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Directing immunomodulation using biomaterials for endogenous regeneration

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Abstract

Stem cell therapy and tissue engineering hold considerable potential for innovative and transformative strategies to repair damaged tissue form and function. Although many approaches are adopting *ex-vivo* expanded cells for transplantation, an alternative is to manipulate the biomaterial-host interactions that recruit the patients' own stem cells endogenously for regeneration. There are several considerations in targeting the biomaterial-host interactions therapeutically, not the least of which is the biomimetic design of extracellular matrix (ECM)-mimicking materials and the administration of navigation cues and small molecules that target specific aspects of the native healing cascades to stimulate homing of endogenous stem cells and, thereafter, their expansion and differentiation. A sequence of coordinated interactions between the local niche cells and implanted biomaterials offers signals and sign posts that may instruct the cells traveling toward the injured tissues. Furthermore, stem cell function is critically influenced by extrinsic signals provided by the niche as well as by the implanted biomaterials. Novel strategies harnessing growth factors and immunological cues to design materials not only can modulate the behavior of stem cells but also can alter innate and adaptive immunity in a controlled manner. We envisage that successful and safe endogenous regeneration will involve at least three aspects, *i.e.*, homing of sufficient stem cells, controlling cell fate determination, and blunting host immune responses to outside biomaterial devices. Improving our understanding of the biological and physicochemical signals of biomimetic biomaterials that govern immunomodulation for *in situ* tissue regeneration, particularly context-dependent macrophage (Mφ) polarization, will lead to a concurrent improvement in clinical outcomes.

Keywords: Biomimetic design; Biomaterials; Endogenous regeneration; Immunomodulation; Macrophage polarization

1. Introduction

Inducing the cell recruitment and cell homing of endogenous stem cells by the design of biomimetic biomaterials is the main strategy of *in situ* tissue engineering design, which is expected to become the focus and core of future *in situ* tissue engineering research [1-3]. In the last 20 years, tissue engineering research has rapidly developed, bringing hope for defect repair and regeneration of various human tissues and organs. However, the translation of tissue engineering and its products into clinical applications is facing enormous difficulties and challenges; the progress achieved in laboratory studies has not yielded true benefits to clinical patients [4,5]. Many key technical problems with respect to the biological behavior of *in vitro* cultured stem cells and their functional modulation after implantation into the body have not been fundamentally resolved [6]. More importantly, no matter which method is applied to implant the *in vitro* stem cells into a body, the clinical admittance requires strict cell culture conditions and complex procedures; consequently, the manpower, materials, and financial resources required have greatly hindered the translation of achievements in tissue engineering research on cell-based design into clinical applications [6,7]. It is foreseeable that this situation will not fundamentally change in the near future; therefore, the search for strategies to accelerate the clinical translation of tissue engineering has great significance and far-reaching influence [2].

Accordingly, research has become increasingly focused on the stimulation and homing of native cells from the patients themselves for *in situ* tissue regeneration, which is generally termed endogenous regeneration [7]. This approach stimulates tissue formation from resident stem or progenitor cells, aiming to enhance the body's potential for self repair and tissue regeneration; such regeneration research is currently the closest to the field of clinical medicine [6]. Multiple stem cells or precursor cells that are available for tissue repair and regeneration, including mesenchymal stem cells (MSCs), are almost ubiquitous in human tissues and organs [8,9]. Studies have shown that these stem cells are involved in wound repair and healing of host tissue through mobilization, migration, recruitment, and homing mechanisms [10,11]. Thus, the design of biomimetic biomaterials offers the possibility to simulate the *in vivo* living microenvironment of the stem cells and induce stem cell homing, thereby avoiding *in vitro* cell culture and transplantation and achieving *in situ* endogenous regeneration of damaged tissue [12,13].

However, homing of effective host resident cells to sites of regeneration poses a significant challenge to tissue engineers and biomaterials scientists because of the low efficiency of cell homing and the difficulties in coordinating cell trafficking, adhesion, proliferation, spreading, and differentiation [14]. After implantation of the biomaterial for cell recruitment, the inflammation and immune response of the host to the material is a particularly important factor affecting tissue regeneration, in which the early oxidative stress of the neutrophils (polymorphonuclear leukocytes, PMNs) and the subsequent phenotype and function of macrophages (Mφs) play key regulatory roles in the outcome of innate inflammation and tissue healing [13,15]. Studies have confirmed that under the synergy of physical, chemical, and biological signals in the microenvironment of cells, Mφs can be activated with the classical pathway (M1) or alternative pathway (M2). M1 Mφs can secrete a series of pro-inflammatory cytokines to hinder the interactions between the material and endogenous tissue and the tissue regeneration, whereas M2 Mφs can engulf the tissue debris and secrete anti-inflammatory cytokines to maintain the stability of the local tissue environment, which is conducive to tissue regeneration [16,17]. In addition, M2 Mφs can secrete a series of growth factors and chemokines to promote the migration

and recruitment of endogenous stem cells [15]. Accordingly, the balance between the classical pathway (M1) and the alternative pathways (M2) in the regulation of Mφs with biomaterials is crucial to the *in vivo* reproducibility of the *in vitro* results of the biomaterials [18]. Due to the lack of three-dimensional (3D) cell culture models, the current *in vitro* assessments of immune system modulation by biomaterials have shown that it is difficult to simulate the *in vivo* cell growth microenvironment, resulting in poor *in vivo* reproducibility of *in vitro* results, which greatly affects the application prospects of the biomaterials for tissue regeneration. With the deepening understanding of the M1-to-M2 transition of Mφs, *in vitro* modulation of Mφ phenotype and function has become possible [19-23]. The modulation of Mφ function to promote tissue regeneration by biomaterial design has been accepted and adopted by an increasing number of biologists, as well as tissue engineers [24-26]. An in-depth exploration of methods to reconstruct the regenerative microenvironment using biomaterials and to stimulate the body's self-healing potential can optimize *in situ* tissue engineering design and provide experimental support for the clinical translation and product development of biomaterials [1-3]. This manuscript reviews the background of *in situ* tissue engineering strategies involving stem cell recruitment and homing. We conduct a critical examination of the perspectives and challenges of this biomaterial-centered strategy with respect to material design and immunomodulation and explore the role of Mφs in such endogenous regenerative processes, as well as the potential biotechnologies and interventions that act cooperatively or synergistically to govern Mφ polarization toward a regulatory or anti-inflammatory phenotype that promotes tissue remodeling and ensures functional outcomes.

2. From *in vitro* engineering to *in situ* regeneration

With advances in the design of biomaterials and the deeper understanding of stem cell biology, research on tissue engineering has undergone an unprecedented, rapid development since the beginning of the 20th century, bringing new hope for defect repair of various human tissues and organs [4,5]. The traditional idea of tissue engineering is the design based on three elements (seed cells, biomaterials, and growth factors). Particularly, studies on the cell source, culture, and modulation of cell traits are the focus and core of this field [6-12]. In recent years, with the emergence of new concepts and methods of biomaterial design, especially since decellularized tissue matrices or whole organs have been used as the scaffold material for tissue engineering, the *in vitro* regeneration of complex tissues and organs is becoming possible [27-29]. At the same time, we are keenly aware that although the technology of *in vitro* isolated and cultured stem cells combined with new biomaterials for tissue regeneration has made significant progress and many achievements have been reported in the treatment of human diseases [30-33], the translation of regeneration and engineering strategies based on cell transplantation from experimental research into clinical treatment of patients will encounter many difficulties. On the one hand, multiple issues regarding stem cell transplantation remain controversial; on the other hand, many "bottleneck" problems in the *in vivo* and *in vitro* modulation of stem cells are still fundamentally unsolved [3,6]. At the technical level, the collection, *in vitro* culture and expansion, and implantation of clinically acceptable cells have many difficulties and challenges; for either autologous or allogeneic cell transplantation, a series of problems in the spread of pathogens, potential tumorigenicity, immune rejection, complex clinical procedures, and admittance difficulties exist to varying degrees [6]. We have reason to believe that tissue engineering products will someday be beneficial to clinical patients. However, as clinicians, we are not only attempting to identify treatments to

serve patients in the future but also hoping to develop simple methods to solve current clinical problems as soon as possible. To apply new technologies and new methods in a wider range of patient groups, especially in the treatment of some non-fatal diseases (*e.g.*, dental diseases), research should be aimed at making the treatments simple, practical, and economical. The proposal of the *in situ* tissue engineering concept has provided an opportunity to accelerate and promote the clinical translation of tissue engineering technology [1-3,34].

In almost any regenerative process after bodily injury, stem cells in the host stem cell niches are mobilized and recruited to the injured site to participate in the tissue repair and regeneration to varying degrees through a series of complex signals [10-13]. In recent years, researchers have found that this self-healing ability can be accelerated and enhanced by therapeutic interventions, thereby providing a new therapeutic strategy [9]. Thus, *in vitro* stem cell culture and transplantation is not the only strategy of tissue engineering research. Simulating, reproducing, and enhancing the self-repair and regeneration process in the injured tissue, as well as recruiting the host's own stem cells to achieve tissue regeneration, namely, endogenous regeneration, is expected to become a new option in tissue engineering research [14,35]. Because endogenous tissue regeneration avoids difficulty in the culture and transplantation of clinical stem cells, it is beneficial to the clinical translation, with a broad application prospect and development space. The tissue engineering research area is currently closest to clinical application [1-3].

Of note, biomaterials play an important role in reconstructing the cell microenvironment with different tissue engineering designs [14]. For *in vitro* tissue engineering design, the materials mainly play the role of template and scaffold (Fig. 1A); in the cell transplantation process, the material can act as the carrier for cell spread and implantation (Fig. 1B); when the tissue defect is repaired using the method of *in situ* tissue engineering, the material must also have the functions of recruiting host cells and inducing stem cell homing (Fig. 1C) [9,14]. Although the study of *in vitro* tissue engineering and cell transplantation is also inseparable from the ideas and concepts in material innovations, *in vivo* tissue engineering relies to a greater extent on the biomimetic and functional design of biomaterials [7,14]. Only with the reconstruction of the *in vivo* microenvironment of cell growth, the biologically controlled release of signaling molecules, the induced recruitment and homing of host cells, and effective cell regulation can endogenous tissue regeneration be truly achieved [14,34].

During the past decade, *in situ* tissue engineering based on biomaterial design has evolved significantly, offering considerable promise to restore form and function to damaged or diseased tissues and organs [1-3]. In our previous investigations, we successfully developed a glycidyl methacrylated dextran (Dex-GMA)/gelatin hydrogel system for controlled delivery of growth factors and for cell/tissue scaffolding (Fig. 2 and 3) [36,37]. Interestingly, a well-designed Dex-GMA/gelatin device can be used to deliver more than one growth factor [38] or intelligently control the release of target cargo [39]. These findings have great potential for incorporating cell homing factors into biomaterials for endogenous regeneration applications [14]. Indeed, there is considerable evidence that biomimetic biomaterials that incorporate cell homing factors support the migration of native reparative cells toward the injury site, and these cells have been demonstrated to facilitate wound healing and tissue regeneration [40-52]. Although the delivery of cell homing factors is a vital consideration [39-42], it is important to choose the proper scaffolding for facilitation of cell migration. In the field of *in situ* tissue engineering, the use of multiphasic biomaterials to replicate the nature of the native tissue is a key challenge and precondition for the organization of single cells into functional tissue. In the context of tissue formation, the 3D

microenvironment and material conformation significantly affects cell behavior and matrix deposition. Thus, a novel radially oriented collagen scaffold that has channels in both vertical and horizontal directions was designed because a cell that infiltrates the scaffold will align with the pore and follow the channels. Loaded with stromal cell-derived factor (SDF-1), this device was shown to be a promising strategy for *in situ* osteochondral regeneration [43]. In another study, a 3D collagen scaffold infiltrated with intrafibrillar silica improved osteoconductive properties and enhanced compressive stress-strain responses and toughness over nonsilicified collagen scaffolds. Thus, materials with well-designed components and structures can facilitate the process of cell homing and lead to a satisfactory regenerative outcome [44]. Despite great breakthroughs in the "cell recruitment design" of endogenously regenerative biomaterial, the majority of studies used *in vitro* cytology. To reproduce these results in animals and ultimately translate them into clinical applications, the following key issues must be solved [14,35]: (1) whether modulating the physical, chemical, and biological properties of the materials with respect to the immune system is conducive to cell recruitment and tissue regeneration and (2) whether the material can reconstruct a regenerative microenvironment to induce cell homing, promote cell proliferation and differentiation, and modulate the formation and vascularization of new tissue, thereby truly achieving *in situ* regeneration.

3. Stem cell homing: road toward *in situ* regeneration

In recent years, the research on *in situ* tissue engineering has made important progress, and the feasibility of the endogenous tissue regeneration strategy has also been demonstrated in different *in vivo* and *in vitro* models [9,53,54]. In 2010, the regeneration of periodontal ligaments and tooth-like structures was demonstrated without cell transplantation but via biomaterials loaded with SDF-1 and bone morphogenetic protein (BMP)-7 (Fig. 4A) [40]. Recently, in an *in situ* pulp revascularization model, SDF-1α-loaded biomaterial improved the *de novo* ingrowth of pulp-like tissues based on endogenous cell homing in pulpectomized mature dog teeth (Fig. 4B) [41]. Dental diseases are far from life threatening and are thus not considered a major target for novel regenerative research, such as stem cell transplantation, due to cost and potential risk. The concept of endogenous regeneration has gained attention in the field of regenerative dentistry [6]. Indeed, studies have shown that with a biomaterials design and the controlled release of multiple growth factors, the wound healing cascade of both pulpal and periodontal tissue can be simulated to promote self-healing and tissue regeneration [35]. Given the natural healing mechanism in the musculoskeletal system, the recruitment of stem cells from the side and the bottom of the defect and their subsequent reparative actions at the damaged site are thought to play a crucial role in osteochondral regeneration [56]. There is mounting evidence that a cell-free approach is practical and effective for bone tissue repair, representing a less complex and costly alternative to contemporary cell transplantation and tissue engineering strategies (Fig. 4C) [42-45,53]. In a milestone study involving cell homing to achieve endogenous cartilage regeneration, Mao and colleagues (2010) used an individually designed biomaterial with the anatomical shape of rabbit articular cartilage and transforming growth factor-beta3 (TGF-β3) to induce the *in situ* regeneration of the entire articular cartilage surface of a rabbit without cell implantation. These authors reported that the function of free movement in the experimental animal was restored within 3-5 weeks after surgery (Fig. 4D) [46]. Continuing their work, this group demonstrated that cartilage regeneration can be achieved by the homing of stem/progenitor cell populations from synovium, bone marrow, or adipose tissue [56];

furthermore, this technique was effective at treating severe neurological diseases, such as stroke, neurodegeneration, and spinal cord lesions (Fig. 4C) [47], facilitating skin wound healing (Fig. 4D) [48], and aiding in renal repair after renovascular disease (Fig. 4E) [49], cardiac repair after myocardial infarction (Fig. 1F) [50], and liver repair after acute/chronic liver diseases (Fig. 4G) [51,52]. Most, if not all, of these studies showed that biomaterials design and the controlled release of growth factors (including cell homing inducers) are critical to reconstruct the *in vivo* microenvironment [14,54].

Cell homing has been well studied, offers new therapeutic options for *in situ* tissue regeneration, and may serve as an alternative or adjunctive strategy to tissue engineering. However, for *in situ* tissue engineering, in-depth understanding is currently lacking with respect to the mechanism and regulation of cell homing, as well as the impact of immunity on cell homing [18]. For the specific method of *in vivo* tissue engineering, the induction of host stem cell homing by biomaterials is the critical first step, which must not be separated from the biological controlled release of cell homing inducers [36,37,57]. Under normal conditions, only with effective controlled release and intervention by biological signaling molecules may the endogenous stem cells migrate and be recruited by amoeboid movement, or with the help of blood flow, finally reaching the defect area to achieve tissue regeneration [14,35,58]. In addition, whether the modulation of the material on the immune system is conducive to cell recruitment and tissue regeneration is the key issue to be solved for *in situ* tissue engineering [15,18]. However, an in-depth and comprehensive understanding is lacking with respect to the reconstruction of the endogenous microenvironment by biomaterials in the process of endogenous regeneration and the regulation of the immune response. Furthermore, the influencing factors, regulatory strategy, and specific molecular mechanism await further investigation [59,60].

Thus far, studies have shown that different signaling molecules, such as SDF-1, TGF-β3, vascular endothelial growth factor (VEGF), BMP-2, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), monocyte chemotactic protein (MCP)-3, granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor/scatter factor (HGF), stem cell factor (SCF), and chemokine growth-regulated oncogene (GRO)-1, can induce varying degrees of stem cell homing for the repair and regeneration of several tissues under different conditions [14,34,61]. Further study, on the one hand, should be committed to developing controlled release carriers and controlled release technologies for different cytokines, thereby meeting the different controlled-release requirements of the various signaling molecules; on the other hand, research and development of the new biomaterial systems should be conducted, with optimization and screening for the signal molecules, as well as exploration of the screening, combination, and controlled release criteria [36,37]. Although some evidence suggests that *in situ* tissue regeneration is based on the recruitment and homing of host stem cells, only a better understanding of the molecular mechanism of cell homing and the related signaling pathways will allow the development of specific biomaterial systems for cell recruitment [1-3]. Although the underlying mechanisms by which stem cells are mobilized into blood from their original niche (*e.g.*, bone marrow), undergo transmigration across the endothelium to specific peripheral tissue, and reach the sites of injury via extravascular interstitial migration are not yet fully elucidated, current evidence suggests that stem cells display certain cellular activities similar to the homing of local mature leukocytes to inflammatory sites, which is known to involve an orchestrated multistep sequence of adhesive and signaling events [10,11,14]. While novel materials possess a tremendous capacity to regulate stem cell homing, it is essential for biomaterial scientists and tissue engineers to understand the basic molecular and cellular principles guiding immunomodulation by biomaterials because the implantation of biomaterials following injury alters the default wound healing response [59,60,63,63]. In particular, the

material design in terms of structural molecular patterns within unique tissue microenvironments for immune regulation and its impacts on *in vivo* cell recruitment and homing are critical for *in situ* tissue engineering strategies.

4. Role of Mφs in endogenous regenerative cascades

Our understanding of native wound healing and regeneration offers guideposts for designing endogenous strategies for therapeutic regeneration. Entrusting the host response to promote successful integration of the implants logically relies upon the *in vitro* design of biomaterials that incorporate one or more biologically active molecules [14]. Recent advances in this area have delivered highly innovative biomimetic scaffolds for use, but without exception, translating these material devices into therapeutics requires an in-depth understanding and investigation of the host response following their *in vivo* implantation [15]. PMNs and Mφs are recognized as the most important cells in the regulation of immune and inflammatory reactions, and *in vivo* and *in vitro* studies have revealed different early oxidative stresses of PMNs, as well as the responses and outcomes of Mφs, around different biomaterials, which may affect the inflammatory and healing processes at the interface [59]. Indeed, both cells also dominate the initial reactions on the interface contacting the tissue after biomaterial implantation. Following the injury response to the implantation of outside biomaterials, the infiltration of the tissue-material area by inflammatory cells is one of the earliest foreign-body reactions (FBRs). PMNs comprise the initial wave of inflammatory cells to enter the site and typically completely fill the implant 8 h after implantation. Monocyte-derived Mφs are recruited to this area shortly after PMN infiltration [64]. In this context, PMNs phagocytose host necrotic bacterial or cellular debris and propagate a pro-inflammatory response, leading to the local recruitment of monocytes and Mφs. Although PMNs may reach elevated levels shortly after implantation injury, the PMN response in terms of the release of T-helper (Th1)-associated pro-inflammatory cytokines and reactive oxygen species (ROS) is generally resolved within 3-4 days, along with a decline in the numbers of PMNs through apoptosis [65]. Thereafter, Mφs represent the predominant immunologic participant in the implantation response, and the interaction between Mφ and the implanted biomaterial greatly affects healing at the material-tissue interface, thus playing an important role in triggering and regulating the endogenous regenerative response [15,66].

Whether endogenous Mφs appear with stable conditions or inflammatory responses is based on significant characteristics such as the distribution of their location, surface markers, function, and migration capability (Fig. 5) [67-69]. Traditionally, the activation of the bactericidal function in Mφs is dependent on interferon-γ (IFN-γ) secreted by the antigen-specific T cells. With the stimulation of IFN-γ, the antigen presentation, secretion of toxic mediators and pro-inflammatory cytokines, and complement-mediated phagocytosis of the Mφs were significantly up-regulated, thus more effectively killing the bacteria and intracellular pathogens. This functional activation is known as "classical Mφ activation." With the discovery of Th1 and Th2 subsets derived from naive CD4(+) T cells and their molecular function, it was found that Th1 can secrete IFN-γ and activate Mφs and CD8+ T cells to promote the cellular immunity and enhance anti-tumor and intracellular pathogen functions. By contrast, through the secretion of interleukin-4 (IL-4) and IL-13, Th2 cells may be involved in the conversion of B class cells and antibody production to promote humoral immunity, as well as activate eosinophils, basophils, and Mφs. With activation by IL-4 and IL-13, the characteristics of Mφs change in a manner

completely different from the classic activation: the surface major histocompatibility complex-II (MHC-II) molecules and mannose receptors are significantly up-regulated, while the respiratory burst and inflammatory cytokines, as well as the synthesis of nitric oxide (NO), are inhibited, the bactericidal function is inhibited, the cell fusion between Mφ and the giant cell formation is increased, and the synthesis of tissue repair media is increased with antiprotozoal enhancement. The IL-4-mediated activation of Mφs is known as "alternative Mφ activation" [70,71]. Thus, Mφs can exhibit significant plasticity, with different physiological functions. Further research has shown that Mφs are a class of plasticized and heterogeneous cells with versatile and changeable immunity. In addition to the classical and alternative activation pathways, Mφs can also be activated by glucocorticoids, IL-10, and other immune complexes in their corresponding pathways, resulting in different phagocytosis and secretion functions, which partially overlap with the characteristics of the alternative activation mediated by IL-4 and IL-13. To date, at least three types of Mφs with alternative activation have been identified. The Mφs with classical activation and the three types of Mφs with alternative activation form the plasticized and diverse Mφ population. Each Mφ type has different phenotypes and functions, playing an important regulatory function in different immune responses and disease/reparative processes [72-75] (Fig. 6A). Among them, the Mφs activated through the classical pathway and the alternative pathway as two extremes of the plasticity modulation of the Mφ function are known as type I Mφs (M1) and type II Mφs (M2), with significantly different surface markers and cytokine secretion [20]. The former type is also known as classically activated Mφs (CAMs), mainly exhibiting pro-inflammatory activity, with the secretion of pro-inflammatory mediators including IL-1β, IL-12, tumor necrosis factor-α (TNF-α), IFN-γ, NO, IL-23, and IL-6, as well as a high expression level of ROS—which generally involve endogenous oxygen radicals and peroxides that likely form radicals related to oxygen metabolism; these pro-inflammatory mediators participate in activating a wide variety of antibacterial mechanisms, including the elimination and clearance of the oxidation reactions of the invaded pathogenic microorganisms. In CAMs, IL-12 is highly expressed, with a low expression level of IL-10. The latter type is also known as alternatively activated Mφs (AAMs). These cells have anti-inflammatory activity, mainly secreting IL-10, showing a high expression level of CD206 and the phagocytosis of tissue debris, clearance of ROS, and maintenance of the local tissue stability [70-73]. AAMs play an important role in wound healing, with the antagonistic effect of CAMs, to inhibit the inflammatory effect by the high expression of IL-10 and the low expression of IL-12. The differentiation process of Mφs toward M1 and M2 is known as Mφ polarization. In the actual situation, Mφs can be located at any stage between the extreme differentiation of M1/M2 and exhibit different phenotypes and functions [74]. Importantly, the Mφ phenotype is plastic and may change from one phenotype to another in response to paracrine and autocrine signals. The currently available analytical data suggest that Mφs may also adopt a transitional cell phenotype with characteristics and functions of both M1 and M2 subsets (Fig. 6B) [76,77]. Based on the important role of Mφs in innate immunity and adaptive immunity, several new modulation strategies based on Mφ polarization have become the emerging fields of biomaterial design in regenerative medicine [15,18,58,73-75]. The key roles of Mφs in functional tissue recovery from damage suggest that biomimetic materials capable of regulating the Mφ response following *in vivo* implantation, ideally in a well-defined, reproducible, and controlled manner, may also present improved regenerative outcomes in therapeutic applications [15].

The significant impact of Mφ regulation on the outcomes of tissue regeneration may play a key role in the *in vivo* reproducibility of *in vitro* results of the implanted material. The conversion between the Mφ subtypes and their regulation have not only promoted the study of clinically relevant diseases but also

provided a new idea for the development of new biomaterials, that is, to control the inflammatory response of the implanted material and induce stem cell recruitment by controlling Mφ subtype conversion in order to, among other things, promote the formation of new tissue [72-75]. After implantation of the biomaterial, an inflammatory response will first occur, and then, the corresponding wound healing and tissue regeneration will begin. In the early stage of material implantation, the blood monocytes can rapidly reach the wound site through chemotaxis stimulated by a variety of inflammatory mediators and then convert into Mφs, which are involved in tissue reorganization and, through the release of chemokines such as MCP-1, contribute to the further recruitment of leukocytes. In this context, Mφs are responsible for eliminating damaged cells and pathogens, being the main phagocytic cells in the initial inflammatory stages (CAMs, M1 Mφs); on the other hand, these cells release a variety of biologically active substances at later stages, which play an important regulatory role in the resolution of inflammation and in promoting the wound healing process and the successful remodeling of the implants (AAMs, M2 Mφs) [71]. With the influence of the foreign object reaction in the body, after inducing protein adsorption and matrix deposition on the surface of the implanted material, M1 Mφs can secrete related cytokines (proinflammatory cytokines) and recruit fibroblasts to form dense fibrous tissue on the surface of the implanted material. The fibrous tissue wrapping the implanted material is not only the main consequence of the reaction to the foreign object in the body but also the underlying cause for separation of the implanted material from the endogenous tissue [78]. Therefore, the study of the impact of different implanted materials on the activity of Mφs will contribute to the further understanding of the healing process at the interface between the implanted material and the endogenous tissue [62].

As a necessary component of efficient and functional tissue repair, M2 Mφs are known to participate in the endogenous host injury response, suggesting a promising strategy to activate or augment self-repair mechanisms via M2 Mφ-directed immunomodulation [15]. Studies have shown that biomaterials have an important impact on the differentiation of monocytes into Mφs, as well as the adhesion, apoptosis, integration, and cytokine secretion of Mφs on the surface of the implanted material, which is regulated by the proteins adsorbed on the surface of the implanted material [59,79]. By designing and preparing biomaterials with different compositions, hydrophilicities, hydrophobicities, surface potentials, surface topologies, and morphologies to interact with various extracellular matrix (ECM) proteins and plasma proteins, the protein adsorption characteristics and adsorption kinetics of the materials were investigated to explore the regulation and mechanism of the materials with respect to Mφs [80-84]. It has been demonstrated that inducing the conversion of Mφs from type M1 to type M2 is an important strategy to reduce the immune response after implantation of the material, induce cell homing, and ensure the interaction between the material and the tissue in favor of cell differentiation and tissue regeneration [85-89]. Unfortunately, much of our current knowledge on the functional plasticity of Mφs is largely based on how soluble factors (*e.g.*, cytokines and chemokines) modulate their phenotypes and function. Mφs are often present in an *in vivo* milieu that delivers a spectrum of microenvironmental cues, including soluble factors. The complexity of the environment underscores the necessity to identify other factors (*e.g.*, substrate topography/stiffness and matrix architecture/composition) that may play a role in coaxing Mφ adhesion, spreading, and polarization in biomaterial design [59]. An in-depth exploration of the molecular mechanism of the modulation of Mφ polarization by biomaterials is not only conducive to research and development of new immunomodulatory biomaterials but also the key issue to be solved to translate "cell-recruiting biomaterials" into large animal experiments and clinical applications.

5. Regulation of Mφ phenotype and function by biomaterials

As detailed above, shortly after implantation, biomaterials are extensively infiltrated by multiple types of immune cells. Through paracrine and autocrine signaling, these cells can evoke and modulate the inflammatory responses that regulate the subsequent healing and regenerative process [68,69]. Among them, Mφs represent the key player in the activation and inhibition of numerous pathways, and the delicate balance between and orchestration of the M1 and M2 phenotypes are central to functional regeneration [15]. The regulation of M1/M2 polarization by substrate topography and composition has long been well recognized, and the type of biomaterials, their pore sizes and fiber diameters are all important parameters that are able to affect Mφ phenotypic profiles [79,80]. In recent years, the development of research on transcriptomics, transgenics, and epigenetics has provided opportunities for investigators to study the molecular mechanisms of the polarization and plasticity of Mφs, in which signaling molecules, transcription factors, microRNA, and histone modification all play an important role. Chemokines are a class of small protein molecules, originally discovered in the inflammatory environment, that interact with chemokine receptors on the cell surface [34]. Chemokines can attract and induce inflammatory white blood cells such as monocytes, activated T cells, and PMNs to the inflammation area [90]. Studies have shown that chemokines and their receptors play a key role in regulatory immune responses; they can mediate specific homing of the tissue with lymphocytes such as T cells and B cells [91] and regulate the migration and survival of hematopoietic stem cells, as well as the formation of blood cells [92]. Chemokines are the important driving molecules for the directional movement of monocytes/Mφs, and they can affect the type and quantity of inflammatory immune cells with local infiltration. It is known that M1/M2 can express different chemokine receptors; therefore, one possible strategy for designing anti-immune biomaterials is to specifically attract a certain subtype of Mφs to reach the pathological site. Typically, TNF-α and IL-6 are highly expressed in M1 Mφs, with a low expression level in M2, while Arginase and FIZZ-1 (a novel cysteine-rich secreted protein associated with pulmonary inflammation) are highly expressed in M2, with low or even no expression in M1. Thus, the Mφ polarization type can be identified by the phenotypic analysis to investigate the diversity of the Mφ functions and dynamic changes in the development of a disease [93].

Mφ polarization is induced and regulated by different cytokines and immune complexes. MCP-1 is a member of the chemokine family, with specific chemotaxis and activation for monocytes. It is an effective mediator that attracts monocytes into the infiltrated tissue and plays an important role in recruiting Mφs to the injured site [94]. More specifically, recent findings suggest that a relatively rapid release of MCP-1 from biomaterials following implantation leads to the early deposition of native-like ECM, which promotes the adhesion/proliferation of host cells and binds important immunoregulatory cytokines (*e.g.*, IL-10), subsequently ensuring favorable tissue remodeling and formation [74,82]. TGF-β1 is also an important mediator of inflammation through the inhibitory regulation of the immune and inflammation response. It also affects tissue-forming cells by regulating their proliferation and differentiation and promoting the formation of new tissue [95]. TNF-α is an inflammatory cytokine produced by activated Mφs, with a promotive effect on inflammation. The binding of different activated molecules onto the surface of Mφs can activate downstream intracellular signaling pathways, causing the function and phenotype maturation of four subtypes of Mφs (M1, M2a, M2b, and M2c). The main signal pathways include [80] signal transducer and activator of transcription (STAT), nuclear factor

kappa-B (NF-κB), peroxisome proliferator-activated receptors (PPARs), suppressors of cytokine signaling (SOCS), liver X receptor (LXR), and CCAAT-enhancer-binding protein (C/EBP)-β, among others [66].

Generally, it is believed that the activation of M2 is mediated by STAT6; SOCS1 can also inhibit M1 polarization and participate in the regulation of M2 activation. Protein jmjd3 is a demethylase containing a Jumonji-C (jmjC) domain. The activation of Toll-like receptors (TLRs) reportedly may up-regulate the expression of jmjd3, and interferon regulatory factor 4 (IRF4) is the target gene of jmjd3. The deficiency of jmjd3 does not affect Mφ polarization in the M1 phenotype but may suppress Mφ polarization in the M2 phenotype [20]. IRF4 negatively regulates the TLR pathway, and the expression level of the M2 molecular marker in the IRF4 knockout Mφs is significantly lower than that in the wild type, suggesting that jmjd3 and IRF4 co-regulate the polarization of M2 Mφs [96]. PPAR is recognized to be related to lipid metabolism and the maturation of M2 Mφs; it is significantly up-regulated in IL-4-induced M2 cells. The synthesis of Arg1 as a functional marker of M2 activation after PPAR knockout is inhibited by more than 50%. Further studies have shown that the binding of a PPARγ/retinoid X receptor (RXR) response element in the essential enhancer region of Arg1 synthesis can significantly promote Arg1 synthesis and M2 polarization, and the axis of IL-4/IL-13/STAT6/PPAR is a necessary signaling pathway for the functional maturation of M2 [97]. Kruppel-like factor 4 (KLF4) is an important transcription factor in the initiation of hematopoiesis. KLF4 not only suppresses M1 polarization by inhibiting NF-κB but also co-functions with STAT6 to promote the activation of M2 Mφs and M2 gene expression [98]. Although the molecular mechanism of the M1/M2 polarization regulation in tissue regeneration remains to be further studied, the immunomodulatory factors in biomimetic biomaterial design are attracting increasing attention and concern [20].

Exploration of the main signaling molecules, transcription factors, and regulatory strategies and methods in Mφ polarization has important significance for biomimetic biomaterial design [69,70]. Diversity and heterogeneity are the important characteristics of mononuclear phagocytes. They are not only inherently variable in phenotype and function but also change corresponding with the microenvironment in a particular direction, which determines the role of multiple functions of the mononuclear phagocytes. With deeper research into the M1-to-M2 transition of Mφs, different levels of exploration have been conducted into the morphology, surface molecules, cytokine secretion, and polarization pathways, as well as the relevant regulatory factors, and new insights into these areas will continue to be generated [99-101]. However, the *in vivo* studies of Mφs are based on the microenvironment of the body, and the construction of an *in vitro* microenvironment simulation model is necessary for reconstructing the tissue regeneration microenvironment to regulate Mφ polarization using the biomaterial. The primary selection based on a single factor for the physical (structure, surface morphology, lightness, hardness, etc.) and chemical (composition of the polymer materials) properties of the materials, as well as the optimization of multiple factors, will definitely elucidate the impact of the biomaterial on Mφs and its mechanism [59]. Of note, the surgical implantation of exogenous biomaterials is commonly associated with an FBR that consists of persistent M1 Mφ activity and increased scar deposition [78-80]. To date, there have been many new studies aimed at modulating biomaterial properties with specially designed surface chemistry and structural characteristics to reduce the persistent pro-inflammatory M1 Mφ response, which have provided important references for the research and development of biomaterials (Fig. 7) [81-89]. Recently, it was also found that in the case of biomaterials for the regeneration of load-bearing structures, mechanical cues exerted on immune cells through mechanotransduction can also directly impact their phenotype and their underlining

genomic profile without impairing the ability of Mφs to synthesize ECM [82]. The complexities of the host immune system can potentially be tailored to use and sense specific cues such as dose, spatial pattern, molecular recognition, physiological location, cell phenotype, and cellular trafficking, which all are known to participate in the activation and kinetics of the innate and adaptive immune responses [66]. Recent investigations clearly indicate that physical and mechanical cues, alone or functioning synergistically with soluble factors in an *in vivo* milieu, can regulate Mφ behavior [59]. Due to the lack of an ideal *in vitro* cell culture model, in-depth research on the regulatory mechanism of the materials (particularly the water-insoluble signal caused by the physical and chemical properties of the material) with respect to Mφs and the key signaling pathways is lacking, which directly limits the clinical translation process of the novel biomimetic biomaterials, functional design, and the related products.

6. Future directions

Although *in situ* tissue engineering is a relatively new field of biomedical research, there has been a rapid growth in the field of biomaterial development over the past few years [14]. The role of the innate immune system of the human body, including Mφs, in the host response to implanted materials has recently received considerable attention [15]. Mφs exhibit strong plasticity and can be polarized to achieve a spectrum of functional phenotypes in different microenvironments. The re-differentiation of Mφs can occur with changes in the microenvironment. This characteristic may enable the Mφs to make a rapid and effective response to changes in the complex microenvironment [71]. Using the materials in different constructions to interact with monocytes/Mφs can further reveal their behaviors of adhesion, differentiation and integration, and apoptosis on the material; furthermore, the changes in structure and morphology of the material after interacting with Mφs can be examined to clarify the possible mechanism of the material degradation mediated by Mφs/foreign object giant cells and to identify the factors having the key role in the adhesion activity and phenotypic development of Mφs among the physical and chemical structural parameters of the material. However, the *in vivo* stem cell microenvironment has never been generated by a single or independent factor on Mφs. Although the impacts of different physical [102-104], chemical [105,106], and controlled release factor [107,108] parameters on the phenotype and function of Mφs are widely recognized, an in-depth study on the integrated immune regulation with multiple factors is lacking. Modification of the material surface using a specific protein or polypeptide with an adhesion sequence can modulate the impact of the biomaterial on the adhesion, activation, integration, and apoptosis of Mφs to elucidate the ligand-receptor interactions in the activation/inhibition of Mφs, as well as the underlying mechanism [109-111]. These studies have explored new design approaches for the development of the desired biomaterial-Mφ interaction to enhance the compatibility of the biomaterials, which have provided a theoretical basis for the research and development of novel biodegradable tissue engineering materials with the potential for clinical application [59].

Scientists have been inspired by immunological principles in nature, and in the current century, they have begun to design biomaterials to clone specific biological processes with extreme precision to control the design of devices toward biomedical applications [18]. However, the role of physical and mechanical properties of biomaterials in the regulation of Mφ phenotype and function requires further investigation [62]. Immune cells are embedded in a complex *in vivo* milieu of more than just soluble factors; thus, the mechanical properties of this microenvironment must be carefully considered in the

design of regenerative biomaterials. From a physical point of view, alterations in the physical and mechanical aspects of the biomaterials may have a profoundly important effect on the properties of the resident stem or immune cells following implantation. The inflammatory processes and reparative cellular responses to the mechanical stress of the material (*e.g.*, adhesivity, matrix stiffness, nanotopography, and external forces), can be integrated into the overall tissue regeneration process [112]. Recently, many reviews have highlighted how the physical cues of implants play vital roles in the classical hallmarks of Mφs and inflammation [18,59, 60,62,112,113]. In addition, the way in which a cell migrates is influenced by the physical properties of its surroundings [113]. Taken together, these data suggest that physical influences of biomaterials remarkably affect the homing and accommodation of both immune and reparative cells. Although fundamental research has confirmed the substantial impact of physical signals on Mφ phenotype and function, a wealth of literature in this field has been generated in 2D cell conditions [59]. Understanding how the physical aspects of the cell microenvironment affect cell migration and Mφ function in physiologically relevant 3D matrices poses a considerable challenge, particularly when trying to understand these cellular events in a complex tissue environment. Therefore, the extrapolation of *in vitro* biomaterial design to *in vivo* situations calls for a more comprehensive understanding of these problems in native biological systems [113-115]. Over the past decade, a close collaboration between material scientists, engineers, and biologists has begun to produce the types of physical parameters that can influence the human immune system. Current studies seek to identify and characterize the mechanics and molecules of structural transitions within materials and cells. The mechanistic aspects of mechanotransduction are beginning to be unraveled with the advent of super-resolution microscopy and single-molecule methods for in vitro nano-manipulation [116,117]. In this context, physical signals derived from matrices are generally transduced into biochemical signaling events that subsequently guide cellular responses, including cell homing and Mφ polarization [118,119]. Mφ and stem cell adaptation to mechanical alterations and their mechano-regulating and mechano-coupling functions appear to be crucial steps in the inflammatory response to implants [15]. Progress in this field necessitates tacit understanding of the physicochemical nanoarchitecture aspects of cell-material interactions. These features can then be exploited and manipulated for the development of the next generation of sophisticated materials [120,121].

When developing biomaterials for cell recruitment, it is also necessary to consider the effect of the cytokines secreted by Mφs on the function and differentiation of homing stem cells. Research on the effect of a single cytokine on stem cells has relatively deepened. The *in vivo* microenvironment and the cytokines secreted by Mφs co-influence stem cells. A rational design of a co-culture model of stem cells and Mφs requires the construction of a relatively stable and independent microenvironment of the material to allow further in-depth study [20,59]. *In vivo*, cells reside in a 3D ECM structure that possesses an intricate, unique, and often fractal topography with a heterogeneous mixture of pores, fibers, and ridges that range from the submillimeter to the nanometer scale. Hence, both biological signaling and mechanical integrity are critical in the design of cell culture substrates and materials. In this context, the bioinspired topographic features of biomaterials may drive immune cells into spatial arrangements and provide topographical cues that regulate their phenotype and function [122]. Although much of the work performed thus far has been on engineered substrate materials, work with 3D systems for cell and tissue manipulation are emerging. The effects observed *in vitro* may very well translate to *in vivo* conditions in the future. In this context, the construction of an *in vitro* cell culture material model (artificial ECM) that introduces different cells into the porous gaps of the material so that it can achieve a designed spatial and temporal distribution is expected to become an important model

for in-depth exploration of the immune cell-stem cell interaction for studying the regulation of Mφ polarization by the physical, chemical, and biological signals of the materials [60,123]. Tissues, organs, and their various components may be conceptualized as specific or distinct medical devices that possess a wide range of mechanical properties and exert physical signals influencing Mφs in a manner similar to engineered biomaterials, which will directly guide the biomimetic and functional design of biomaterials.

Much work remains to be done to elucidate the underlying mechanism for the physical and mechanical regulation of Mφ phenotype and function. In terms of mechanobiology, the pathways between the nuclei, cytoskeleton, and potential transcription factors and the links between adhesion, cytoskeletal dynamics, and activation are still largely unknown [59]. With the development of modern means and methods of detection, the tools for the *in vivo* monitoring of Mφ phenotype and function allow further *in vivo* reproducibility of the results of *in vitro* studies, along with improvements in the design of immune modulation for cell-recruitment biomaterials [124-126]. Based on the design of biomimetic biomaterials, the focus on the regulation of Mφs by the physicochemical and biological properties of materials in *in vitro* research of material models, as well as the related molecular mechanisms, will provide data support for immune modulation with biomimetic biomaterials in the future. At the same time, *in vitro* exploration of the design parameters of biomaterials for cell recruitment and the reproduction of the results of *in vitro* studies in *in vivo* mouse models of Mφ polarization will help predict functionality of *in vitro* material design, thereby enriching the theoretical basis of endogenous regeneration and providing a reference and ideas for various *in vitro* tissue engineering projects, particularly those related to scaffold design [59]. Most likely, more than one Mφ phenotype is needed for complex processes, such as endogenous wound healing and regeneration. Therefore, the key to future developments may lie in the timely transition of Mφs from one phenotype to another, which presents a formidable challenge to biologists, biomaterials scientists, and tissue engineers. Using both biological and physicochemical influences to maintain functional plasticity and coax Mφs to attain beneficial phenotypes in a timely manner may be the most promising strategy for real therapeutic outcomes.

7. Conclusions

Decades of efforts from different research groups have brought immunomodulating biomaterial research to an important crossroads. The significant impact of the physical and chemical signals of biomaterials on Mφs and stem cells has reached a unanimous agreement. Constructing a 3D *in vitro* microenvironment for cell growth—as well as exploring the independent and synergic regulation of Mφs, the mutual influence of the physicochemical properties of the materials (chemical composition, surface morphology, and mechanical properties), and the biological signals using the means and research methods of molecular biology and immunology to reveal the key factors and molecular mechanisms of the regenerative microenvironment involved in positive immune modulation (especially the physicochemical properties)—is the focus and core of the biomimetic design of future biomimetic biomaterials. Using Mφ polarization-controlled animal models and new means and methods of *in vivo* detection to observe the impacts of the biomaterial on the Mφ M1/M2 polarization and cytokine secretion involved in immune modulation of cell homing and tissue regeneration may provide not only new ideas for the construction of endogenous regenerative microenvironments, the stimulation of the self-healing potential, and the optimization of the *in situ* tissue engineering design but also experimental

support for the clinical translation and product development of biomaterials. In the near future, new insights into material chemistry and conformation will translate into the development of novel biomaterials that can precisely modulate endogenous wound healing processes. These advancements hold great promise for tissue engineering and therapeutic applications.

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Captions

Fig. 1. Roles of biomaterials in tissue engineering and regenerative medicine (illustration is not to scale). (**A**) Biomaterials can serve as a template for the regeneration of new tissue, typically to fill specific anatomic defects (green). (**B**) Biomaterials can serve as carriers/vehicles for the delivery of stem cells, typically to localize transplanted cells at sites of tissue injury; to maintain stem cell viability, proliferation, and differentiation; and to promote outward cell migration at an appropriate stage of differentiation, *i.e*., to disperse differentiated cells from a material niche into regenerating new tissues. (**C**) Biomaterials can serve as an artificial niche, which is used to recruit host stem cells from a local cell niche neighboring a tissue defect independent of blood flow or from a distal central cell niche (*e.g*., bone marrow) with the aid of blood flow for endogenous tissue regeneration, thus avoiding the need for transplanting exogenously expanded cells.

Fig. 2. The development of microparticle drug delivery systems based on glycidyl methacrylate-derivatized dextran (Dex-GMA)/gelatin for delivering a wide range of therapeutic agents. (**A**) A representative scanning electron microscopy (SEM) image showing a microparticle with corrugated surfaces; (**B**) a representative SEM image showing a microparticle with smooth surfaces.

Fig. 3. The creation of a macroscale drug delivery platform based on glycidyl methacrylate-derivatized dextran (Dex-GMA) and gelatin materials with cabin structures that potentially exert control over the spatiotemporal presentation of molecular and cellular payloads. (**A**) A representative scanning electron microscopy (SEM) image showing the corrugated shell; (**B**) a representative SEM image showing the cabin core, with each cabin possessing its own interconnected porous structures.

Fig. 4. Schematic representations of the potential therapeutic applications for endogenous regenerative techniques based on the recruitment and homing of host resident stem cells (illustration is not to scale). The examples listed include odontogenic tissue regeneration, *e.g*., regeneration of dental pulp tissue (**A**) and periodontal structures (**B**); musculoskeletal regeneration, *e.g*., regeneration of bone (**C**) and cartilage/tendons (**D**); nerve regeneration (**E**); skin regeneration (**F**); kidney repair (**G**); treatment of heart disease (**H**); and therapy for liver fibrosis (**I**).

Fig. 5. Schematic representation of source of monocytes (illustration is not to scale). Monocytes originate in the bone marrow hematopoietic stem cells (HSCs) [68,71]. In response to macrophage colony-stimulating factor, HSCs undergo multiple differentiation steps. First, the cells divide into monoblasts and then into a monocyte lineage (*e.g*., pro-monocytes) before becoming monocytes. The monocytes exit the bone marrow and are released into the bloodstream, where they circulate for several days and are thought to differentiate into a phenotypically distinct cell subset before entering tissues for the replenishment of tissue-resident Mφs. In response to injury, monocytes can efficiently infiltrate inflammatory sites and respond to environmental cues by adopting either classically activated (M1) pro-inflammatory Mφs or alternatively activated (M2) wound healing/regulatory phenotypes. The M2 phenotype can be further subcategorized into M2a, M2b, and M2c, based on their specific yet overlapping functions. Although the Mφ phenotype spectrum is characterized by the classical M1 Mφs and by the alternative M2 Mφs, there are many other recognized Mφ subsets between the two Mφ designations.

Fig. 6. Schematic representation of the phenotype and function of descendant macrophages (Mφs) (illustration is not

to scale). (**A**) A description of the common inducers that influence the phenotypic polarization and activation of Mφs into recognized Mφ subsets (M1, M2a, M2b, and M2c), including interleukin (IL) family members, G-protein coupled receptor (GPCR) ligands, toll-like receptor (TLR), lipopolysaccharide (LPS), interferon gamma (IFN)-γ, and tumor necrosis (TNF)-α, and their cytokine and effector molecules, including IL family members, transforming growth factor (TGF)–β, TNF-α, reactive nitrogen intermediates (RNI), intracellular nitric oxide (NO), and reactive oxygen intermediates (ROI). (**B**) Through paracrine and autocrine signals, tissue-resident Mφs represent a dynamic plasticity. They can give rise to Mφs with distinct phenotypes (*e.g.*, M1 and M2 subsets). The activated Mφs can occasionally change back into inactivated Mφs. Plastic Mφs are presumed to adopt a transitional phenotype with the characteristics and functions of both M1 and M2 Mφs [66].

Fig. 7. Schematic representation of the biomaterials design in terms of immunomodulation for *in situ* tissue engineering (selected strategies; illustration is not to scale). (**A**) Surface modifications of the chemistry features of the materials. (**B**) Modifications of the physical and mechanical features (*e.g.*, nanotopography, stiffness, porosity, and/or structure) of the materials. (**C**) Coating of materials with extracellular matrix-mimicking biomacromolecules (*e.g.*, collagen and proteoglycans) that modulate immune cell behavior through the provision of natural binding sites for cell adhesion receptors (*e.g.*, integrins) and/or the interaction with endogenous cytokines and growth factors. (**D**) Functionalization of materials through the incorporation of bioactive molecules (*e.g.*, integrin adhesion sites). (**E**) Functionalization of materials through the incorporation of growth factors. (**F**) Functionalization of materials through the incorporation of other pharmaceuticals (e.g., anti-inflammatory mediators) [70].

Fig.1.

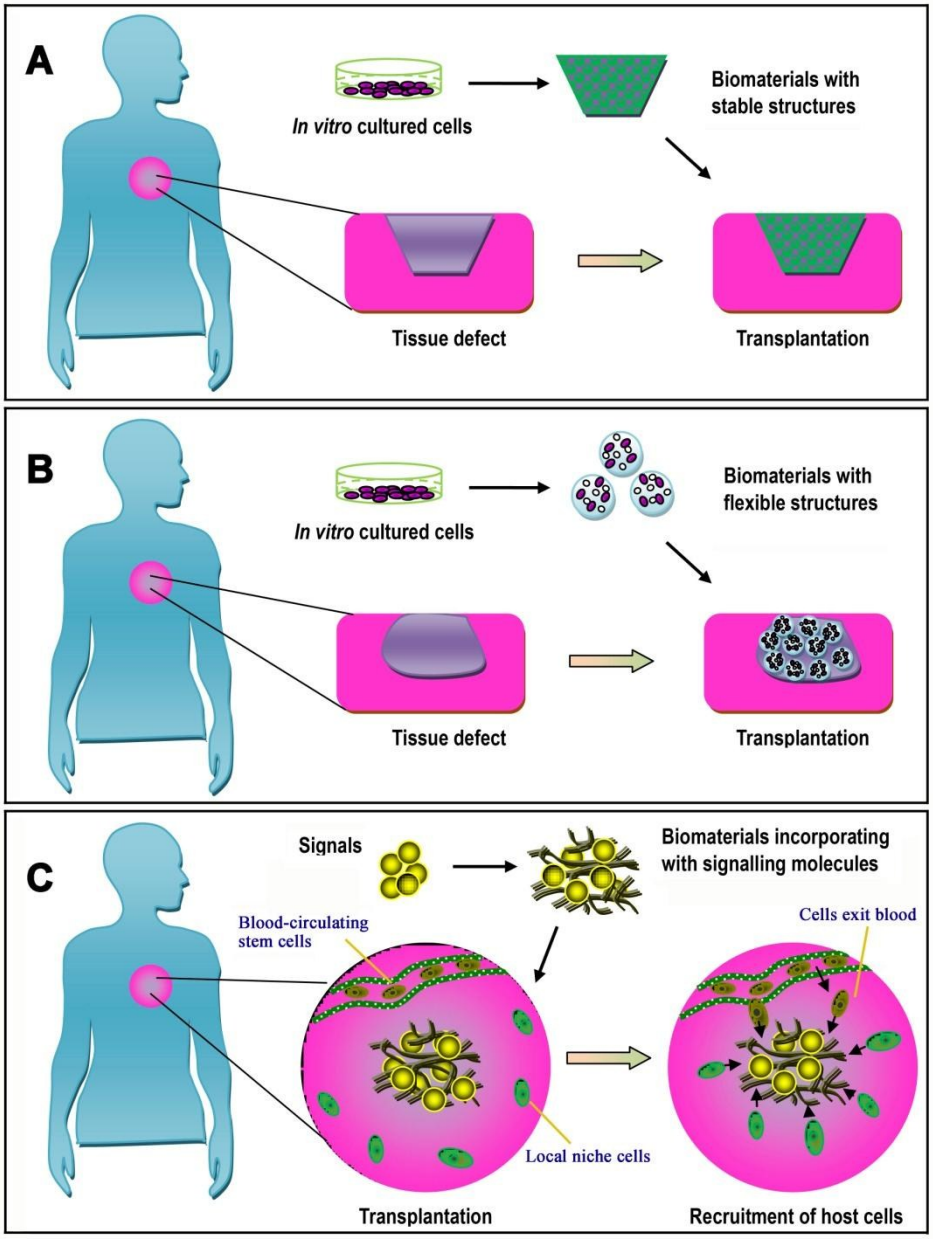
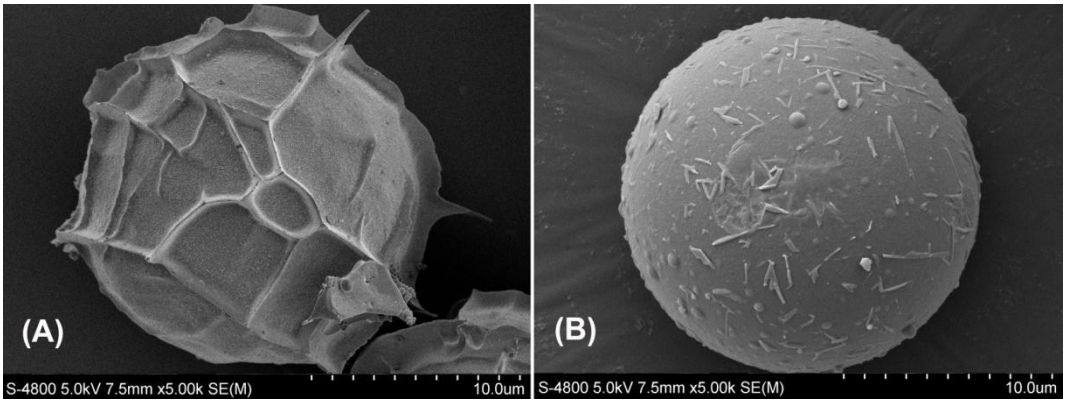


Fig.2



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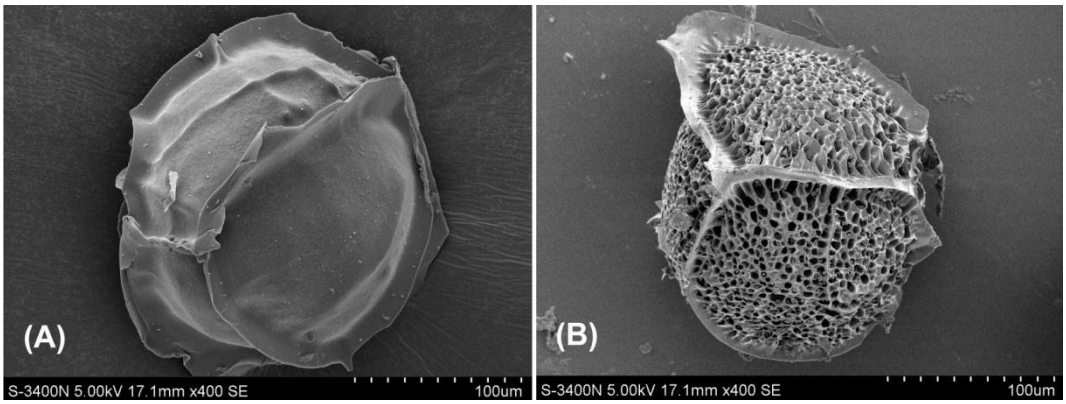

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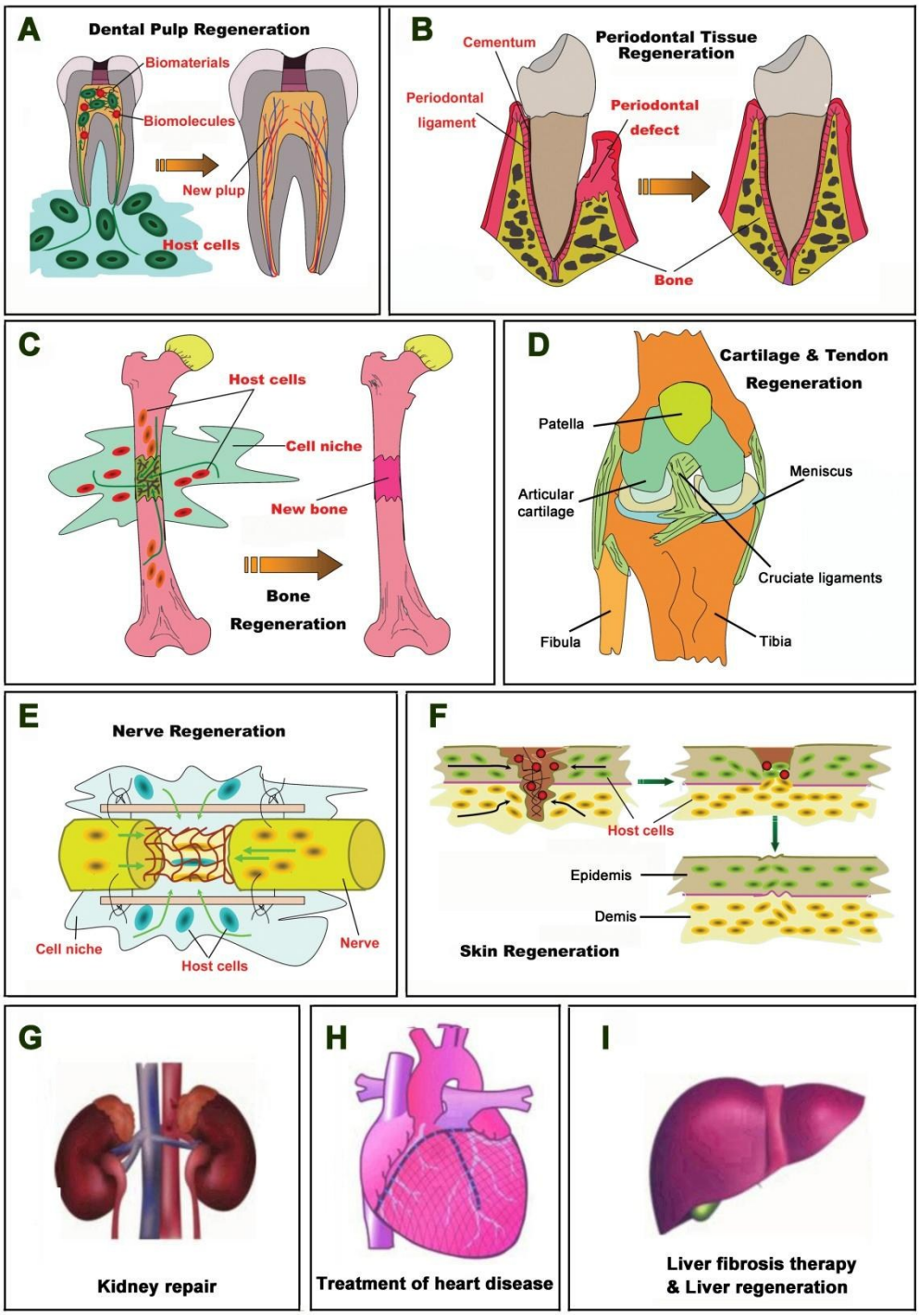

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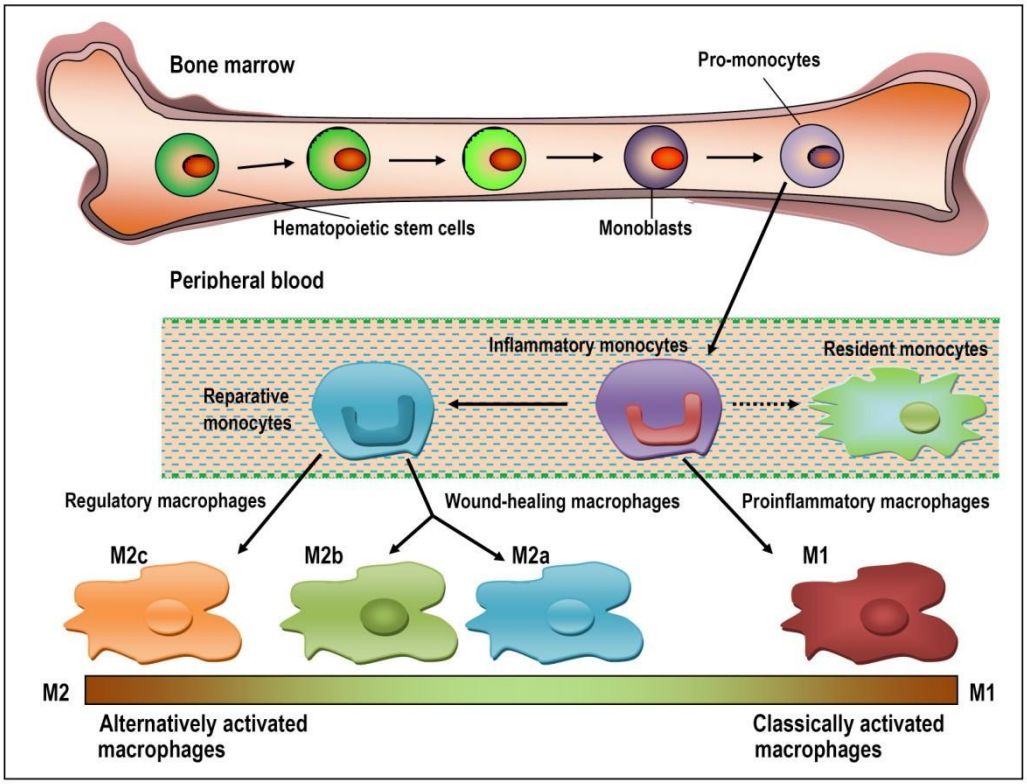

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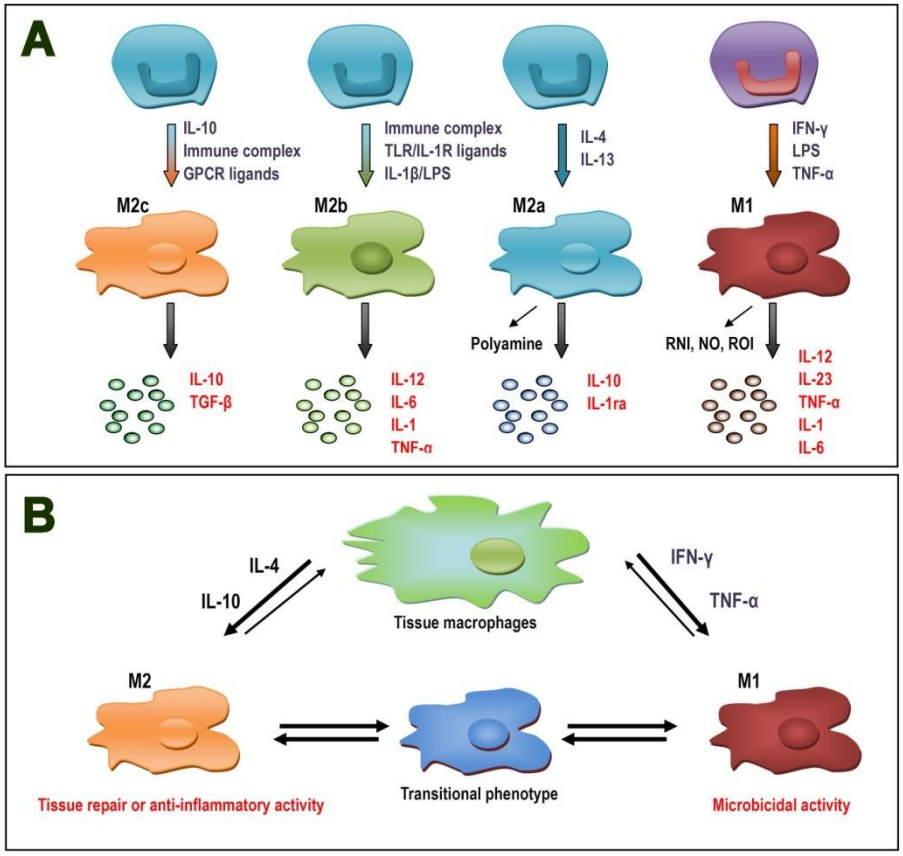

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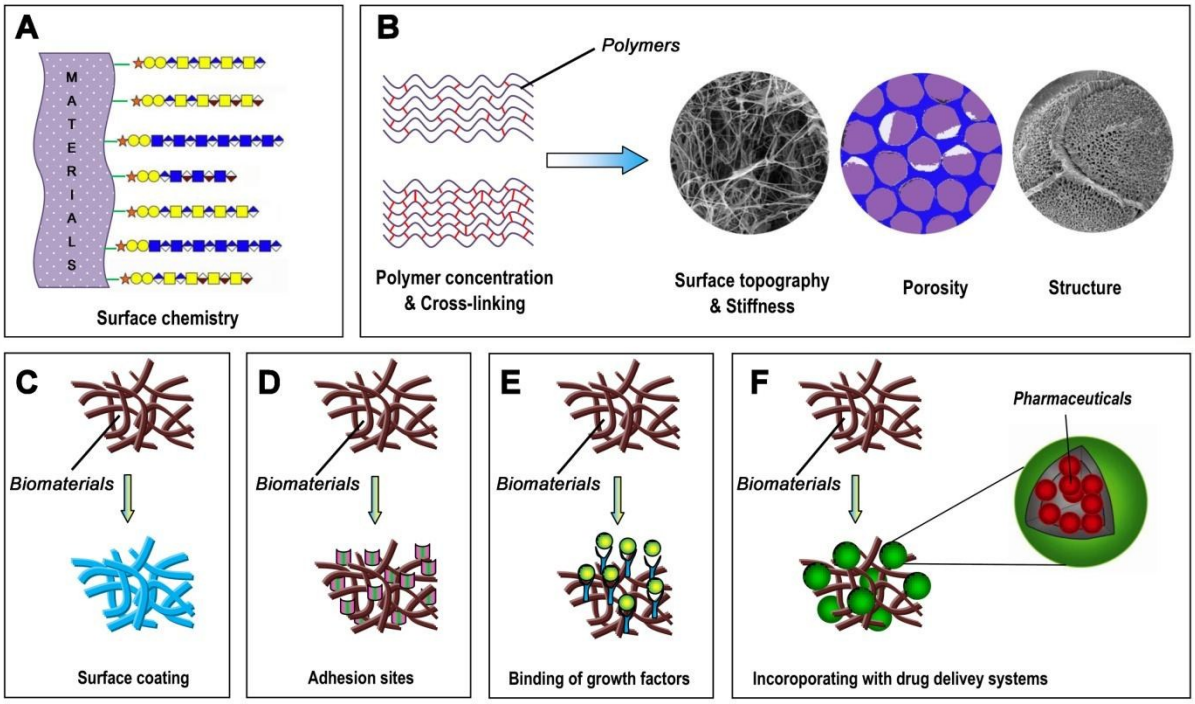
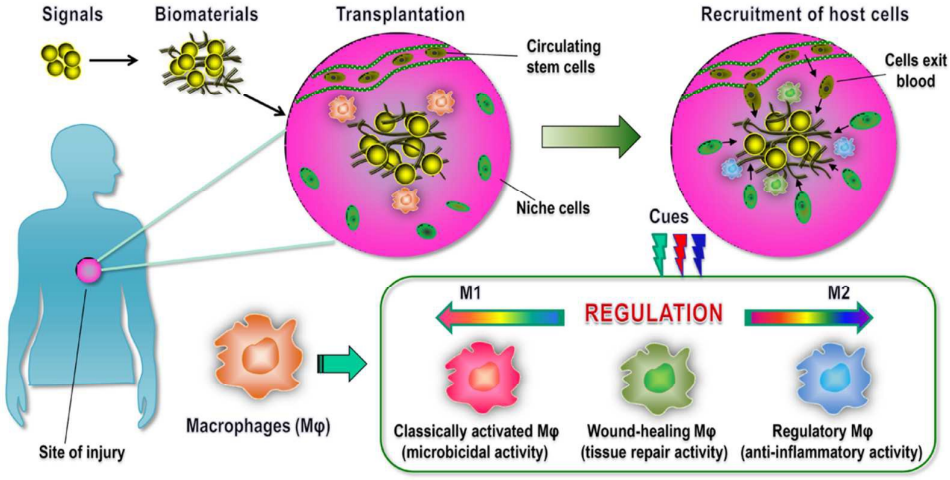


A table of contents entry



Biomaterials recreated an artificial biochemical and mechanical niche at the implanted site that coaxed polarized macrophages to display a spectrum of functional phenotypes that are required for stem cell homing and endogenous regeneration.