Engineered biomaterials for in situ tissue regeneration

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Abstract | In situ tissue regeneration harnesses the body's regenerative potential to control cell functions for tissue repair. The design of biomaterials for in situ tissue engineering requires precise control over biophysical and biochemical cues to direct endogenous cells to the site of injury. These cues are required to induce regeneration by modulating the extracellular microenvironment or driving cellular reprogramming. In this Review, we outline two biomaterials approaches to control the regenerative capacity of the body for tissue-specific regeneration. The first approach includes the use of bioresponsive materials with an ability to direct endogenous cells, including immune cells and progenitor or stem cells, to facilitate tissue healing, integration and regeneration. The second approach focuses on in situ cellular reprogramming via delivery of transcription factors, RNA-based therapeutics, in vivo gene editing and biomaterials-driven epigenetic transformation. In addition, we highlight tools for engineering the next generation of biomaterials to modulate in situ tissue regeneration. Overall, leveraging the regenerative potential of the human body via engineered biomaterials is a simple and effective approach to replace injured or diseased tissues.

All organisms, including humans, are capable of regeneration mediated by molecular processes, which are directed by the gene-expression programme that controls renewal, restoration and growth. Recent advances in regenerative medicine leverage the innate regenerative potential of the mammalian body to generate complex tissue structures. The approach of using the body's regenerative abilities, in combination with engineered biomaterials, is known as in situ tissue regeneration. Specifically, engineered biomaterials, loaded with bioactive cues, can be used to direct endogenous progenitor or stem cells to the site of an injury and aid the healing of damaged tissues. During this process, biomaterials provide a structural framework to facilitate the attachment and migration of host stem and progenitor cells, and drive the differentiation of these cells into tissue-specific cell types.

The modern concept of tissue engineering was introduced by Langer and Vacanti[1] in 1993. Since then, a range of synthetic biomaterials with tunable biophysical and biochemical characteristics have been fabricated. For optimizing the use of cells, protocols have been developed to isolate and expand cells under specific in vitro conditions, populate synthetic scaffolds and obtain cell-laden scaffolds that can be implanted back into the body. More recently, the concept of cellular reprogramming fundamentally changed the course of regenerative medicine[2]. With this approach, terminally differentiated cells, such as skin cells, can be directly converted into a pluripotent state through the delivery of cell-fate-changing transcription factors. Thus, this technology provides an unlimited source of progenitor cells that can be directly reprogrammed (transdifferentiated) to specific lineages by expression of a transcriptional 'code'[3,4]. Additionally, there have been notable recent advances in therapeutic delivery to control and direct tissue regeneration (for example, the conjugation of proteins and small molecules without losing bioactivity[5] and on-demand delivery for precise release of biochemical cues[6]).

Regeneration of damaged tissue can be achieved through two tissue-engineering approaches — ex vivo and in situ. In ex vivo tissue engineering, scaffolds are combined with cells and biomolecules outside the body to obtain cell-laden tissue constructs for implantation (FIG. 1a). This approach relies on the generation of biologically relevant constructs in vitro to recapitulate the native tissue functions[7]. However, ex vivo tissue engineering has notable limitations. These include donor-tissue morbidity, the need for large quantities of immune-acceptable cells to populate synthetic scaffolds and challenges owing to extensive in vitro cell expansion under non-native conditions, such as the lack of reliable and reproducible cell sources and the loss of cellular phenotype. Furthermore, the autocrine and paracrine signalling effects for ex vivo tissue engineering are difficult to recapitulate.

Such disadvantages have motivated the use of in situ tissue regeneration (FIG. 1b), which leverages the body's innate regenerative potential, while eliminating the

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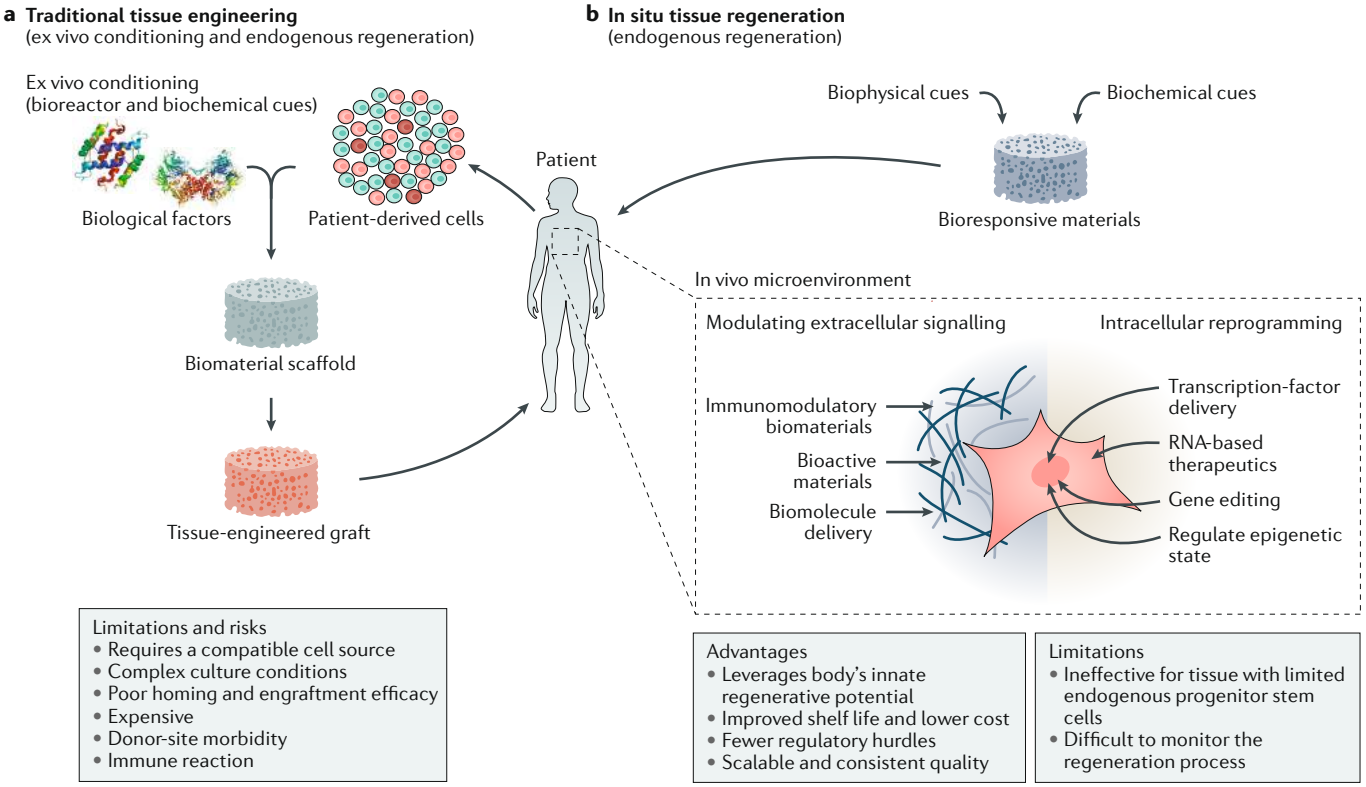


Fig. 1 | **In situ tissue engineering. a** | Traditional tissue-engineering approaches require the pre-seeding of engineered scaffolds and ex vivo conditioning before implantation into the body. **b** | In situ tissue regeneration uses bioresponsive materials that harness the innate regenerative ability of the body. These materials are loaded with biochemical and biophysical cues to recruit endogenous cells for tissue healing.

need for ex vivo cell manipulation. There are several approaches to in situ tissue engineering, including biomaterials that can be loaded with bioactive cues, to guide functional restoration to the site of injury. These approaches are relatively simple and eliminate the need for harvested cells, thus, reducing regulatory hurdles. In addition, complex cell culture conditions are needed to obtain functional tissues ex vivo, which are not needed for in situ approaches. Lastly, the shelf life of synthetic scaffolds surpasses that of cell-laden scaffolds. Thus, in situ approaches are more favourable than ex situ approaches for clinical translation.

Despite progress in tissue engineering and regenerative medicine, few technologies have been translated into the clinic. This is mostly because of our limited ability to understand and control the regenerative process, resulting in suboptimal technologies. Only a few ex vivo biomaterials systems are approved for clinical use, including Apligraf (keratinocytes and fibroblast in a collagen matrix for diabetic foot ulcers) and Dermagraft (human fibroblast with extracellular matrix (ECM) and biomaterial scaffolds). Most of these biomaterials systems provide temporary solutions or alternatives for simple tissues, such as skin, which lack complex cellular organization. In addition, some tissues, such as cartilage, cardiac and the central nervous system, do not regenerate because of a limited supply of endogenous cells. In such a scenario, ex vivo approaches can provide better outcomes than in situ approaches.

More in situ biomaterials systems are available for clinical use than ex vivo systems. These include the INFUSE Bone Graft for orthopaedic or dental applications, NeuraGen and Neurotube for nerve conduits and GORE-TEX for vascular grafts. Compared with ex vivo systems, in situ biomaterials systems face fewer regulatory hurdles, owing to their lack of cellular components. However, biomaterials for in situ tissue regeneration have the ability to interact with and alter the in vivo microenvironment. This results in the need for regulatory approval for all aspects of safety and performance, including host–tissue receptivity, effect on gene expression and signalling, short-term and long-term effects on the local microenvironment (including inflammation, foreign-body response, fibrosis or rejection) and safety or therapeutic effects of the degradation products. Accordingly, the additional verification and validation tests for biomaterials-based in situ regeneration require substantially more effort and resources than for bioinert scaffolds or devices.

This Review outlines recent developments for in situ tissue regeneration. First, we discuss the innate regenerative potential of the body, including the role of tissue-specific stem cell niches in local tissue regeneration, factors influencing the kinetics of endogenous cell mobilization and homing, and the role of the immune response in tissue repair. Second, we discuss the biophysical and biochemical characteristics of engineered biomaterials in directing endogenous cells to the implant site and priming

them to perform tissue-specific functions. Finally, we discuss biomaterials-driven approaches to harness the innate regenerative potential of the body. In particular, we focus on tissue regeneration directed via extracellular signals provided by biomaterials and scaffolds, and reprogramming of endogenous cells via intracellular delivery of reprogramming factors using biomaterials.

Innate regeneration

Regeneration is a coordinated process of cell growth and differentiation, and tissue morphogenesis. This process involves the generation of billions of cells, including a highly evolved feedback loop to eliminate potentially damaged or unfit cells. This regenerative process is one of the most complex biological phenomena, involving many cell types, growth factors, cytokines and metabolites. This can be broadly divided into three distinct but overlapping stages of tissue regeneration: inflammation (acute and chronic), neo-tissue formation and tissue remodelling[8,9]. During the first stage, the immune system performs a multitude of tasks, including wound debridement and the release of chemokines, metabolites and growth factors (FIG. 2). Inflammatory cells clean up dead cells and infectious organisms, thus, reducing local inflammation and initiating a tissue-repair response. In the second stage, the migration and proliferation of endogenous progenitor and stem cells to the site of injury leads to the replacement of damaged ECM and the formation of vascularized networks. The final stage is tissue remodelling, which can extend over a long period of time, in some cases, up to two years. In this stage, the biophysical integrity of the newly formed ECM is improved through reorganization, degradation and resynthesis of the tissue.

The degree and duration of this repair response depend on the tissue location and development stage. For example, fetal tissue has the potential to completely recreate damaged tissue. However, postnatally, the regenerative capacity of the body is substantially reduced and followed by some degree of fibrosis — a non-functioning mass of collagenous connective tissue (that is, a scar). The immune system has a crucial role in repairing

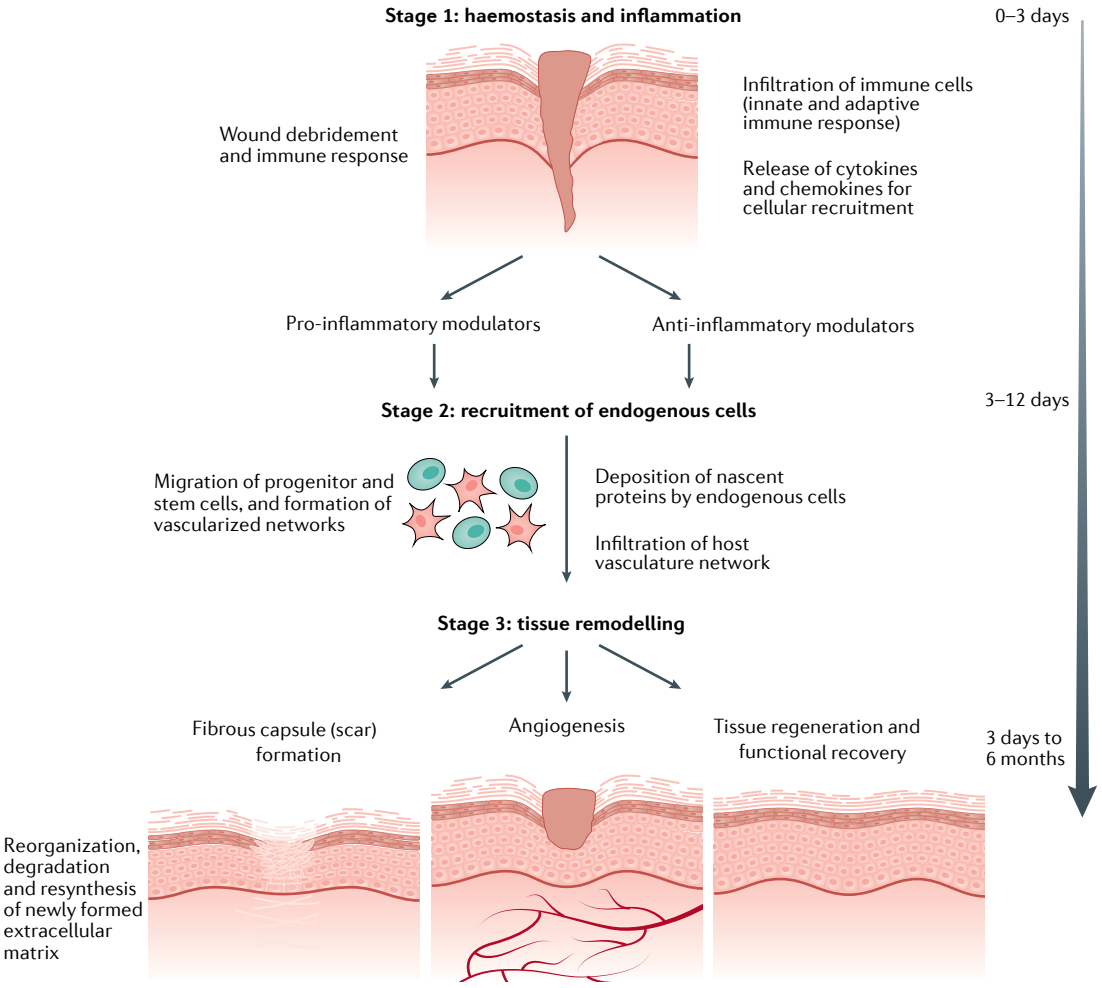


Fig. 2 | **Innate regeneration.** Innate regeneration of the body can be classified into three major stages: haemostasis and inflammation; neo-tissue formation owing to recruitment of endogenous cells; and tissue remodelling of newly formed extracellular matrix. The immune response has an important role in healing. Tissue injury results in the recruitment of neutrophils, monocytes and macrophages, which have immune-modulatory effects, the balance among which governs the regeneration process.

tissue and often determines the degree of scarring, as well as the structure and function of the restored tissue. Although the loss of regenerative capacity inversely correlates with the development of the immune system across different species, it is not clear why the regenerative potential of tissues differs within the same species. These differences are often attributed to the variation in types of immune responses, kinetics and duration, as well as the type of immune cells involved. This results in a range of outcomes, from incomplete healing (such as scar formation) to complete regeneration.

The immune response to tissue injury can happen in both a positive and a negative manner, depending on the type of tissue, as well as its maturity. First, a tissue injury is detected by tissue-resident macrophages, owing to the release of damage-associated molecular patterns and pathogen-associated molecular patterns. Soon after the injury, circulating immune cells, such as neutrophils, are recruited to the site of injury, followed by monocyte and macrophage recruitment. Immune cells are broadly divided into two categories: pro-inflammatory (including monocytes, M1 macrophages and T helper (T_H) cells) and anti-inflammatory (such as M2 macrophages and T_H2 cells). Pro-inflammatory cells are responsible for debridement, whereas anti-inflammatory cells are important for remodelling the ECM, blood-vessel maturation and differentiation of endogenous stem cells. The balance between these two types of immune cell dictates the degree of tissue regeneration. As our understanding of the immune response increases, in situ biomaterials-based approaches to direct the immune response towards tissue repair and regeneration are becoming attractive[10,11].

In the case of dysregulated tissue repair or the absence of endogenous stem cells, it is possible to transdifferentiate mature cells into pluripotent stem cells using cellular reprogramming. Several recent studies have highlighted the use of combinatorial transcriptional 'code' for in situ cellular reprogramming[3,4]. This approach can replenish the endogenous stem-cell population and contribute to tissue repair. Leveraging endogenous cells for in situ regeneration also mitigates one of the major risks associated with cell-based therapies — immune rejection of transplanted (exogenous) cells. However, it is also important to note that not all tissues have a sufficient number of endogenous cells. For example, cartilage, cardiac and nerve tissues have limited quantities of endogenous stem and progenitor cells, which makes it difficult to regenerate them using conventional in situ approaches.

Overall, based on our understanding of the body's innate regeneration potential, a range of biomaterials-based approaches can be developed to leverage immune responses and to recruit endogenous stem cells. For example, biomaterials can be used to modulate different immune components (cytokines, cells) during the tissue-repair process. In addition, they can be used to facilitate the homing of endogenous progenitor and stem cells using chemotactic signalling to facilitate tissue regeneration. Moreover, smart and responsive biomaterials can be designed by integrating immune-mediated mechanisms with the homing of endogenous cells.

Characteristics of biomaterials

The range of scaffolds used for in situ tissue regeneration includes monolithic, microporous, nanoparticles, fibrous, hydrogels and 3D-printed scaffolds (FIG. 3a). Biomaterials used to fabricate scaffolds can be polymers, ceramics, metals and composites. These materials derived from synthetic, natural or a combination of sources must respond to biological signals and interact with the immune system and endogenous cells to stimulate regeneration. These responsive biomaterials can interact with the body through their biophysical and biochemical properties, which can alter local tissue microenvironments by modulating the immune system and controlling the kinetics and degree of healing from endogenous cells (from scarring to total regeneration).

The biophysical characteristics of biomaterials, such as stiffness, structure, topography and degradation, can alter the local tissue microenvironments through intracellular and intercellular signalling (FIG. 3b). These changes in tissue microenvironment include altering the pH or temperature, and controlling the presence of enzymes, cells, ions or radical species. The matrix stiffness dictates the adhesion, spreading and fate of stem cells[12,13]. For example, stiffer surfaces promote adhesion and spreading of bone marrow cells, which directs stem cells towards osteogenic lineages, whereas a softer matrix facilitates a round-shaped morphology of stem cells and promotes chondrogenic differentiation[12]. The porosity of the scaffold dictates cellular infiltration, as interconnected pore networks can facilitate the transport of nutrients, oxygen and waste products. Porosity also promotes vascularization of scaffolds by facilitating angiogenesis[14]. Similarly, topological features, such as the presence of patterned surfaces (high surface roughness), can promote or suppress cell adhesion and cell fate[15]. In situ degradation of biomaterials is desired for tissue regeneration. The degradation rate should match the rate of tissue generation for optimal tissue growth. Some biomaterials, like collagen or gelatin, can be degraded using cell enzymes for scaffold remodelling and deposition of neo-tissue. Because biomaterial degradation results in the loss of mechanical stiffness, the newly formed tissue should sustain load transfer. Finally, if the biophysical characteristics of biomaterials are not matched to those of the tissue, suboptimal healing can result in poor functionality of regenerated tissue, tissue loss and implant loosening. Overall, the biophysical properties of biomaterials can be tuned to dictate cellular fate and to modulate the in vivo microenvironment.

The biochemical characteristics of biomaterials include the release of signalling biomolecules, such as proteins, small molecules in the form of drugs, as well as degradation and dissolution of the scaffold (FIG. 3c). Biochemical cues can be used to activate specific signalling pathways or a set of genes to direct and control cellular responses. For example, signalling factors released from responsive biomaterials can trigger the activation of cell-receptor proteins, which control processes such as protein transport into the cell, cell morphology and other signalling pathways. Biomaterials can stimulate angiogenesis in vivo[16], for example, by sequestering

pro-angiogenic growth factors within scaffolds[17,18]. Sequestering or attachment of these signalling molecules to biomaterials can result in sustained activation of cell-surface receptors and subsequent downstream signalling in contrast with exogenous (unbound) delivery of growth factors[19]. Degradation of biomaterials can also release signalling ions that can alter the local microenvironment. For example, calcium can trigger calcium-sensing receptors, which are important for cell proliferation, differentiation and chemotaxis[20]. Moreover, the release of ions from calcium-phosphate-based biomaterials can activate endogenous cells to differentiation towards bone lineages[21,22]. Overall, sequestering growth factors or releasing mineral ions from biomaterials can alter the tissue microenvironment, further modulating regenerative processes.

The biophysical and biochemical characteristics of biomaterials, as well as the implantation site, need to be considered and optimized for the intended application. For example, in the regeneration of cartilage tissue, endogenous cells should assume a round-shaped morphology, whereas in bone regeneration, biomaterials should facilitate cell adhesion and cells should have a spindle-shaped morphology. Similarly, angiogenesis is preferred for the regeneration of vascularized tissues or organs such as the heart, muscle, kidney, liver and lung, but should be suppressed during regeneration of avascular tissues, such as cartilage and cornea. It is also important to consider the availability of endogenous stem and progenitor cells in specific tissue types, which can alter the regenerative process. Thus, the biophysical and biochemical characteristics of the biomaterial need

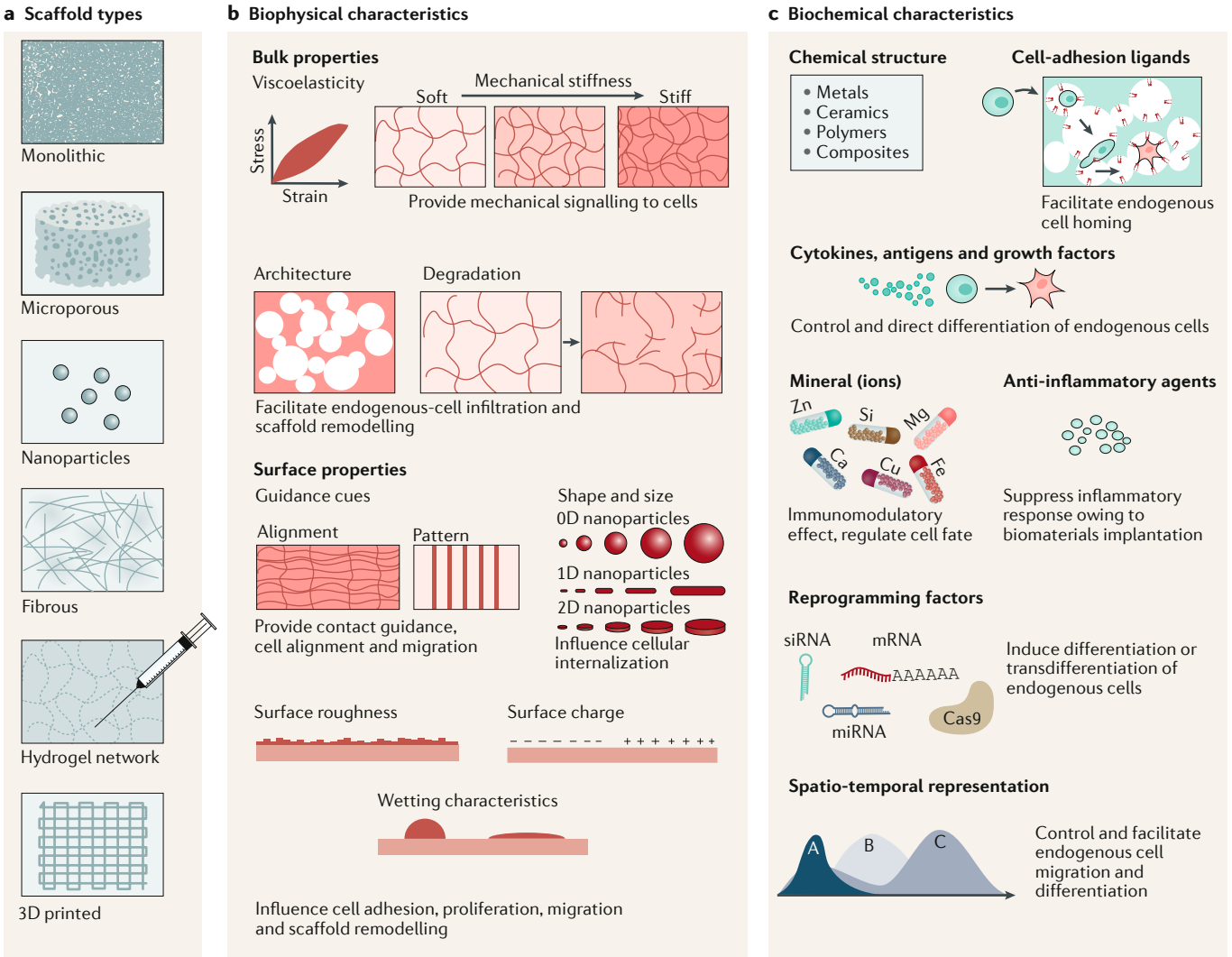


Fig. 3 | **Approaches to engineer biomaterials for in situ tissue regeneration.** **a** | A range of biomaterial scaffolds, including monolithic, microporous, nanoparticles, fibrous, hydrogels and 3D-printed scaffolds, has been developed to harnesses the innate regenerative capacity of the body. The characteristics of these biomaterials can be classified as either biophysical or biochemical. Both these characteristics function in synergy to facilitate tissue healing. **b** | Biophysical characteristics include bulk properties such as viscoelasticity, stiffness, architecture and degradation, as well as surface properties such as guidance cues, shape and size of nanomaterials, roughness, charge and wetting characteristics of biomaterials. These biophysical characteristics can control a range of cellular functions, including adhesion, migration, proliferation and differentiation. **c** | Biochemical characteristics include the chemical structure of biomaterials, as well as the presence of signalling biomolecules, such as proteins, minerals, small-molecule drugs and reprogramming factors. mRNA, messenger RNA; miRNA, microRNA; siRNA, small interfering RNA.

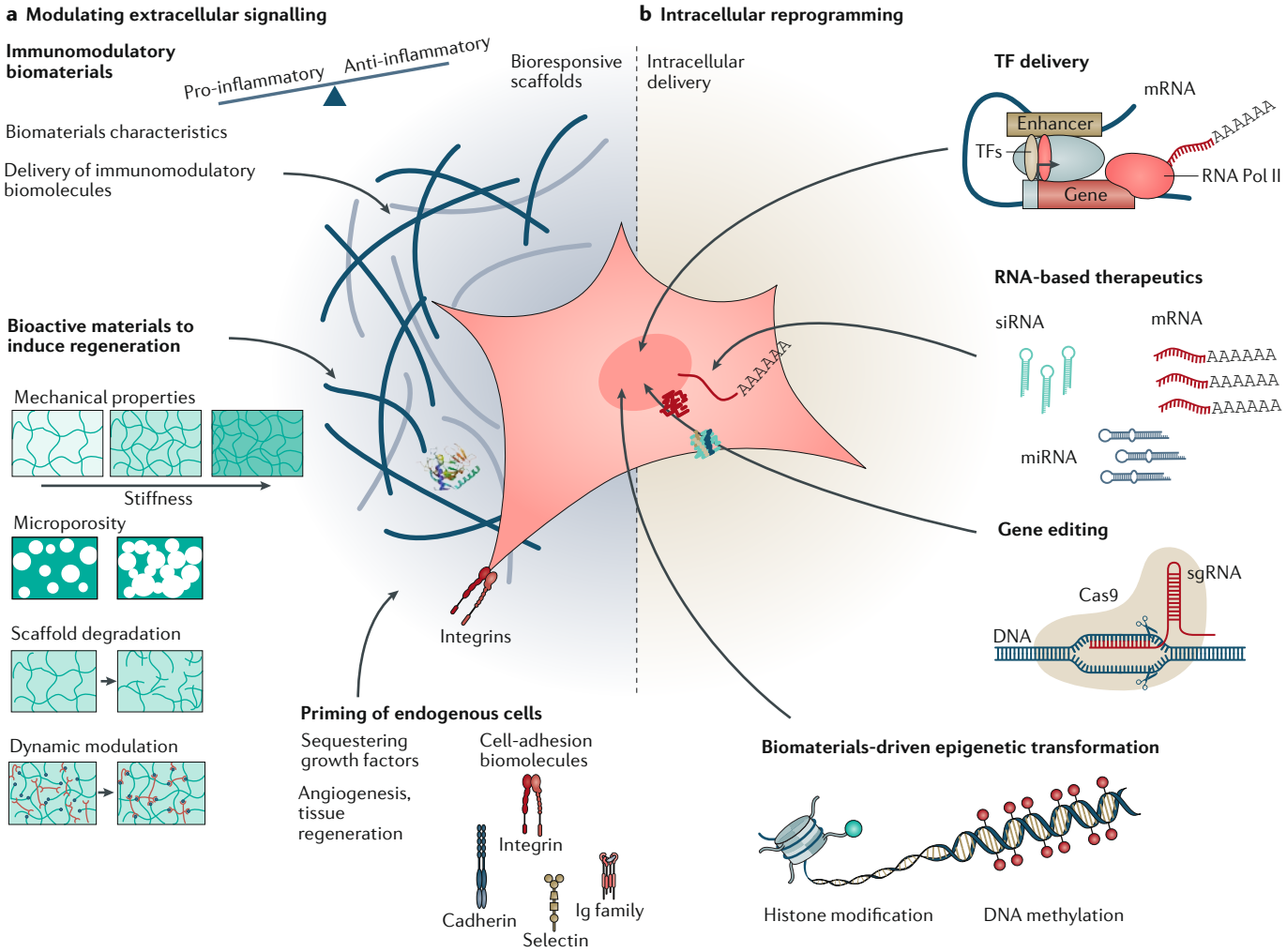


Fig. 4 | **Two approaches for in situ tissue regeneration. a** | The first approach manipulates the extracellular microenvironment to direct endogenous cells to stimulate tissue regeneration. Specifically, biomaterials with appropriate biophysical and biochemical cues modulate the immune response, facilitate the homing of endogenous cells, tissue ingrowth and the development of functional tissue. **b** | The second approach focuses on the direct reprogramming of endogenous cells, by intracellular delivery of biomolecules to activate or silence target genes at various levels. miRNA, microRNA; mRNA, messenger RNA; RNA Pol II, RNA polymerase II; sgRNA, single-guide RNA; siRNA, small interfering RNA; TF, transcription factor.

to be designed to target the tissue microenvironments and promote healthy regeneration.

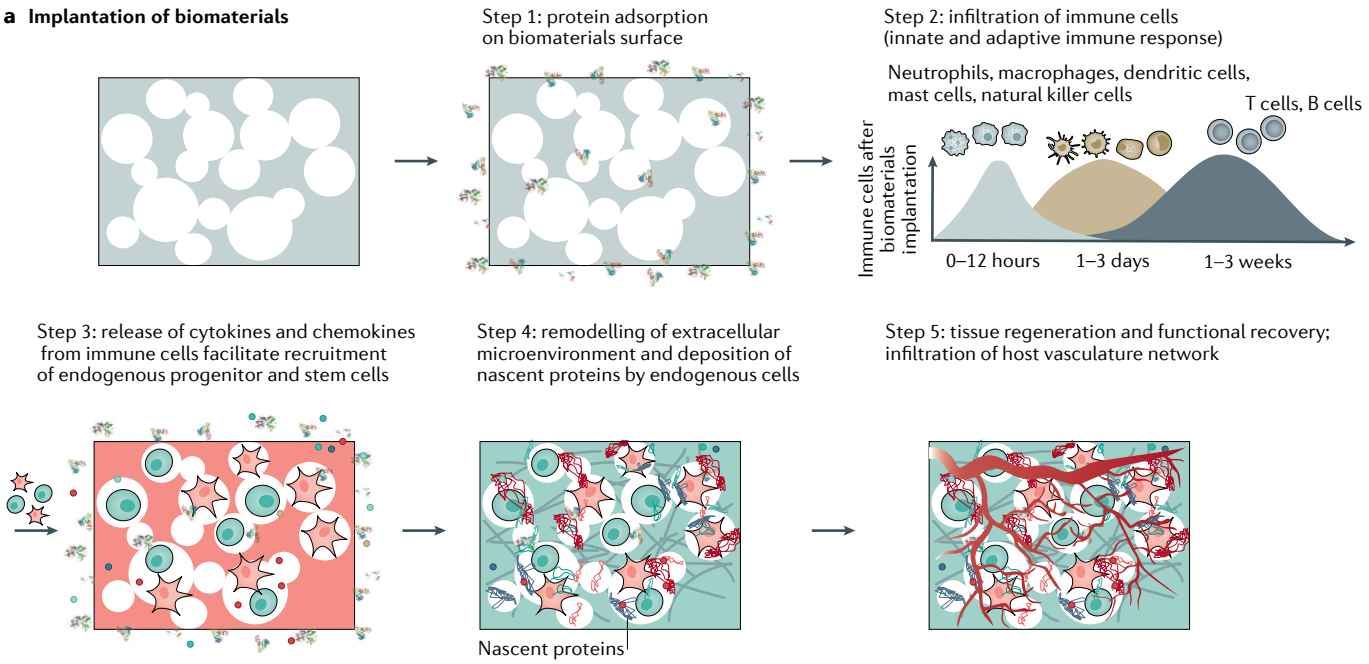
Modulating extracellular signalling

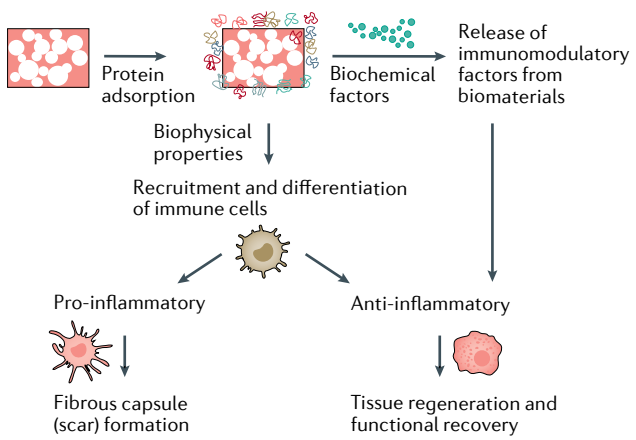
Broadly, in situ tissue regeneration can be classified as either stimulating endogenous cells via extracellular signals or reprogramming cells directly via intracellular interactions of biomolecules. In the first approach, tissue regeneration is stimulated by priming cells via extracellular modes, such as through modulating the biophysical and biochemical characteristics of the biomaterial (FIG. 4a). In the second approach, tissue regeneration is achieved by direct manipulation of the cellular gene-expression programme through cellular reprogramming (FIG. 4b). We review the first of these approaches in this section.

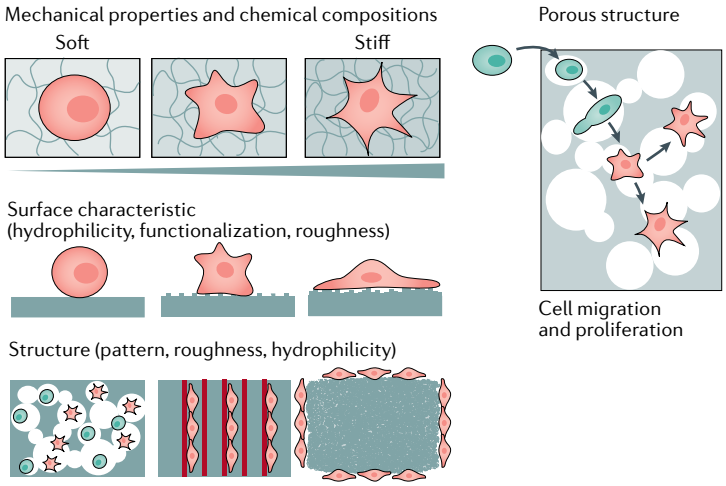
When a biomaterial scaffold is implanted, a range of serum proteins are adsorbed, altering its surface characteristics (FIG. 5a). Endogenous immune cells attach to the adsorbed protein and respond by initiating either pro-inflammatory or anti-inflammatory responses through the release of cytokines and chemokines[23,24]. The release of these molecules leads to the recruitment of endogenous progenitor and stem cells within the scaffold, which is a crucial step during in situ tissue regeneration. After initial attachment, these cells synthesize and deposit nascent proteins on the biomaterial surface, which dictates cellular fate and continuously remodels the local ECM through the secretion of matrix metalloproteinases[25,26]. This newly deposited ECM meditates bidirectional signalling between biomaterials and endogenous cells. These bidirectional interactions can be modulated by controlling the biophysical and biochemical properties of the implanted biomaterials, directly influencing cellular responses and local tissue microenvironments. The infiltration of host vasculature and biophysical characteristics of the newly formed tissue dictates the functional recovery.

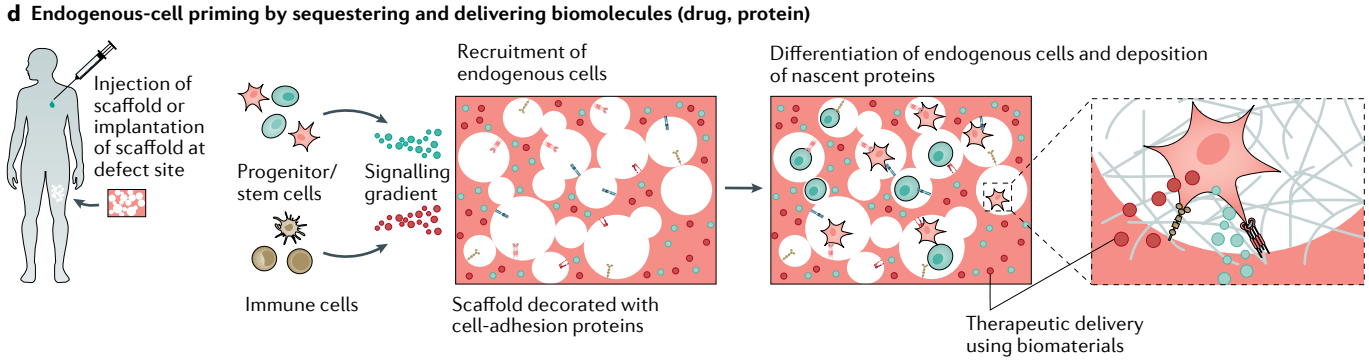
Immunomodulatory biomaterials

The balance between pro-inflammatory and anti-inflammatory immune cells dictates the degree of inflammation and tissue regeneration. Biomaterials can direct immune responses through the recruitment of specific immune cells (FIG. 5b). These responses can be manipulated via the biomaterial's mechanical properties, chemical composition or hydrophobicity, surface chemistry and roughness, and structure[27,28]. Additionally, degraded products of biomaterials or the sustained









release of immunomodulatory biomolecules can also alter the local immune microenvironment by recruiting specific immune cells.

Effect of biophysical properties on the immune response. Mechanical properties such as stiffness and viscoelasticity are important in directing the immune response[29–31]. For example, hydrogels with higher stiffnesses (~840 kPa) were shown to stimulate the production of both pro-inflammatory and anti-inflammatory cytokines, in contrast with hydrogels with lower stiffness[32] (~130 kPa). This study also demonstrated that softer hydrogels (~130 kPa) suppress inflammatory responses and result in an overall lower foreign-body reaction in vivo. In another study, T cells were able to discriminate between the wide range of stiffness found in the body and modulate their responses accordingly[31]. A range of key functions, including migration, gene expression, cytokine secretion, metabolism and cell-cycle progression, become more active on stiff surfaces (~100 kPa) compared with softer surfaces (~0.5 kPa). This is attributed to the presence of T cell receptors, which act as a mechanosensor. Thus, controlling the mechanical stiffness of biomaterials is a facile approach to modulate the immune response.

The surface properties of biomaterials, such as the hydrophilicity and surface charge, control the immune response via the adsorption of proteins. For example, hydrophilic scaffolds promote adsorption of albumin, resulting in the production of anti-inflammatory cytokines derived from local macrophages[33]. By contrast, hydrophobic scaffolds facilitate the adsorption of immunoglobulins and stimulate the production of pro-inflammatory signals from macrophages. Positively charged biomaterials have been shown to activate the inflammasomes, which stimulate a pro-inflammatory signalling cascade, to a higher degree than negatively charged biomaterials[34]. Interestingly, antigen-presenting cells also avoid cellular uptake of negatively charged particles, thus, eliminating antibody and T cell responses[35]. Other charged materials, such as zwitterionic-based biomaterials, have been shown to activate monocytes and dendritic cells, which subsequently modulate macrophage polarization[36]. There is untapped potential to modulate immune cell phenotypes by altering the surface hydrophilicity, charge, roughness and chemical functionalization for in situ tissue regeneration.

Porous scaffolds facilitate macrophage infiltration, pro-regenerative microenvironments and blood-vessel invasion. In a recent example, non-porous and porous scaffolds were implanted subcutaneously and immune response and cellular infiltration was assessed[37]. The non-porous scaffolds showed minimal cellular infiltration and led to the formation of a thick fibrous capsule, whereas the porous scaffolds showed high cellular infiltration, blood-vessel formation and collagen-rich ECM deposition. In the porous scaffold, macrophages that adhered to the pores became pro-inflammatory M1 macrophages; outside the porous scaffold, anti-inflammatory M2 macrophages were enriched. Such behaviour was not observed for the non-porous scaffolds. Although the porosity of scaffolds can influence the immune response to induce and control tissue regeneration, it can decrease the mechanical stability of the implant. Thus, for in situ regeneration of tissue with structural functions, such as bone, the porosity and mechanical properties of the scaffold need to be optimized.

Effect of biochemical properties on the immune response. The chemical structure of the biomaterial influences immune-cell recruitment and subsequent immune response[38,39]. For example, in a recent study, in vivo implantation of biomaterials with different chemical structures (silk and polypropylene) showed markedly different immune responses[40]. At 3 weeks post-implantation, polypropylene stimulated more macrophages, monocytes and neutrophils in the innate system, and more T_H1 and cytotoxic T cells in the adaptive system[40]. In another study, the type of biomaterial — synthetic or natural — has been shown to induce very different immune microenvironments[41]. Synthetic biomaterials (for example, poly(ethylene) glycol (PEG)) facilitate a large and chronic neutrophil infiltrate, which results in pro-inflammatory responses in the wound microenvironment. By contrast, natural biomaterials result in an anti-inflammatory response owing to infiltration of M2 macrophages and upregulation of T_H2-associated genes such as *Il4*, *Il13*, *Arg1*, *Chil3*, *Gata3* and *Cd163*. These studies highlight that the biochemical characteristics of biomaterials can be used to target and modulate the immune response to enhance tissue repair and regeneration.

Sequestering or sustained delivery of immunomodulatory biomolecules from biomaterials can alter the local immune microenvironment and stimulate in situ tissue regeneration. Local delivery of pro-resolving mediators (for example, resolvins, protectins, lipoxins and maresins), inhibitors for pro-inflammatory signals (for example, anti-TNF or inhibitor of NF-κB) or anti-inflammatory cytokines (for example, IL-4 and IL-10) can improve healing outcomes[42]. In a recent study, delivery of anti-inflammatory and anti-fibrotic cytokine (IL-10) prevented and reversed pulmonary fibrosis in mice[43]. The IL-10 was sequestered to hyaluronan and heparin-based hydrogels for direct delivery to the lung via inhalation, which resulted in a significant reduction in TGFβ1 activation. In a similar manner, an injectable hydrogel–microgel composite was used to deliver IL-10

Fig. 5 | **In situ tissue regeneration by modulating the extracellular microenvironment. a** | Soon after biomaterial implantation, adsorption of serum protein dictates the immune response. Depending on the biophysical and biochemical characteristics of the biomaterial, immune cells initiate either a pro-inflammatory or anti-inflammatory response. The release of cytokines and chemokines from immune cells helps in the recruitment of endogenous progenitor stem cells. These endogenous cells synthesize and deposit nascent proteins, which is followed by the infiltration of host vascularization and functional recovery. **b** | The biophysical and biochemical cues of biomaterials can direct immune responses through the recruitment of specific immune cells. The release of immunomodulatory factors can alter the local immune microenvironment to facilitate tissue regeneration. **c** | Biophysical characteristics of biomaterials, such as mechanical stiffness, microporous structure, surface roughness and degradation, recruit specific endogenous cells and promote lineage-specific differentiation. **d** | Endogenous cells, including immune cells and progenitor and stem cells, can be recruited by the presentation of specific biomolecules, such as cytokines, cell-adhesion proteins and growth factors. Sustained release of these signals can facilitate rapid recruitment, migration and infiltration of endogenous cells to promote tissue healing and functional recovery.

after myocardial infarction[44]. Localized delivery of IL-10 reduced macrophage density near the infarct site, resulting in a reduction in scar formation and improvement in ejection fraction and cardiac output. Interestingly, at 4 weeks post-delivery of IL-10, a significant increase in the size of vascular structure was observed[44]. In a different approach, nanoparticles were used for the localized delivery of anti-inflammatory proteins (interleukin-1 receptor antagonist) for treating osteoarthritis, an inflammatory diseasse[45].

Bioactive materials

Cells sense and react to the biophysical characteristics of biomaterials as they anchor and pull on their surrounding synthetic or natural ECM (FIG. 5c). This process is mostly driven by transmembrane receptors, including integrins, cadherins, the immunoglobulin superfamily, cell-adhesion molecules, syndecans, selectins on the cell surface, cytoskeleton components (such as microtubules and microfilaments) and intermediate filaments. The combination of transmembrane receptors and cytoskeleton components is important in signal-transduction pathways that regulate the cell cycle, cytoskeleton organization, cell fate and intracellular transport. Recent studies have shown that cells actively modify their extracellular microenvironment by secreting a proteinaceous ECM and degrading the surrounding microenvironment[25,46]. These secreted nascent proteins are important determinants of cell viability, proliferation and differentiation[46]. For example, proteolytically degradable hydrogels facilitate deposition of ECM protein by cell spreading and osteogenic differentiation of human mesenchymal stem cells (MSCs)[25]. By contrast, the inhibition of cells interacting with deposited ECM facilitates adipogenic differentiation of human MSCs.

Cells sense the mechanical properties of biomaterials. The mechanical characteristics of biomaterials influence cellular attachment, migration, proliferation and differentiation. Early in vitro work demonstrated the role of stiffness in controlling adhesion, spreading and differentiation of stem cells in 2D culture conditions[12]. Stem cells cultured under the same conditions but seeded on a soft matrix (0.1–1 kPa) promoted neurogenic differentiation, matrices with a medium stiffness (8–17 kPa) promoted myogenic differentiation and matrices with high stiffness (25–40 kPa) promoted osteogenic differentiation. In 3D culture conditions, the effect of matrix stiffness was observed on cell fate[47]. Interestingly, no significant difference in cell morphology or cell protrusion was observed as a consequence of matrix rigidity, in contrast with the results from 2D culture. Instead, the matrix stiffness was demonstrated to control the molecular interface between cells and the matrix via integrin binding, which dictates cellular processes. In another approach, the effect of matrix stiffness and structure was delineated using nanoparticles as crosslinking agents, resulting in a tenfold change in matrix stiffness without affecting polymer concentration or microstructure[48]. The matrix stiffness controlled the cell morphology and protrusion in the 3D microenvironment.

To demonstrate the effect of matrix stiffness on in vivo bone regeneration, decellularized bone scaffolds with different stiffness (but the same microstructure) were coated with collagen–hydroxyapatite composite[49]. Subcutaneous implantation of these scaffolds demonstrated that they attract endogenous stem cells. Although the exact origin of these cells is not known, they remodel the microporous scaffolds by depositing their own ECM. Higher scaffold stiffness increased the production of osteo-related proteins, such as osteocalcin and osteopontin, and the extent of vascularization, indicating strong coupling between vascular development and bone formation. Overall, this study demonstrated that the matrix stiffness could be sensed by endogenous stem cells and facilitated deposition of tissue-specific ECM.

In a similar approach, the effect of matrix stiffness was also shown to influence angiogenesis[50]. Matrices with intermediate stiffness (800 Pa) showed enhanced cellular infiltration, angiogenesis and expression of vascular endothelial growth factor receptor 2 (VEGFR2) compared with more elastic (700 Pa) or stiff (900 kPa) gels. Interestingly, the optimal stiffness to facilitate angiogenesis, under in vivo conditions, was much lower than that under 2D in vitro conditions (4 kPa). In an in vivo microenvironment, cyclic mechanical stimulation can also induce angiogenesis, leading to enhanced bone regeneration, as demonstrated in a segmental defect model[51].

Microporosity aids cell migration and tissue ingrowth. The scaffold microstructure and surface topology influence cellular adhesion, infiltration and lineage-specific differentiation. For example, microporous scaffolds facilitate human MSC adhesion and promote osteogenic differentiation[15]. Similarly, the surface topography of biomaterials also influences osteogenic differentiation of human MSCs[52]. Moreover, the microstructure and surface topography promote cytoskeletal organization through modulation of integrin clustering and focal adhesion assemblies, resulting in integrin-mediated mechanotransduction to dictate cell fate.

Microporous scaffolds allow vascularization and tissue ingrowth by providing an interconnected porous network for cellular migration and tissue integration. For example, in one study, microporous scaffolds were fabricated through the assembly of homogenous hydrogel microparticles (that is, microgels) with an enzyme-mediated annealing process[14]. These microgels could subsequently be loaded with cells or bioactive cues to direct migration and modulate the ECM. Under in vitro conditions, they facilitated cell migration, proliferation and formation of a 3D cellular network within the scaffold. Under in vivo conditions, the scaffold was rapidly integrated within the host tissue via formation of vascular networks. Both endothelial cells and pericytes were present within microporous scaffolds, indicating the formation of a stable vascularized network. Interestingly, the microporous scaffold promoted the infiltration of inflammatory cells and showed lower apparent inflammatory response than non-porous scaffolds. The microporous scaffolds also demonstrated extensive wound re-epithelialization and formation of subcutaneous tissue compared with non-porous

scaffolds. This study demonstrates that in situ tissue regeneration can be accomplished in the absence of exogenous growth factors.

Scaffold degradation for cell and tissue infiltration. Degradation is important for both the biophysical and the biochemical responses of scaffolds in vivo. The scaffold's mechanical stability needs to provide a 3D framework for cell and tissue infiltration post-implantation. Subsequently, scaffolds should be broken down and completely resorbed to facilitate load transfer to neo-tissue formation and functional recovery. The mechanical integrity and degradation of the scaffold are strongly interdependent, requiring a fine balance to inhibit scar formation and to direct in situ tissue regeneration. The degradation kinetics of scaffolds depend on various parameters, including structure (for example, pore size and distribution), chemical functionalization and biological milieu. In addition, degradation by-products modulate the local tissue microenvironment through the recruitment of endogenous cells, including immune cells, and progenitor and stem cells. By stimulating anti-inflammatory cells via degradation products, remodelling of the ECM, vascularization of scaffolds and differentiation of endogenous stem cells can be facilitated for immune-mediated regenerative approaches. Thus, the controlled degradation of biomaterial scaffolds is needed to direct in situ tissue regeneration.

Rapid degradation of biomaterials should be avoided because newly formed tissue requires time to remodel and to sustain in vivo forces. By contrast, slow-degrading scaffolds provide prolonged structural support but impair the regeneration process by promoting fibrosis. Thus, it is important to match the scaffold's resorption rate with the formation of neo-tissue to accomplish structural as well as functional regeneration. For example, in bone regeneration, implanting a non-resorbable scaffold, such as hydroxyapatite (HAp), is not advantageous because HAp is inert and does not degrade readily, thereby, limiting neo-tissue formation to replace the scaffold. However, incorporation of β-tricalcium phosphate (β-TCP) within HAp scaffolds can improve the resorption rate of this scaffold formulation[53]. In this way, HAp/β-TCP scaffolds provide the same structural support as pure HAp implants but increase the degradability over time, allowing for native bone tissue to eventually replace the implant.

Degradation by-products can be controlled to further modulate the surrounding environment. For example, bioactive mineral ions, such as magnesium and calcium, can be incorporated within HAp/β-TCP scaffolds and released as the scaffold degrades, thereby, stimulating bone regeneration[54]. Both HAp/β-TCP and HAp/β-TCP/Mg had higher bone formation than HAp alone in a segmental-bone-defect model in rabbits. Specifically, scaffolds loaded with Mg demonstrated enhanced scaffold vascularization, which leads to stronger bone formation. In addition, limited bone integration was observed in HAp, owing to its limited bioactivity.

Degradation by-products can also modulate immune responses that facilitate in situ tissue regeneration. For example, β-TCP scaffolds release calcium ions, inducing a phenotypic change in macrophages from pro-inflammatory M1 to anti-inflammatory M2 (REF.[55]). This leads to the release of anti-inflammatory cytokines (IL-10, IL-1Ra) and osteoinductive molecules (BMP2). The release of BMP2 stimulates SMAD-dependent and SMAD-independent pathways of endogenous stem and progenitor cells towards osteogenic lineages and facilitates the deposition of mineralized ECM. Similarly, the release of phosphate ions from ceramic scaffolds has been shown to have a synergistic effect with calcium ions to stimulate genes associated with matrix mineralization, including osteopontin and matrix gla protein, through the ERK1/2 pathway[56]. In a recent study, the release of silicon, and magnesium and calcium ions from ceramic scaffolds was shown to cause local immunomodulatory effects that promoted regeneration[57].

Overall, these studies demonstrate that scaffold degradation is required to support tissue ingrowth and facilitate transfer of mechanical load to neo-tissue. In addition, the degradation products, such as mineral ions, can stimulate in situ tissue regeneration by modulating the local tissue microenvironment and recruiting endogenous cells.

Dynamic modulation of biomaterials. A range of dynamic biomaterials can be developed with biophysical and biochemical characteristics that can be remotely tuned[6]. For example, the physiochemical properties of photoresponsive biomaterials can be dynamically controlled via exposure to light. This approach provides a tool to either alter the ECM or release specific biomolecules to control cellular functions in a user-defined manner. In another approach, cells seeded on a soft matrix (3 kPa) resulted in limited spreading; however, as soon as the matrix stiffness was increased, the cells responded to this dynamic stiffening (from ~3 to 30 kPa), evidenced by an increase in cell area and enhanced cytoskeletal organization[58]. Interestingly, the temporal changes in matrix stiffness also directed the differentiation of human MSCs. Similarly, bioorthogonal photochemical reactions have been used to pattern gels with specific biochemical signalling to direct the cellular functions[59]. In another approach, biomaterials were programmed to release therapeutic biomolecules in a user-defined manner[60]. The release was controlled by exposure to external or internal stimuli, such as light, enzymes or pH. These studies have provided proof of concept for responsive materials and can potentially be used for in situ tissue regeneration.

Priming of endogenous cells

The rapid recruitment, migration and infiltration of endogenous stem cells is crucial for promoting in situ tissue regeneration. This can be achieved using biomaterials decorated with biomolecules, such as cell-adhesion proteins and growth factors (FIG. 5d). In this section, we discuss scaffold designs to recruit endogenous cells with biomolecules.

Cell-adhesion biomolecules. Cell-adhesion proteins are present in native ECM and are important in determining cell shape, function and tissue integrity. Cells recognize these ECM proteins via cell-surface receptors, including

integrins. Integrins are heterodimeric transmembrane proteins consisting of α- and β-subunits, and bind to the ECM and cellular cytoskeleton to provide biomechanical and biochemical signalling. In one study, by promoting specific integrin (α3/α5β1) binding on biomaterials, differentiation of human MSCs towards osteogenic lineage was achieved, demonstrating that cellular phenotype can be controlled using biomaterials with specific cellular adhesive interactions[61]. Clustering of integrins, as a consequence of ECM binding, promotes cell survival and proliferation, owing to activation of focal adhesion kinase, phosphoinositide 3-kinase/protein kinase B and/or mitogen-activated protein kinase pathways[62]. By engineering scaffolds with these cell-adhesion proteins, it is possible to promote adhesion, migration and differentiation of endogenous cells. For example, integrin-binding peptides (Arg-Gly-Asp) have been used to control cell adhesion on biomaterials surfaces[63].

Biomaterial scaffolds decorated with integrin-binding sites also promote tissue regeneration by facilitating angiogenesis. This was recently demonstrated using hyaluronic-acid hydrogels containing fibronectin fragments and VEGF-loaded nanocapsules[64]. Fibronectin, which has integrin-binding domains and growth-factor-binding regions, improved the sprouting of vessels on the surface and within the hydrogel. The type of integrins engaged during tissue regeneration determines the type of vasculature formed. Specifically, α3/α5β1 integrin interaction corresponded to space-filling and non-tortuous, non-leaky blood vessels; by contrast, engaging αvβ3 integrin resulted in the formation of dense and tortuous blood vessels, which are leaky. In a similar study, a poly(ethyl acrylate)/fibronectin scaffold loaded with VEGF promoted vasculogenesis within the porous scaffolds[65]. Overall, the two studies showed that integrin stimulation is crucial to promote the formation of vasculature network.

Sequestering growth factors. Biomaterials that present growth factors can be used to trigger in situ tissue regeneration. For example, a synthetic bone graft (collagen sponge) was loaded with the therapeutic growth factor recombinant human bone morphogenetic protein 2 (rhBMP2)[66] and applied in spinal-fusion, tibial-fracture and sinus-augmentation procedures[67]. As rhBMP2 has a short half-life of 7–16 min in vivo owing to proteolysis[68], supraphysiological doses of rhBMP2 (1.5 mg ml^{-1})[66] are needed for in situ bone regeneration. However, recent clinical studies have established adverse effects of such high doses of rhBMP2, including osteolysis, swelling and heterotopic ossification[66,69,70]. These adverse effects are attributed to poor localization and rapid release of rhBMP2 from the scaffolds[71]. There are several approaches currently underway to overcome these limitations[72–75].

Biomaterials loaded with nanoparticles that sequester therapeutic proteins have been investigated for in situ tissue regeneration. One example of this is synthetic 2D nanoclay[75,76]. In vivo, scaffolds decorated with nanoclay/rhBMP2 were shown to stimulate in situ bone regeneration and reduce the effective concentration of protein 10–100-fold[75]. Moreover, sequestering of VEGF within nanoclay-based biomaterials has been shown to promote angiogenesis in vitro and in vivo[17]. In a similar study, nanoclay-based gels were investigated to deliver stem-cell secretome to stimulate angiogenesis after myocardial infarction[77]. Secretomes are cocktails of therapeutic biomolecules (for example, growth factors and exosomes) produced by stem cells under ex vivo conditions. The delivery of a secretome using nanoclay showed improved heart function, as demonstrated by higher ejection fractions than for untreated controls. This is mainly attributed to the formation of new blood vessels within the infarcted heart, owing to secretome localization. These studies demonstrated that nanoclay-based biomaterials can sequester biomolecules for prolonged durations and direct in situ tissue regeneration.

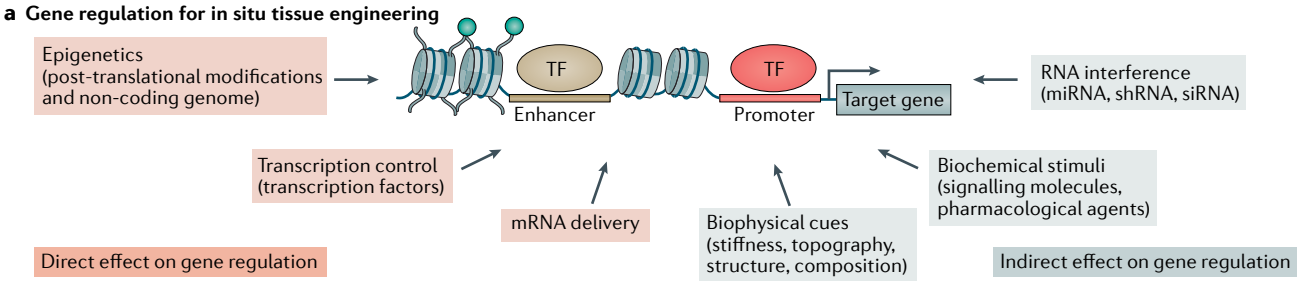
Intracellular reprogramming

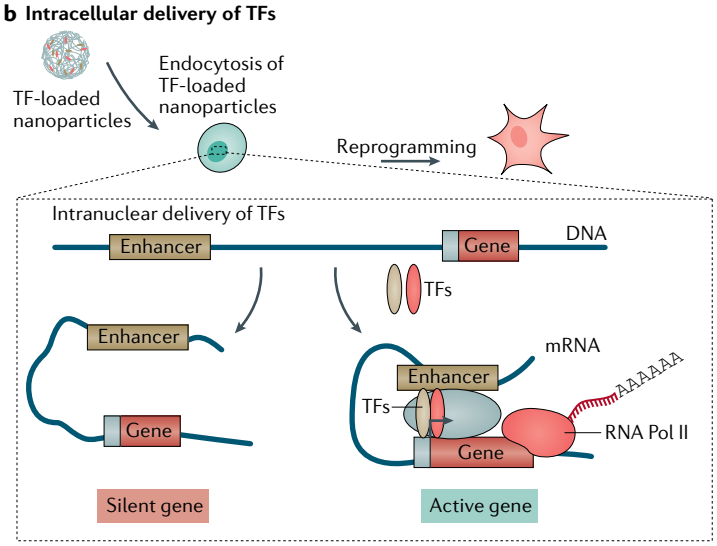
Different cell types contain the same genetic material (DNA), with the cell fate and identity being tightly controlled by the genes that are expressed. For the expression of any gene, the cis-regulatory regions (promoters and enhancers) are occupied by lineage-determining and signal-dependent transcription factors. These transcription factors allow for the recruitment of chromatin-remodelling complexes, leading to epigenetic modifications that allow the DNA to loosen and become accessible by other essential cofactors and, eventually, by RNA polymerase II, which transcribes the DNA into RNA. Thus, a large extent of regulation happens during transcription. In addition, the level of transcription is regulated by long-range interactions of promoters with enhancers. In a distinct cell type, gene expression level is controlled by intrinsic and extrinsic conditions (FIG. 6a). These include the epigenetic state (post-translational modifications of histone proteins and epigenetic modifications of the non-coding genome by the chromatin-remodelling complexes), transcription control (by regulatory transcription factors and pause–release control of RNA polymerase II), RNA processing (capping, splicing, alternative cleavage and polyadenylation), translational control (factors defining the translation efficiency of the RNAs), microenvironment (biophysical and biochemical cues) and external factors (light, stress, signalling molecules).

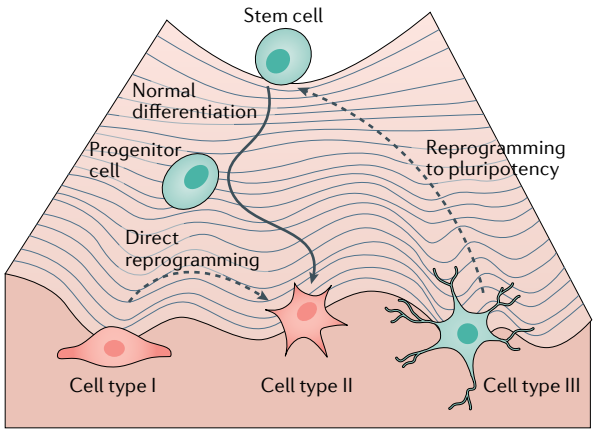
There are five major existing approaches to cellular reprogramming: overexpression of lineage-determining transcription factors; silencing (downregulation) the expression of specific genes through the delivery of small biomolecules, such as microRNA or pharmacological drugs; delivery of mRNA; genetic reprogramming using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9); and epigenetic modifications via the biophysical characteristics of materials or delivery of biochemical cues. In this section, we detail each of these approaches and discuss the challenges and potential solutions to translate these technologies to the clinic for in situ cellular reprogramming.

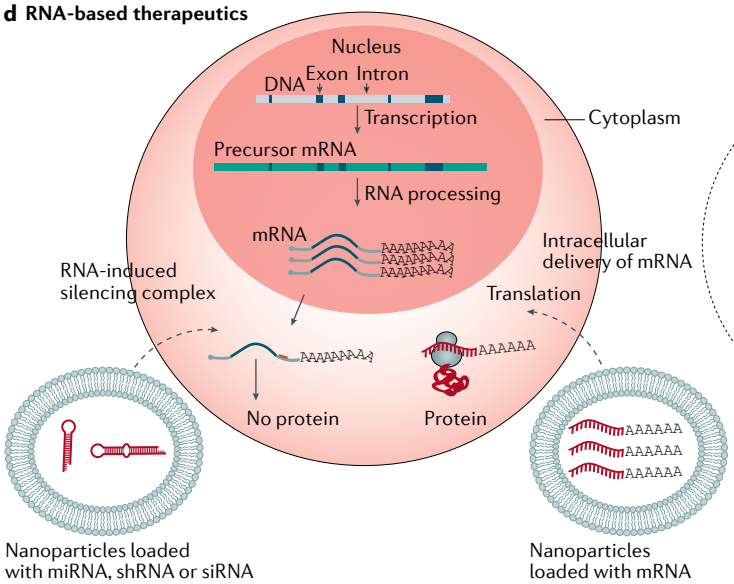
Delivery of transcription factors

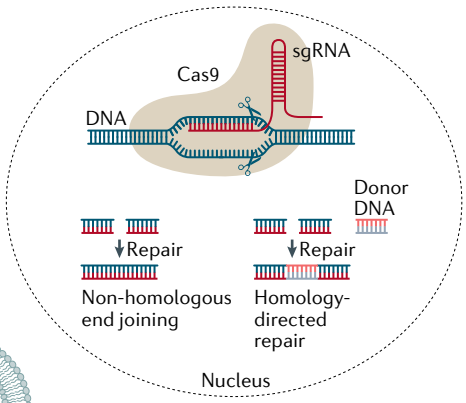
The exogenous delivery of lineage-determining transcription factors can reprogramme the cell state and induce lineage-specific differentiation (FIG. 6b). However,


a Gene regulation for in situ tissue engineering
Epigenetics (post-translational modifications and non-coding genome)
TF
TF
Enhancer
Promoter
Target gene
RNA interference (miRNA, shRNA, siRNA)
Transcription control (transcription factors)
mRNA delivery
Biophysical cues (stiffness, topography, structure, composition)
Biochemical stimuli (signalling molecules, pharmacological agents)
Direct effect on gene regulation
Indirect effect on gene regulation


b Intracellular delivery of TFs
TF-loaded nanoparticles
Endocytosis of TF-loaded nanoparticles
Reprogramming
Intranuclear delivery of TFs
Enhancer
Gene
DNA
TFs
Enhancer
Gene
mRNA
Enhancer
TFs
Gene
RNA Pol II
Silent gene
Active gene


c Cellular reprogramming
Stem cell
Normal differentiation
Progenitor cell
Reprogramming to pluripotency
Direct reprogramming
Cell type I
Cell type II
Cell type III


d RNA-based therapeutics
Nucleus
DNA
Exon
Intron
Transcription
Cytoplasm
Precursor mRNA
RNA processing
mRNA
RNA-induced silencing complex
Intracellular delivery of mRNA
Translation
No protein
Protein
Nanoparticles loaded with miRNA, shRNA or siRNA
Nanoparticles loaded with mRNA


e Gene editing using nanoparticles
Cas9
sgRNA
DNA
Donor DNA
Repair
Repair
Non-homologous end joining
Homology-directed repair
Nucleus
Cas9 form
Plasmid, mRNA, protein + sgRNA
Delivery approach
Lipid nanoparticles, liposomes, exosomes, gold nanoparticles
Electrospun scaffolds

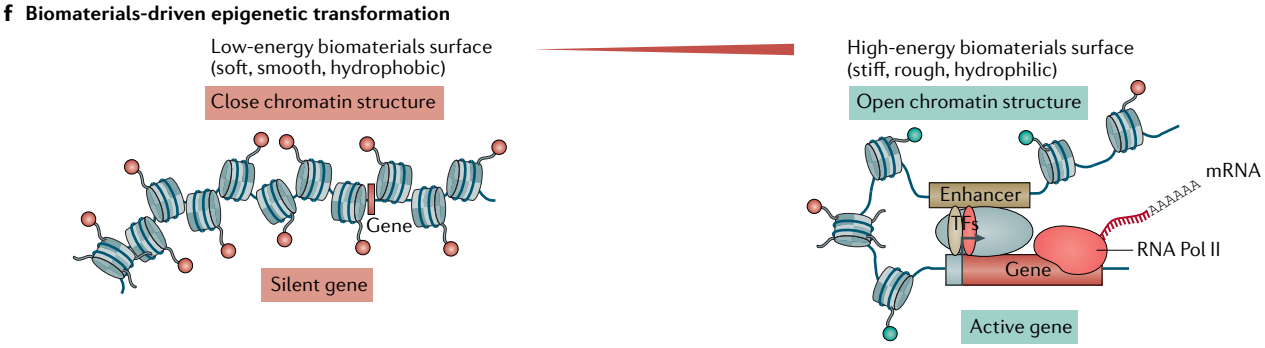

f Biomaterials-driven epigenetic transformation
Low-energy biomaterials surface (soft, smooth, hydrophobic)
Close chromatin structure
Gene
Silent gene
High-energy biomaterials surface (stiff, rough, hydrophilic)
Open chromatin structure
Enhancer
TFs
mRNA
RNA Pol II
Gene
Active gene

Fig. 6 | **In situ cellular reprogramming for tissue regeneration. a** | Gene expression is regulated at several stages and the expression levels are controlled by many factors, including epigenetics, transcriptional control, RNA processing, the biophysical and biochemical microenvironment, and external stimuli. A range of biomaterials-based approaches can be designed for gene regulation. **b** | Intracellular delivery of transcription factors (TFs) to reprogramme cells from one cell type to another has the potential to remodel chromatin to activate and silence specific gene-expression programmes. **c** | Revised Waddington model for cellular reprogramming. Cells can be reprogrammed from one type to other by the expression of pioneer transcription factors. **d** | RNA-based therapeutics for protein expression and gene silencing. RNA interference can be achieved via intracellular delivery of microRNA (miRNA), short hairpin RNA (shRNA) or small interfering RNA (siRNA). Delivery of messenger RNA (mRNA) can be used to promote the production of specific proteins to stimulate tissue healing. **e** | Biomaterials for the in vivo delivery of the gene-editing tool CRISPR–Cas9. Nanoparticles are loaded with guide RNA, donor DNA and Cas9 protein to achieve homology-directed repair. **f** | Biophysical and biochemical cues from biomaterials can induce epigenetic modifications, including DNA methylation and histone deacetylation. These epigenetic modifications directly regulate gene expression and determine cell identity. RNA Pol II, RNA polymerase II; sgRNA, single-guide RNA.

challenges of this approach are to preserve the integrity and activity of these proteins. Several approaches have been developed to overcome this challenge using retroviral, lentiviral, adenoviruses and/or plasmids by integration of transgene in the host genome. For example, somatic cells can be reprogrammed towards pluripotency (known as transdifferentiation) by the expression of four master transcription factors, OCT4, SOX2, KLF4 and MYC, using retroviral transduction[2]. The concept of transdifferentiation is depicted using the modified Waddington 'epigenetic landscape' (FIG. 6c). Transdifferentiation is a promising strategy for cases such as osteoarthritis, neurodegenerative diseases and myocardial infarctions, in which damaged tissue is not regenerated, owing to a lack of migration of endogenous stem cells. Although these approaches can induce the expression of these lineage-determining transcription factors via the transcription of incorporated viral DNA, there are concerns of unexpected genetic modifications within target cells by these exogenous sequences[78]. If these concerns can be addressed by designing a different carrier for transcription factors, the potential of this approach for reprogramming endogenous cells could be realized.

Synthetic nanoparticles, injectable hydrogels, electrospun scaffolds and microspheres have been used for intracellular delivery. Among these, synthetic nanomaterials have good stability, high biocompatibility, long shelf life and high loading efficiency, which are desirable for cellular reprogramming[79,80]. For example, one approach taken for passive delivery of a transcription factor is a bioinert polymeric nanocapsule consisting of PEG[81]. Using in situ polymerization, transcription factor myoblast determination protein 1 (MYOD1) was encapsulated within PEG nanoparticles. The nanoparticles demonstrated intracellular delivery of MYOD1 in myoblast cells, which subsequently translocated to the nuclei and initiated myogenic differentiation into skeletal, cardiac or smooth-muscle cells. The results were similar to those for cells transfected with MYOD1 plasmid using Lipofectamine. Thus, transcription factors can be delivered to direct the differentiation of cells ex vivo, without permanent inclusion of extracellular genetic material into the host genome.

Although passive delivery of nanoparticles can be used for intracellular delivery, this technique has a lower yield than active targeting. Addressing this limitation, DNA-assembled recombinant transcription factors (DARTs) were developed to facilitate the active uptake of nanoparticles[82]. These DARTs are multifunctional oligonucleotides that enter the liver via galactose-mediated endocytosis. Endosomal disruption and release into the cytosol of hepatocytes is triggered by hydrolysis of pH-sensitive acetal linkages within the DARTs. In vivo, DARTs were used to deliver transcription factor nuclear erythroid 2-related factor 2 (NRF2) and protect mice from acetaminophen-induced liver injury. The efficacy of NRF2 delivery was validated through monitoring of downstream genes, for example, HO1 and NQO1. Overall, this study demonstrated the feasibility of in situ delivery of transcription factors using nanoparticles.

Artificial transcription factors can be assembled using nanoparticles by synthesizing the key functional domains of transcription factors. For example, gold nanoparticles can be decorated with DNA-binding domains, nuclear localization signals and activation domains to mimic the functional units of transcription factors[83,84]. The nuclear localization signal facilitates nuclear import of the transcription factor. Once inside the nucleus, the DNA-binding domains bind to complementary DNA sequences and the activation domains sequester transcriptional machinery, such as RNA polymerase II, mediator complexes and other transcription factors. Although in vivo study is yet to be performed, in vitro study highlighted the ability of these synthetic nanoparticles to direct differentiation of adipose stem cells towards myocytes for muscle development[84].

The selection of transcription factors for cellular reprogramming is a major challenge, with current approaches based on trial and error. Recent advances in machine learning, omics approaches and systems biology can help in addressing this challenge. Targeted delivery of transcription factors to damaged tissue remains another challenge, as most systemically delivered nanoparticles accumulate in the liver, kidneys, spleen and lungs. Off-target delivery of transcription factors can have unintended consequences, which is why local delivery though injectable biomaterials is preferred. In addition, the delivery of a combination of key transcription factors involved in specific-lineage-defining processes is more efficient than delivery of a single transcription factor; however, this adds complexity to the biomaterials design for delivery of multiple transcription factors. Other challenges, such as low reprogramming efficiencies, off-target effects and long-term fate of reprogrammed cells, need to be overcome to translate this technology into the clinic.

RNAi-based therapeutics

RNA interference (RNAi), which silences the expression of specific genes, can be achieved via the intracellular delivery of microRNAs (miRNAs) or small interfering RNAs (siRNAs) (FIG. 6d). However, it is difficult to deliver RNAi molecules efficiently. These nucleic-acid biomolecules are negatively charged, owing to their phosphate backbone and, thus, cannot diffuse across negatively

charged phospholipid cell membranes. Moreover, RNAi biomolecules degrade under physiological conditions. For example, during endocytosis, RNAi molecules are subjected to low pH, which results in their rapid degradation. Although viral delivery vectors are extensively used for expression of RNAi molecules, this is accompanied by the risk of mutagenesis and immunogenicity. Therefore, the use of synthetic biomaterials, such as liposomes, polymeric nanoparticles, hydrogels, nanofibres and microporous scaffolds, are being investigated.

miRNAs are short, non-coding RNAs that are transcribed in the nucleus by RNA polymerase II and are preprocessed to bind mRNA via base pairing of complementary sequences. This binding leads to gene silencing by repression of translation and degradation of mRNA, thereby, regulating the cellular gene-expression levels. Overexpression or inhibition of miRNAs can simultaneously regulate the expression of several growth factors. miRNAs have an important role in angiogenesis, the immune response and tissue regeneration. For example, miR-26a delivered using a hyaluronan–heparin–gelatin hydrogel stimulated angiogenesis and bone regeneration in vivo[85]. An increase in vascular volume of more than threefold was observed in implanted scaffolds after the localized delivery of miRNA. Interestingly, 3 months post-implantation, complete healing of mice calvarial defects was observed for miRNA treatment, whereas only partial healing was observed in the control group (hydrogel only).

In a recent study, electrospun scaffolds were shown to provide contact guidance to endogenous cells, whereas micellar nanoparticles loaded with miR-222 were dispersed in collagen hydrogels[86]. The aligned fibrous hydrogel scaffold contained NT3 — a neurotrophic factor that promotes survival of neurons and the growth of axons — and supported in vivo axonal remyelination. Interestingly, incorporation of miR-222 resulted in robust neurite ingrowth in contrast with scaffolds without miR-222, but did not influence the microglia and astrocyte morphology. The enhanced axon regeneration and remyelination in vivo highlights the potential of combining biophysical and biochemical approaches to achieve in situ tissue regeneration.

siRNAs are exogenous, double-stranded RNA that are processed by the cellular machinery to bind to and cleave their distinct target mRNA, resulting in gene silencing. A distinction between siRNAs and miRNAs is their specificity: siRNAs are highly specific with only one mRNA target (full complementarity is essential), whereas miRNAs have many targets (only 2–7 nucleotide seed complementarity is required). Although siRNA-based therapeutics are extensively being investigated for the treatment of cancer, relatively few reports have focused on their application in regenerative medicine. In an early study, noggin siRNA (noggin is a BMP antagonist) and rhBMP2 were co-delivered using a synthetic hydrogel to the dorsal muscle pouch of mice, resulting in ectopic bone formation[87]; there was no effect of delivering only siRNA on bone regeneration. In another study on bone regeneration in rat calvarial defects, noggin siRNA and miRNA-20a (to inhibit PPAR-γ, a negative regulator of BMP2-mediated osteogenesis) were co-delivered[88]. As these siRNA and miRNA must be delivered in the cytoplasm to regulate mRNA levels, they provide a simpler approach than gene editing.

Stimulation of protein translation

Recent development in improving the stability and inherent immunogenicity of mRNA using chemical modifications has produced a new wave of interest in developing mRNA-based therapeutics[89–91]. Mature mRNA that is transported from the nucleus to the cytosol is translated into protein by the ribosomal apparatus. Thus, a range of polymeric and inorganic nanoparticles can be used for intracellular delivery of mRNA (FIG. 6d). As intranuclear delivery is not required, mRNA delivery has a major advantage over plasmid DNA (pDNA) delivery. Moreover, direct mRNA delivery does not require permanent inclusion of the genetic material into the host genome, greatly reducing the likelihood of adverse effects. In addition, mRNA delivery to the cytoplasm can stimulate protein production, as it is more potent in non-dividing or slowly dividing cells. By contrast, pDNA needs to be delivered in the nucleus and is more efficient in proliferating cells. In a recent study, pDNA and mRNA delivery were compared for ocular applications using cationic polymer nanoparticles[92]. The encapsulation of mRNA in polymeric nanoparticles substantially improves its stability and reduces its immunogenicity. Under in vitro conditions, substantially higher protein production was obtained from mRNA delivery than pDNA delivery. Interestingly, chemical modification of mRNA resulted in a more than 1,800-fold increase in protein production compared with pDNA delivery. In vivo mRNA translation was only observed when delivered using nanoparticles (that is, bolus delivery did not show any protein production).

In a similar study, lipid nanoparticles were used to deliver mRNA to produce therapeutic proteins for the treatment of genetic diseases[93]. To improve the delivery efficacy, a library of lipid nanoparticles with various formulations was prepared. Nanoparticles containing oxidized cholesterol were highly efficient in the delivery of mRNA to endothelial Kupffer cells but not to hepatocytes[93]. Although the exact mechanism for selective uptake of nanoparticles by certain cells is not well understood, it is attributed to the formation of nanoparticles-specific protein coronas. Importantly, this work demonstrated that selective delivery of therapeutic mRNA in vivo can stimulate endogenous protein production.

In vivo gene editing

Endogenous gene regulation can be achieved through direct genome editing using engineered nucleases, or 'molecular scissors', such as meganucleases, zinc-finger nucleases, transcription activator-like effector nucleases (TALENs) and CRISPR–Cas9 (REFS[94–96]). These nucleases create site-specific double-strand breaks at selected locations in the genome, which are repaired through two mechanisms: non-homologous end joining, which results in gene silencing by causing indel mutations, and homology-directed repair (HDR), which repairs gene mutations or activates specific genes (FIG. 6e).

Among these nucleases, CRISPR–Cas9 is a promising approach for in situ tissue regeneration, owing to its facile design and fabrication, high efficiency, widespread use and adaptation for different cell types, and limited off-target effect.

CRISPR–Cas9 gene editing commonly involves delivery via adeno-associated viruses[97,98]. However, virus-based delivery is associated with immunogenic complications, off-target effects and low efficiency. As an alternative, gold nanoparticles loaded with guide RNA, donor DNA and Cas9 protein have been demonstrated for non-viral CRISPR therapeutics[99]. In this approach, the nanoparticles were decorated with densely packed DNA, which were used to load donor DNA and Cas9 protein. This nanoparticle complex was coated with cationic polymer to facilitate endosomal escape after cellular internalization. In vitro studies confirmed HDR in human embryonic stem cells. Injection of the loaded nanoparticle in the muscle tissue of mice resulted in gene editing near the injection site. Moreover, in a mouse model of Duchenne muscular dystrophy, the dystrophin gene mutation was corrected and the expression of dystrophin protein was restored. The editing efficacy was 5% and 1% with and without cardiotoxin, respectively (cardiotoxin results in muscle damage that activates endogenous HDR mechanisms; however, it is not used in clinical settings). Although the low editing efficiency (1%) partially rescued muscle function, there was a lack of full, functional recovery. Interestingly, multiple injections of these nanoparticles did not show any adverse effect, indicating safe usage for in situ gene editing. More broadly, this demonstrates a strong potential for the use of synthetic nanomaterials for in vivo gene editing for tissue regeneration.

Other types of synthetic nanoparticles have also been used for in vivo gene editing. These include lipid nanoparticles[100], exosome-liposomes[101], PEGylated helical polypeptide nanoparticles[102], lipid-coated gold nanoparticles[103] and lipid-coated PEG-PLGA nanoparticles[103]. However, as with the example above, delivery using these nanoparticles has the limitations of low editing efficiency and off-target gene editing. To overcome these limitations, biomaterials implantation has been used. For example, electrospun scaffolds were recently decorated with Cas9 and single-guide-RNA complex using a bioadhesive coating[104]. Although the in vivo efficacy of this approach was not demonstrated, the use of biomaterial scaffolds can potentially facilitate localized gene editing for in situ tissue regeneration.

Biomaterials regulate epigenetic state

Epigenetic modifications directly alter the accessibility of DNA and the structure of chromatin, thereby, regulating gene expression. The best-studied epigenetic modifications are DNA methylation and histone protein modifications, which directly regulate gene expression levels and define the cell identity. The addition of methyl groups to DNA affects gene transcription by occluding the binding of transcription factors. Similarly, post-translational histone modifications play a fundamental role in defining the chromatin structure and controlling the expression of DNA. These epigenetic modifications are catalysed by enzymatic proteins and can be reversed by another set of enzymes. The reversible nature of these modifications makes them a pragmatic candidate that can be potentially leveraged for situ tissue regeneration. Indeed, biomaterials have been used to control the chromatin configuration and epigenetic mechanisms in cells (FIG. 6f).

Biophysical characteristics of biomaterials, such as mechanical stiffness, surface roughness, patterning and wetting characteristics, have been shown to influence the epigenetic state of cells. For example, soft biomaterials promote a dense and transcriptionally inactive heterochromatin structure[105]. By contrast, stiff surfaces result in euchromatin, which is open and transcriptionally active. Earlier studies have shown that stem cells seeded on stiff biomaterials enhanced differentiation towards osteogenic lineage[12,13]. These studies showed that the interaction between biomaterials and the cytoskeleton is important in defining cell shape[12,13]. Other studies highlighted the role of biophysical cues to modulate the chromatin state and reprogramming efficacy by changing histone H3 acetylation and methylation[106,107]. For example, cells seeded on microgrooves or nanofibrous scaffolds have higher reprogramming efficacy than cells seeded on a smooth surface[106]. This is attributed to the open chromatin structure of cells seeded on patterned surfaces facilitating the interaction between DNA and reprogramming factors. Nanopatterned surfaces have also been shown to influence the DNA methylation pattern of embryonic stem cells[108]. These epigenetic changes promote differentiation of embryonic stem cells towards MSCs and osteogenic progenitor cells. In another study, soft and smooth surfaces reduced cell adhesion, impaired nuclear organization and increased histone deacetylase (HDAC) activity[109]. By contrast, rough surfaces facilitate cell adhesion, histone acetylation and chromatin remodelling, thereby, promoting osteogenic differentiation of stem cells. A recent study reported that nanomaterials can induce epigenetic modifications to regulate cell function[110]. For example, nano-HAp was shown to facilitate DNA methylation of the *ALP* gene[110]. Although these studies highlight the use of biophysical cues to induce epigenetic modifications, in vivo validation is still required.

During cellular reprogramming, the epigenetic state of cells is reset. The use of biochemical cues, such as pharmacological agents, can facilitate or suppress cellular reprogramming by directly influencing the epigenetic state. A range of epigenetic drugs are currently used in the clinic for the treatment of various types of cancer[111,112]. These drugs facilitate transcription by inhibiting HDACs. In a recent study, human MSCs treated with epigenetic regulating molecules (such as gemcitabine, decitabine, I-CBP112, chidamide and SIRT1/2 inhibitor IV) induced osteogenic differentiation[113]. Notably, gemcitabine and decitabine improved osteogenic efficacy by more than fivefold in stem cells obtained from aged human donors. This study highlights the role of epigenetics and nucleosome remodelling in cellular differentiation and their potential use for in situ tissue regeneration.

A combination of topographical cues provided by the scaffold structure and biochemical cues provided by

the release of epigenetic drugs from synthetic scaffolds can promote tissue healing. In a recent study, electrospun poly(L-lactic acid) (PLLA) scaffolds were decorated with HDAC inhibitor trichostatin A to facilitate regeneration of the Achilles tendon[114]. The aligned fibrous scaffolds facilitate cellular alignment and the degradation of PLLA scaffolds results in release of trichostatin A, which regulates stem cells undergoing tenogenesis. After optimization of the biophysical and biochemical characteristics of the scaffold, there was a substantial increase in the expression of tendon-specific transcription factor and tendon-related ECM (that is, collagen fibrils) in vivo. Although localized delivery of trichostatin A is effective in stimulating in situ tissue regeneration, its potential off-site effects need to be evaluated.

Outlook

The past few years have witnessed widespread advances in the development of biomaterials to control and direct the innate regenerative potential of the body (FIG. 7). In this section, we highlight opportunities for further development.

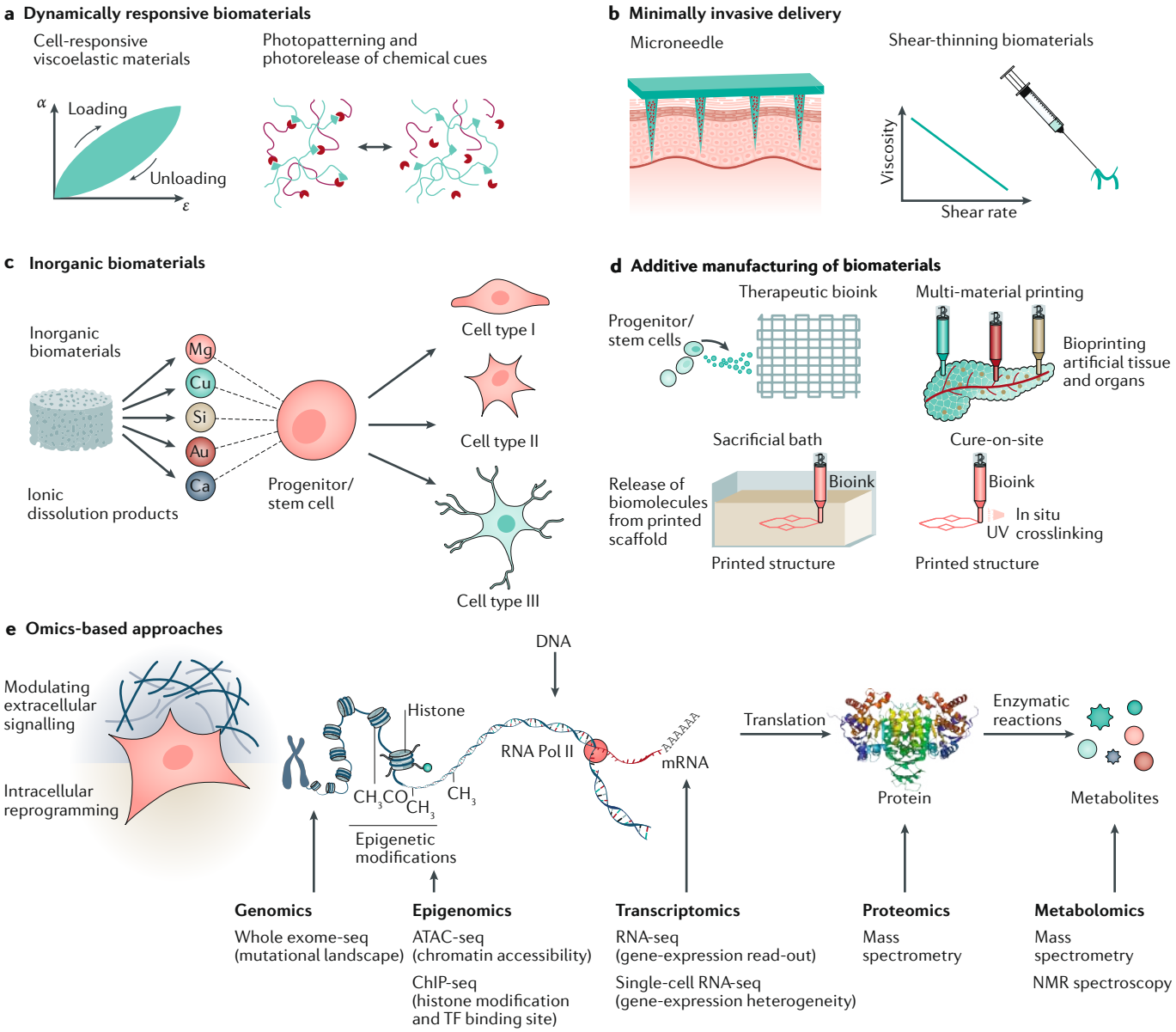


Fig. 7 | **Emerging trends in the development of engineered biomaterials for tissue regeneration. a** | Dynamic biomaterials can fine-tune healing responses after implantation in a non-invasive manner. **b** | Using minimally invasive approaches including microneedles and shear-thinning biomaterials, reprogramming or regenerating factors can be delivered locally, without damaging the surrounding, healthy tissue. **c** | Mineral-based biomaterials can direct cellular functions in the absence of therapeutic proteins and other bioactive cues by the release of mineral ions. **d** | Additive-manufacturing approaches can be used to design complex tissue structures, which can provide biophysical and biochemical cues to direct in situ tissue regeneration. These approaches include therapeutics bioinks, multi-material printing, use of a sacrificial bath and cure-on-site methods. **e** | The use of omics-based approaches can be used to optimize and validate the design of next-generation bioresponsive materials by understanding cell–biomaterials interactions in relation to genes (genomics), methylation and histone modification (epigenomics), mRNAs (transcriptomics), proteins (proteomics) and metabolites (metabolomics). RNA Pol II, RNA polymerase II; TF, transcription factor.

Dynamically responsive biomaterials

Dynamic biomaterials allows on-demand modulation of biophysical and biochemical properties[115–118]. These properties can be used to direct cellular function and control immune responses towards tissue healing. Two main functions of dynamic biomaterials are to control cellular response via alternation in biophysical characteristics and to release biomolecules on demand (FIG. 7a). For example, fast-relaxing biomaterials encourage stem-cell adhesion, proliferation and osteogenic differentiation, which is mediated via integrin, RGD clustering, actomyosin and nuclear localization of YAP[116]. A dynamic change in architecture of implanted biomaterials has also been shown to facilitate tissue healing in situ[117]. Future prospects exist in designing biomaterials with an inherent ability to form pores upon implantation to attract endogenous cells. The rate of pore formation can be used to control migration, proliferation and differentiation of endogenous progenitor and stem cells. In addition to microenvironment alterations, dynamically responsive biomaterials can be used to mask or activate biochemical cues, such as ECM proteins or growth factors, to manipulate cell signalling at specific time points. Specifically, local uncaging of photodegradable biomaterials loaded with cell-adhesion peptide and therapeutic growth factors can facilitate the invasion of stem and progenitor cells, which is necessary to stimulate in situ tissue regeneration.

Another area of future biomaterials development will focus on incorporating independent and different dynamic responses within the same materials system. Photopatterning and photorelease of more than one protein can be achieved by engineering biorthogonal reactive handles. For example, sortase-tag-enhanced protein ligation can be used to engineer proteins with linker molecules in a site-specific manner for photopatterning within PEG hydrogels[118]. The modified protein retained bioactivity in vitro, demonstrating a unique approach to pattern and release biomolecules in a spatio-temporal manner. Although these emerging approaches are promising, only a few of them have demonstrated in vivo efficacy. We expect that studies will focus on designing and validating new approaches to control the dynamic interplay of matrix properties for in situ tissue regeneration.

Minimally invasive delivery

Minimally invasive delivery of therapeutic agents is an emerging approach to accelerate tissue healing and recovery. Two main approaches have been investigated for the delivery of functional regenerating agents: microneedles and injectable biomaterials (FIG. 7b). The advantages of these approaches include reduced discomfort, preserved therapeutic activity by overcoming filtration from the liver, and sustained and localized delivery for enhanced therapeutic efficacies. Microneedles can be used to penetrate the skin and deliver therapeutic factors to the dermis[119,120]. For example, in a recent study, hair-derived keratin was used to engineer a microneedle-based transdermal patch local delivery of stem-cell-derived exosomes and a small-molecule drug (UK5099) that can activate hair follicle stem cells[121]. In another study, microneedle-based patches were used to deliver therapeutics for heart regeneration after an acute myocardial infarction[122]. Despite significant progress in microneedle-based technologies, the delivery of macromolecules is still challenging. Specifically, the lack of control over the pharmacokinetics and pharmacodynamics is a bottleneck for clinical translational. In addition, challenges related to the mechanical integrity and safety of microneedles need to be investigated. In the future, we expect the development of microneedle-based approaches for intracellular reprogramming of endogenous cells, as well as for controlling the immune response. Another area of development is tissue-specific delivery of microneedles, which will result in high-efficiency drug delivery, outperforming conventional drug-delivery approaches. Developing microneedles with an ability to sequentially release multiple therapeutics in a predetermined manner is needed for in situ tissue regeneration.

Injectable biomaterials can be used to deliver payloads in and around damaged tissue without disrupting the tissue integrity and surrounding microenvironment. Two approaches have been explored for minimally invasive injection: in situ gelation of biomaterials post-delivery and the use of shear-thinning biomaterials. In situ gelation can be achieved via physical or chemical crosslinking mechanisms after injection of precursor solutions[123]. Shear-thinning biomaterials deform during injection and quickly self-recover after injection, preserving the mechanical integrity of biomaterials and defect-specific fit into the injection site[124–127]. Both approaches can be used for localized delivery of therapeutics to modulate the local tissue microenvironment and stimulate in situ regeneration. In the future, we expect the development of biomaterials that can deliver therapeutics on demand, using either external stimuli or local cues. In addition, most of the injectable biomaterials are not mechanically stiff and lack self-healing characteristics, and, thus, cannot be used for tissues that undergo mechanical remodelling. Smart injectable biomaterials that can dynamically respond to mechanical loading, as well as the extracellular microenvironment, are expected to be developed. It will soon be feasible to design shear-thinning and self-healing biomaterials, in combination with the appropriate biophysical and biochemical cues, to stimulate tissue healing and growth.

Mineral-based biomaterials

Inorganic elements, such as minerals, regulate a multitude of the body's biological functions[128] and can be used to direct in situ tissue regeneration by directing cellular functions (FIG. 7c). In the context of tissue regeneration, recent work demonstrated the potential of nanoscale mineralized structures as an alternative to growth factors[129–131]. Nanoscale mineral structures regulate cellular activity to an extent comparable to growth factors through indirect effects on matrix properties, including stiffness, cell adhesivity, nanostructure and degradability. Most of these inorganic biomaterials provide external stimuli to cells that transduce into electrochemical activity, which profoundly affect cell morphology, proliferation, gene expression and,

ultimately, differentiation[131–134]. Despite these promising studies, there has been a lack of research focused on understanding the underlying molecular mechanisms. Such an understanding could create opportunities for designing clinically relevant therapeutics based on inorganic biomaterials for in situ tissue regeneration. Interestingly, these inorganic biomaterials have a natural tendency to dissociate into ions, which can be easily cleared by the body and have a minimum size effect compared with growth factor. In the future, we expect to see new developments using inorganic biomaterials to recruit endogenous cells, as well as to control immune response. Multiple inorganic elements, including gold, zinc, magnesium and strontium, have anti-inflammatory characteristics and can be used for immunomodulation. Incorporation of inorganic biomaterials within a polymer network is expected to stimulate in situ tissue regeneration by providing biophysical as well as biochemical cues. There is, therefore, untapped potential to leverage minerals and mineral-based biomaterials to direct intracellular signalling and gene expression to stimulate in situ tissue regeneration.

Additive manufacturing

The layer-by-layer deposition of biomaterials using additive-manufacturing approaches provides precise spatio-temporal control over biophysical and biochemical cues. For example, 3D printing enables specific deposition of materials into custom shapes and patterns to replicate complex tissue architectures, which is not possible using conventional techniques[135]. Such precise deposition of materials to recapitulate the tissue-level macrostructure and microstructure can facilitate the migration of endogenous cells and deposition of ECM to accelerate tissue healing. Incorporation of therapeutic biomolecules within these printed structures can be used to aid the homing of endogenous cells (FIG. 7d). In addition a range of biomaterials, or inks, required for 3D printing, crosslinking approaches have been developed to obtain scaffolds with high print fidelity, biocompatibility, mechanical stability and biofunctionality[136,137]. By specifically leveraging the biophysical and biochemical characteristics of biomaterials, a range of high-performance inks has been designed to control and direct cell functions[138–140].

Scaffold vascularization is a major limitation preventing the clinical translation of biomaterials constructs for in situ tissue regeneration. Prefabricated vascular networks with specific biochemical cues, such as pro-angiogenic molecules, can stimulate rapid angiogenesis and promote scaffold vascularization by recruiting endogenous cells. However, these prefabricated vascular structures have limited ability to anastomose with the host vascular network, which needs to be overcome to impart functionality within the implanted constructs. It is expected that, by incorporating specific biochemical cues such as adhesion ligands and therapeutic molecules within these 3D-printed structures, integration with host blood vessels can be facilitated. Another area of development is the realization of a fast multi-material 3D printer that can enable the spatial deposition of biophysical and biochemical cues.

Some of the complex micrometre-size features are difficult to recapitulate using biomaterials. Specifically, not all biomaterials are shear thinning and recoverable from extrusion stresses to permit the fabrication of high-fidelity constructs. To overcome these limitations, approaches such as printing within a sacrificial bath[141,142], crosslinking the ink during deposition on the printer bed[143] and addition of shear-thinning additives[144,145] have been developed. However, new crosslinking mechanisms and gelation kinetics, compatible with 3D-printed technology, need to be developed. The use of click chemistry to dynamically modulate biomaterials properties, as well as use of microgels as inks to 3D print macroporous structures, is expected to surge. Overall, we expect to see advances in the areas of ink development and additive-manufacturing technologies to leverage biophysical and biochemical cues to stimulate in situ tissue regeneration.

Omics-based approaches

Recent biomaterials research has focused on engineering bioinstructive and bioresponsive materials. The biological performance of these materials is difficult to assess using traditional low-throughput-screening techniques. By contrast, the recent emergence of 'omics' techniques allows for detailed characterization and understanding of the regenerative potential of biomaterials[146]. Specifically, the use of genomics, epigenomics, transcriptomics, proteomics and metabolomics can provide an unbiased global perspective of cell–biomaterial interactions at the gene, epigenetic, mRNA, protein and metabolic levels (FIG. 7e). In addition, these approaches can be used to gain insight into in vivo responses of various biomaterials, which currently relies heavily on imaging-based methodologies. Single-cell sequencing of engineered cells provides unparalleled ability to measure the precision and accuracy of cellular reprogramming and detect inefficiencies in biomaterials-based in situ approaches[147]. With the growing compendium of data for the effect of biomaterials properties on gene expression, it will soon be feasible to use artificial-intelligence-based approaches to learn and predict the potential outcome of biomaterials with specific properties.

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