such as IFN-γ and TNF-α, stimulating macrophages to enhance phagocytic activity, antigen presentation, and pro-inflammatory M1 polarization. Conversely, Th2 cells release cytokines like IL-4 and IL-13, promoting an alternative activation state in macrophages known as M2 polarization, associated with tissue repair and inflammation resolution.

The reciprocal interplay between Th1 and M1 macrophages reinforces their pro-inflammatory functions, creating a positive feedback loop that amplifies the immune response. Similar dynamics exist between Th2 cells and M2 macrophages, emphasizing their mutual influence in pro-regenerative responses. The sequence of activation may vary, but generally, $CD4^+$ T cell activation precedes macrophage activation. Importantly, the macrophage-T cell interplay is dynamic and context-dependent, influenced by factors such as the microenvironment, pathogen type, and individual immune responses. The oversimplified Th1/M1 and Th2/M2 paradigm fails to capture the nuanced reality of macrophage polarization and T cell responses.

As discussed, a key indicator for successful biomaterials in regenerative tissues lies in investigating T cell immune responses over extended *in vivo* conditions (e.g., up to 21 days) [114,144]. The distinction between Type I (Th1) and Type II (Th2) foreign body reactions reveals valuable insights. Successful tissue regeneration, as observed with optimized degradable hydrogels or dECM, correlates with a Type II

**Table 3**
Summary of cellular crosstalks in the biomaterial-modulated immune responses between adaptive and innate immune cells.

| Refs | Adaptive immune cells | Innate immune cells | Biomaterials and matrix cues (All 4.5 section) | Biological functions impacted by adaptive immune cells |
|---|---|---|---|---|
| [114] | $CD4^+$ T cells | M2 macrophages | dECM composition (mechanical and chemical cues, not determined) | $CD4^+$ T cells are necessary for M2 macrophage recruitment for tissue (muscle) regeneration |
| [144] | $CD4^+CD25+Foxp3+$ Tregs | Monocytes/ macrophages (to M2) | Possibly mechanical and chemical cues from biomaterials | Direct Tregs coculturing with Monocytes/macrophage diminish proinflammatory response under LPS |
| [147] | $CD4^+CD25+Foxp3+$ Tregs | M2 macrophages | Possibly mechanical and chemical cues from biomaterials | IL-10 and TGF-β secreted by M2 macrophage accelerate Tregs induction |
| [148] | $CD4^+CD25+Foxp3+$ Tregs | Macrophages | ROS from biomaterials | ROS from macrophage induce Tregs |
| [124] | B cells | Macrophages and neutrophils | biomaterial interface (mechanical and chemical cues) | B cell-deficient mice exhibited reduced fibrosis at the biomaterial interface, accompanied by a significant decrease in localized macrophages and neutrophils, in a CXCL13-dependent manner. |
| [51] | B cells | Macrophages (Th2 response) | Degradability of functional B cells (IgG1) targeting d-amino acid of hydrogel | High-degradable d-amino acid incorporated hydrogel, targeting B cells immunity, enhance Th2 tissue repair. |

reaction involving individual macrophages rather than multi-nucleated giant cells. The interplay between Th2 and M2 macrophages is implicated in achieving regenerative outcomes. In detail, biomaterial scaffolds induce a Th2 response, characterized by increased expression of IL-4, which is critical for polarizing macrophages towards a pro-regenerative M2-like phenotype. This interaction is evidenced by the upregulation of M2 macrophage markers such as CD206 and genes like Arg1 and Retnla in the presence of scaffolds from normal mouse with adaptive immunity. The interaction between adaptive and innate immunity in this process is revealed by Rag1−/− mice, which lack mature T and B cells. In these mice, the scaffold-mediated upregulation of IL-4 and M2 macrophage markers is lost, indicating that the adaptive immune system is necessary for the pro-regenerative macrophage polarization. Furthermore, the study shows that $CD4^+$ T cells, specifically those dependent on mTORC2 signaling for TH2 differentiation, are critical for this process. When Rag1−/− mice are reconstituted with wild-type $CD4^+$ T cells, the M2 macrophage phenotype is rescued, but this rescue fails when TH2-deficient T cells (Rictor−/−) are used. These findings underscore the essential role of T cell-regenerative macrophage (M2) interactions, mediated by TH2 cells and IL-4 signaling, in creating a pro-regenerative immune microenvironment in response to biomaterial scaffolds. This highlights the intricate interplay between $CD4^+$ T cells, macrophages, and biomaterials in orchestrating regenerative responses.

The subset of $CD4^+$ T cells exhibiting regulatory characteristics, known as $CD4^+CD25^+Foxp3^+$ Tregs, constitutes 5–10 % of peripheral $CD4^+$ T cells in the circulation. As explained, these Tregs have emerged as crucial regulators of immune responses to biomaterials. Of note, the interaction between Tregs and macrophages significantly influences the host immune response. Co-culturing macrophages/monocytes and Tregs have been shown to diminish the production of proinflammatory cytokines (IL-1β, IL-6, IL-8, MIP-1α, TNF-α) in response to LPS stimulation, concomitant with an increased expression of the regenerative markers (CD206 and CD163) and reduced expression of the proinflammatory markers (HLA-DR, NFκB) on macrophages [144]. Mechanistically, the inhibition of proinflammatory cytokine production by Tregs primarily relies on cell contact-dependent induction of soluble factors, suggesting that the biophysical cues of biomaterials could play a crucial role in modulating these interactions by influencing cell-cell contacts and the subsequent release of immunomodulatory factors [145].

Another interaction with Tregs involves the down-regulation of monocyte survival via the Fas/Fas ligand pathway [146]. Conversely, macrophages also influence the activity of Tregs. The induction of Tregs is well-established to be driven by IL-10 and TGF-β, primarily released by M2 macrophages. In this scenario, the emerging concept of regulatory macrophages (Mregs) introduces a novel perspective, as Mregs contribute to the induction of Tregs [147]. This induction has been linked to the production of ROS, and disruption of the NADPH-oxidase complex, involved in ROS production, impedes the induction of Tregs by macrophages, subsequently affecting immune tolerance [148]. Recent *in vivo* studies involving biomaterial implantation have demonstrated that Treg depletion leads to fibrosis or the formation of a surrounding capsule after biomaterial implantation. This outcome is accompanied by an exaggerated activation of macrophages, underscoring the interactive role between $Foxp3^+$ Tregs and macrophages in determining the reaction to biomaterials [124,126,149]. These findings shed light on the intricate interplay between Tregs and macrophages, crucial in shaping the host response to biomaterials.

As explained, the regulatory role of B cells in innate immunity, particularly their influence on macrophages, has been investigated through a biomaterial implantation such as alginate microspheres [124]. Mice with a B cell knock-out exhibited reduced fibrosis in subcutaneous implants compared to their immune-competent counterparts. Concurrently, a significant decrease in localized macrophages and neutrophils was observed at the biomaterial interface. Neutralizing CXCL13, responsible for recruiting B cells to the inflammation site, further supported the notion that B cells contribute to fibrosis, potentially through their capacity to modulate macrophages via the secretion of pro-inflammatory cytokines or antibodies. To further elucidate interactive roles, drug-induced depletion studies against macrophages or neutrophils were undertaken. Depleting macrophages in wild-type mice using clodrosome, as opposed to neutrophils, mimicked the anti-fibrotic effects observed in B-cell knock-out mice. This effect was also noted with the neutralization of CXCL13, emphasizing its role in B cell recruitment, particularly in areas enriched with macrophages near the biomaterial. This implies a reciprocal interaction between B cells and macrophages governing biomaterial-induced fibrosis.

Such anti-fibrotic effects of a macrophage-targeting colony stimulating factor-1 receptor (CSF1R) inhibitor, attributed to its overexpression in fibrotic tissues, positioned macrophages as upstream regulators of B cell recruitment near biomaterials, influencing fibrosis. B cells, influenced by various cytokines released from macrophages (e.g., IL-4, IL-6, and IL-21), were shown to uptake antigens as APCs with a diverse array of TLRs. This led to the production of pro-inflammatory cytokines, including IL-1, IL-6, TNF-α, and IFN-γ. In turn, B cells stimulated macrophages, enhancing their phagocytic activity, polarization, and cytokine secretion. While B cells were traditionally considered adaptive immune cells due to negligible gene expression changes, their impact on biomaterial immune responses, particularly in fibrosis, suggests an innate immune modulatory role. This parallels the interactive functions of B cells observed in the cancer microenvironment [150], underscoring their versatile contributions to immune regulation.

In another illustrative example supporting the interactive relationship between B cells and macrophages, a subcutaneously implanted D-

MAP prompted a tissue-regenerative Th2 "tissue repair" response characterized by the presence of functional B cells (IgG1) targeting D-amino acid [51]. Significantly, this response was conspicuously absent in the *in vivo* less-degradable chiral counterpart, L-MAP, indicating a selective induction of IgG1 against D-amino acid. Simultaneously, a notable infiltration of macrophages ($CD11b^{+}$) into the implanted D-MAP hydrogel was observed. The absence of IgG1 against L-amino acid and the diminished macrophage infiltration in non-adaptive immunity mice further underscored the pivotal role of B cells in modulating the macrophage response to implanted biomaterials. This compelling evidence reinforces the understanding that B cells play a crucial role in bridging innate and adaptive immunity, thereby shaping the biological response of biomaterials.

## 5. Conclusions and outlook

The adaptive immune system stands as a pivotal ruler not only for maintaining tissue homeostasis and combating diseases but also for regulating the intricate processes of tissue repair and regeneration. This review extensively discusses the influence of adaptive immunity on biomaterials in the context of tissue repair and regeneration following injury. While numerous biomaterial properties have been tailored to govern cell fate, only a subset of parameters, such as roughness, stiffness, and composition, have been controlled to specifically investigate adaptive immunity, particularly involving T cells and B cells.

As contemporary cancer treatments increasingly spotlight the significance of T cells in immunotherapies, there is a growing emphasis on understanding the role of biomaterials in shaping adaptive immunity. Unfavorable clinical outcomes arising from biomaterial implantation across diverse fields, including plastic surgery, dermatology, orthopedics, and dentistry, often manifest as fibrosis, abnormal immune responses, and degradation. While innate immunity alone has proven insufficient for predicting outcomes, an in-depth understanding of adaptive immune responses to biomaterials holds the potential to rectify inadvertent biomaterial failures in clinical settings. This involves unveiling the intricate cross-talk between innate and adaptive immunity, as well as elucidating direct adaptive immune responses in both cellular and humoral immunity [151]. Such insights are positioned to enhance the success of regenerative approaches employing biomaterials.

Moreover, multifunctional biomaterials capable of modulating both innate and adaptive immune responses in a synergistic or sequential manner could lead to more effective immunomodulatory therapies across various applications. Personalized medicine based on biomaterials offers promising prospects for patients with systemic autoimmune conditions, severe pre-existing immune responses, or allergies, such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes, sepsis, pulmonary tuberculosis, and other infectious diseases. This can be achieved through a deep understanding of immunology and the advancement of biomaterials. Major pharmaceutical and biomedical companies are investing substantial R&D resources to develop optimal carriers and biomaterial systems. At this point, recognizing the impact of adaptive immunity is crucial for determining the overall effectiveness of biomaterials. Future therapies may be realized through an immune chip system integrating both innate and adaptive immunity.

Despite the promising findings discussed in this review, several limitations and challenges remain in clinical translations. A major challenge is the intricate nature of the immune system and the potential for unforeseen interactions between biomaterials and various immune cells in human patients, raising concerns about the safety of adaptive-immunity-controlled biomaterials. Patient-specific responses to biomaterials present a significant hurdle, necessitating personalized approaches in biomaterial design and application. While animal models provide valuable insights, they may not fully replicate the complexities of the human immune system, making it difficult to predict clinical outcomes based solely on animal studies. Furthermore, the long-term safety and efficacy of biomaterials designed to modulate adaptive immunity need thorough evaluation, as chronic stimulation or suppression of adaptive immune responses could lead to adverse effects such as increased susceptibility to infections or the development of autoimmune disorders. A review of notable failures in biomaterial-based therapies could provide valuable lessons and guide future research directions. Another challenge lies in the manufacturing, scale-up, and cost-effectiveness of biomaterials with precise control over their physicochemical properties, which is essential for achieving consistent and reproducible immunomodulatory effects in clinical settings. The regulatory landscape for biomaterials that actively modulate adaptive immune responses is still evolving, and navigating the complex pathways to clinical translation can be a significant hurdle.

Clinical trials involving adaptive immunity-modulating biomaterials are in their early stages, with a focus on efficacy, safety and feasibility. The trials face unique challenges in design and execution, given the complexity of immune responses and the potential for long-term effects. Regulatory considerations for the advanced biomaterials are also evolving, with agencies like the FDA developing new guidelines for immunomodulatory products that include both biomaterials and cellular components. Ethical considerations, particularly regarding long-term immunological effects and the potential for unintended consequences, need careful attention. The development of standardized testing methodologies for assessing biomaterial-immune interactions is also crucial for ensuring reproducibility and facilitating regulatory approval.

The use of predictive modeling, including machine learning approaches, is emerging as a powerful tool in biomaterial design and in predicting immune responses. These computational methods can help streamline the development process and potentially reduce the need for extensive animal testing. Additionally, nano-scale materials offer unique opportunities for modulating adaptive immunity, with their ability to target specific immune cell subsets and tissues. However, the long-term effects of these nanomaterials on the immune system require thorough investigation. Emerging technologies, such as organ-on-a-chip platforms and advanced 3D or live imaging techniques in cells and tissues, hold promise for better understanding the complex interactions between biomaterials and the immune system in a physiologically relevant context.

Addressing these limitations and challenges will require close, interdisciplinary collaborations among biomaterials researchers, immunologists, clinicians, and regulatory agencies to ensure the safe and effective translation of biomaterial-based immunomodulatory therapies into clinical practice. This collaborative approach, combined with advanced computational modeling and nanotechnology, holds the promise of accelerating the development of safe and effective adaptive immunity-modulating biomaterials for a wide range of clinical applications.

The potential of biomaterials to modulate (auto)immune responses and integrate with existing immunotherapy strategies represents an exciting area for future research. The COVID-19 pandemic has highlighted the need for optimal vaccination platforms, benefiting from a comprehensive understanding of adaptive immunity against biomaterial carriers. As a prospective avenue, previously unexplored matrix properties, such as size, topology, stiffness, stress-relaxing, and viscoelasticity, either in isolation or combination, holds promise in influencing *in vivo* adaptive immunity involving T and B cells. Future research should focus on designing materials that effectively integrate with and modulate the local tissue environment, recognizing its importance in shaping immune responses. By merging existing findings with future insights, investigations into adaptive immunity-biomaterial interactions are positioned to reveal innovative strategies and designs for biomaterial development, particularly targeting diseases and injuries that manifest immunological characteristics aligned with either the innate or adaptive facets of the immune system.

## Ethics proof and consent to participate

All the authors contributed significantly to writing this review article, and agree to the content and being listed as authors on the manuscript.

## CRediT authorship contribution statement

**Jung-Hwan Lee:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Seong-Jin Shin:** Writing – review & editing, Writing – original draft. **Jun Hee Lee:** Writing – review & editing. **Jonathan C. Knowles:** Writing – review & editing. **Hae-Hyoung Lee:** Writing – review & editing, Funding acquisition. **Hae-Won Kim:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

## Declaration of competing interest

Kim HW is an editorial board member for Bioactive Materials and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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