# Biomaterials for cell transplantation

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Abstract | Cell transplantation holds immense potential for reversing diseases that are currently incurable and for regenerating tissues. However, poor cell survival, cell aggregation and lack of cell integration into the host tissue constitute major challenges for the clinical translation of cell transplantation approaches. Biomaterials can influence cell behaviour in vitro and in vivo. The mechanical and biochemical properties of biomaterials can be tailored to affect cell survival, differentiation and migration. Therefore, the integration of advanced material design with stem cell biology may hold the key to improving the efficacy of cell transplantation. In this Review, we discuss biomaterial design strategies for their potential to influence the fate of transplanted cells and to manipulate the host microenvironment. We examine how biomaterial properties can be modulated to improve transplanted cell survival, differentiation and cell engraftment and how the host tissue can be manipulated for cell transplantation by inducing plasticity and vascularization. Finally, we emphasize the importance of the host immune cells for tissue repair and cell transplantation and discuss strategies to tune the immune response through modulating the mechanical properties, architecture, chemistry and functionalization of biomaterials.

Adult tissues do not regenerate after injury or degeneration[1], with the exception of the gut epithelium[2], cornea[3], skin[4] and liver[5]. Regenerative medicine approaches applying cell transplantation for the regeneration of tissues have shown promise in the laboratory; however, with the notable exception of haematopoietic stem cell (HSC) transplantation[6,7], such approaches have had only minimal success in the clinic thus far. The main hurdles limiting the clinical translation of cell transplantation are cell survival, migration and integration[8]. The vast majority of transplanted cells die irrespective of the tissue type[9,10]. The few surviving cells often remain at the injection site and sometimes proliferate, but most fail to properly integrate with the host tissue and contribute to functional recovery. Key experiments in the bone marrow and central nervous system (CNS) demonstrated[11–15] that the major limitations are the survival and integration of transplanted cells and the barriers presented by the host tissue environment. Moreover, the optimal cell type for successful transplantation and tissue regeneration remains elusive. Transplanting immature stem cells or progenitor cells can lead to better survival during the dissociation and transplantation stages[16,17] but provides less control over the ultimate lineage commitment of the transplanted cells than transplantation of mature cell types.

Combining cells with biomaterials offers an avenue to address the challenges of cell survival, migration and integration[18,19]. In this Review, we discuss material-based approaches to improve the functional outcome of cell transplantation. We do not aim to provide a comprehensive list of all biomaterials used in the literature but rather to conceptualize the different strategies, highlighting key examples (TABLE 1). We examine strategies that improve transplanted cell survival and integration, control differentiation, influence local angiogenesis and modulate the immune response (FIG. 1). We describe approaches affecting transplanted cells and strategies aimed at influencing the host tissue niche, with the ultimate goal of improving regeneration. We mainly focus on in vivo studies and limit the discussion of in vitro experiments to a few key studies that demonstrate important biological principles.

## Influencing transplanted cells

### *Improving cell survival*

Whether transplanted cells are expected to replace lost tissue or support endogenous repair through secreting regenerative factors, their long-term survival is a prerequisite for their successful application. However, cell survival during and after transplantation constitutes a major challenge. The majority of injected cells die within hours or days after transplantation[9], substantially limiting the efficacy of such therapies. There are three main mechanisms that have been identified to contribute to cell death during and after transplantation: mechanical forces exerted on the cells during the injection process; anchorage-dependent cell death and lack of growth factors; and insufficient support from the degenerative host tissue, including limited access to vasculature. Biomaterials can be applied to address all three challenges to improve transplanted cell survival.

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Table 1 | **Biomaterial design for cell transplantation**

| Property | Approach | Refs |
|---|---|---|
| Injectability | Shear-thinning materials | 21,24 |
| Rapid gelation in vivo | Secondary crosslinking in materials responsive to temperature, light or pH | 25,75,77,78 |
| Cell adhesion | Materials composed of natural ECM factors | 25,36 |
| | Modification with peptides that mimic ECM proteins | 40,41,45 |
| Growth and differentiation factor support | Chemical or physical incorporation of factors in the material | 52,58,88 |
| Cell deployment | Porosity | 87,93 |
| | Modification with peptides that mimic ECM proteins | 87,94 |
| | Viscoelasticity | 98 |
| Mechanical cues | Secondary crosslinking in materials responsive to temperature, light or pH | 25,75,77,78 |
| | Separation of bulk and microscale properties | 79,80,84,87 |
| | Magnetically responsive materials | 109,123 |
| Vascularization | Release of pro-angiogenic factors | 272–275 |
| | Matrix-bound VEGF | 164,169 |
| | Porosity | 171,172,175 |
| | Pro-angiogenic materials | 176,178 |
| | Vascular cell delivery | 188,192 |
| Inflammatory response | Backbone selection | 202,211 |
| | Hydrophilicity | 228,234 |
| | Shape and size | 244,246 |
| | Ligand immobilization | 249,250 |

ECM, extracellular matrix; VEGF, vascular endothelial growth factor.

***Reducing mechanical stress.*** The effect of mechanical stress during the cell injection process is an often underappreciated but significant reason for cell death[20–22]. Cells injected in a Newtonian fluid solution, such as saline, are exposed to shear and extensional forces within the syringe[23]. The velocity is higher at the centre of the syringe than near the walls, owing to the flow resistance exerted at the wall/fluid interface. Furthermore, the needle diameter is typically smaller than the syringe diameter, which leads to a sharp increase in extensional force at the syringe/needle interface. This increase in force results in cell membrane rupture and rapid necrotic cell death[21] and can trigger apoptotic processes, which can lead to further cell death after injection[20,22]. These effects are particularly pronounced in sensitive cell types such as mature neurons[17]. Injectable, shear-thinning biomaterials, such as alginate[21] or hyaluronic acid[24] (HA), can overcome this issue. Shear-thinning biomaterials form a lubricating layer on the interior of the syringe walls, thus diminishing the resistance to flow, which results in relatively equal velocities at the centre and the edges of the syringe during the injection process, known as plug flow, which reduces shear stress. The biomaterial network provides mechanical protection from extensional forces to the cells, which leads to an increase in cell viability after injection[20,21].

Fast gelation after injection limits cellular backflow, which is caused by the interstitial pressure of the tissue. This increases the total amount of cellular material remaining in the tissue after transplantation. For example, the absolute number of transplanted neural stem and progenitor cells (NSPCs) in brain tissue immediately after injection is threefold higher when the cells are injected in a physical blend of HA and methylcellulose (MC) (HAMC) than in saline[25]. HAMC can also be used to improve the distribution of retinal stem and progenitor cells (RSPCs) after injection into the retina because the cell distribution in a syringe is better in HAMC than in saline[26]. Similarly, mesenchymal stromal cells (MSCs) can be delivered in an alginate hydrogel, which increases cell retention to 60% of the starting population 24 h after injection into a rat heart, compared with 9% in saline controls[27]. Alternatively, depending on the tissue type, a more solid implant may be required to bridge the void created by injury, as is the case for volumetric musculoskeletal injuries or transection of the spinal cord[28,29]. Polymers such as poly(lactic-co-glycolic acid) (PLGA)[30] or chitosan[31] form macroscale, rigid scaffolds of controlled shape that can be loaded with specific cell types and implanted into the target tissue to keep transplanted cells in place.

***Preventing anoikis.*** With the notable exception of the haematopoietic system, cells need to adhere to matrix to survive. The issue of anchorage-dependent cell death, or anoikis[32], can be addressed using biomaterials for cell transplantation. The pro-survival signal of adhesion is mediated by integrins, which are cell surface receptors that bind to components of the extracellular matrix (ECM)[33]. ECM binding induces integrin

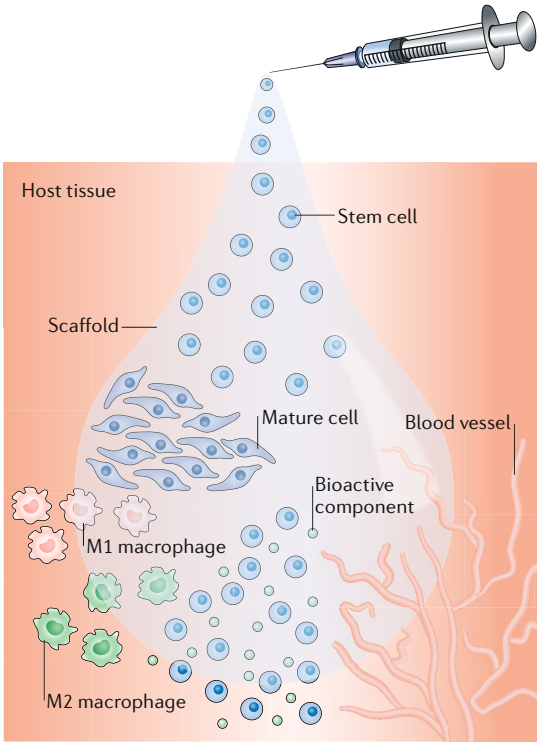


Fig. 1 | **Biomaterials for cell transplantation.** Biomaterials can improve transplanted cell survival, influence transplanted cell fate and integration through the incorporation of bioactive cues, and modulate the host immune response (M1 versus M2 macrophages) as well as angiogenic responses.

clustering, activates focal adhesion kinase and stimulates phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein (MAP) kinase pathways to promote cell survival[34]. Anoikis is triggered in cell suspensions after detachment from the culture plate before transplantation and during the acute post-transplantation phase. Biomaterials comprising molecules that are part of an ECM and that function as integrin ligands, such as collagen, laminin, fibronectin or HA, can prevent anoikis. For example, cell injection in HAMC substantially improves cell survival after transplantation in the brain and retina compared with injection in saline, leading to functional recovery in models of stroke and blindness, respectively[25]. This pro-survival effect of HAMC is partly mediated by the binding of HA to specific cell surface receptors (CD44) and consequent activation of pro-survival signals[35]. Similarly, the use of an injectable scaffold consisting of collagen I and laminin leads to a more than fivefold increase in NSPC survival after transplantation in a mouse model of traumatic brain injury compared with injection in media[36]. Moreover, behavioural recovery is only achieved in animals transplanted with NSPCs in scaffolds.

Synthetic materials can also be modified with integrin-binding domains to mimic ECM-mediated cell signalling events. The best studied example is tripeptide arginine-glycine-aspartic acid (RGD), which binds to at least eight different integrin complexes[37,38], which recognize various ECM molecules including fibronectin and vitronectin. Short peptides, such as RGD, are more stable than their full-length protein counterparts and amenable for the precise spatial and conformational functionalization of materials using flanking amino acid sequences and defined conjugation chemistry[39]. The application of RGD-modified alginate hydrogels resulted in a marked increase in the survival of MSCs in vitro and improved bone growth in vivo after implantation in the mouse subcutaneous space compared with unmodified alginate hydrogels[40]. RGD-modified materials can be used in multiple tissue types, including heart[27], brain[41] and spinal cord[42]. Similar to RGD, the laminin-derived peptides IKVAV and YIGSR and the collagen-derived peptide GFOGER can be incorporated into biomaterials to improve cell adhesion and survival[43–45]. To maximize cell survival, the integrin-binding peptide needs to be tailored to the target cell population and reflect the cell receptors expressed on the surface of the cells as well as the tissue-specific ECM. For example, laminin-mimetic peptides can be used for neural cell transplantation[46], and collagen-mimetic peptides can be employed in the musculoskeletal system[47].

***Growth factor support.*** Maximizing transplanted cell survival requires the replenishing of growth factors that are lost in degenerating tissues. Most growth factors induce survival or proliferation by binding to receptor tyrosine kinases, leading to downstream activation of the PI3K/AKT and MAP kinase pathways[48]. Growth factor concentrations in tissues decrease throughout life[49,50]. Biomaterials can not only act as cell carriers but also constitute a matrix for the attachment and controlled release of growth factors[51] to provide high concentrations and prolonged exposure to growth factors in the immediate vicinity of the transplanted cells. For example, the delivery of myoblasts in alginate hydrogels to an injured mouse muscle leads to an increase in muscle mass and shrinkage of the defect only if hepatocyte growth factor and fibroblast growth factor 2, both involved in muscle regeneration, are co-delivered in the hydrogels[52].

The ideal duration of growth factor release is determined by the balance between maximizing transplanted cell survival and minimizing the potential for tissue side effects caused by unnecessarily long exposure. For example, prolonged exposure to transforming growth factor-α (TGFα) led to epithelial hyperplasia and tumorigenesis in the mouse liver and mammary gland[53–55]. Bone morphogenetic protein (BMP) 2 has been extensively used for osteoinduction and after spinal fusion in the clinic[56]. However, its use has been associated with an array of clinical side effects, ranging from ectopic bone formation to life-threatening spine swelling[57]. Excessive dosing is believed to be a main contributor to these side effects[57]. The temporal release profile of growth factors can be tuned by modulating their immobilization strategy on the biomaterial. Physical blending of the factor in the material often results in rapid release, whereas immobilization of the factor through exploiting protein–protein interactions

(or chemical bonds) can delay (or effectively inhibit) the release, depending on the dissociation constant of the binding partners. For example, an injectable composite scaffold composed of poly(L-lactic acid) (PLLA) nanofibres inside a xyloglucan hydrogel can be used to deliver dopaminergic neurons and glial-derived neurotrophic factor (GDNF) in a mouse model of Parkinson disease[58]. GDNF can be either blended within the scaffold before transplantation, covalently immobilized on the PLLA nanofibres using sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) chemistry or incorporated through a combination of the two. Interestingly, the combination of blended and immobilized GDNF results in an approximately threefold increase in transplanted cell survival compared with cell transplantation without a material, which cannot be achieved if animals are injected with materials with only blended or immobilized GDNF. The improved cell survival might be attributable to a combination of the effects of short-term release of the blended GDNF and long-term release of the immobilized protein.

The growth factor immobilization method can also affect downstream signalling. For example, epidermal growth factor (EGF) signalling transduction occurs both on the cell surface and in endosomes, owing to continuous internalization and recycling of the EGF receptor[59]. Immobilizing EGF on a biomaterial prevents its internalization and therefore spatially concentrates signalling to the cell surface. Chemical tethering of EGF to a poly(methyl methacrylate)-graft-poly(ethylene oxide) polymer induces stronger activation of the MAP kinase pathway in MSCs compared with soluble EGF, even if saturating concentrations of soluble EGF are used[60]. Activation of the MAP kinase pathway further results in superior protection of MSCs from FAS ligand-induced apoptosis. The technology of EGF tethering has also been employed on β-tricalcium phosphate (TCP) scaffolds to transplant human MSCs in the mouse quadriceps perifascial space, leading to an approximate threefold increase in cell survival 21 days after transplantation compared with TCP scaffolds without EGF[61].

Biomaterials can be harnessed to mitigate transplanted cell death through many mechanisms. Interestingly, combining the different strategies within a single material can lead to effects that are greater than the sum of their parts. For example, co-immobilizing growth factors and integrin-binding domains, such as the RGD peptide, on the same polymer chain can potentiate the bioactivity of the material, suggesting that integrins and receptor kinases can be synergistically activated[62,63].

### *Controlling transplanted cell differentiation*

Instead of using fully matured differentiated progeny, immature stem or progenitor cells and committed progeny can be transplanted into the host tissue. These cell types typically show better cell survival during the dissociation and transplantation stages[16,17]. Biomaterials can provide both mechanical and biochemical signals to control stem and progenitor cell fate to obtain the desired terminal cell type.

***Mechanical material properties.*** Stem cell differentiation, maturation and morphogenesis are influenced by the mechanical properties of the ECM[64]. For example, substrate stiffness affects the differentiation of various types of stem cells in vitro[64]. Material scaffolds with the mechanical properties of the tissue of interest are more likely to lead to the formation of the correct cell lineage. For example, MSCs cultured on stiff, medium and soft hydrogels exhibit increased expression of osteogenic, myogenic and neurogenic lineage markers, respectively[65]. Similarly, NSPC differentiation into the three CNS lineages (neurons, oligodendrocytes and astrocytes) can be achieved by culturing NSPCs on hydrogels of varying stiffness[66]. In the majority of these in vitro studies, the cells were seeded on top of the scaffolds and not encapsulated within the materials. However, culture dimensionality is known to affect cell spreading[67]. For example, culture dimensionality can impact the activation of the yes-associated protein (YAP) and transcriptional co-activator with the PDZ-binding motif (TAZ) signalling pathway[68], which is involved in mechanosensation[69–71]. Cell spreading and YAP nuclear localization increase if MSCs are cultured on top of stiff HA-based hydrogels but decrease if MSCs are encapsulated within the hydrogels. MSCs cultured within stiff, highly crosslinked hydrogels do not spread and lack nuclear YAP, which can be partially reversed if the hydrogels are crosslinked by matrix metalloproteinase-degradable peptides that allow encapsulated cells to remodel their microenvironment.

The mechanical properties of organs change during development and disease[72]. Scaffolds with dynamic mechanical properties that can change over time can be employed to mimic this process and study its effect on cell differentiation. For example, the stiffness of HA hydrogels during crosslinking through click chemistry can be regulated by the length of the crosslinker chain[73]. The crosslinking reaction in these gradually stiffening gels can be optimized to mimic the stiffening process in the developing chicken myocardium for the culture of embryonic chicken heart cells, enabling their differentiation into mature cardiomyocytes. A variety of other hydrogels with time-controlled mechanical properties have been developed using a combination of diverse crosslink chemistries and incorporation of stimuli-responsive functionalities[18]. However, these materials have been used only to mimic disease progression or organ development in vitro thus far and have not yet found an application in cell transplantation.

Unfortunately, the knowledge acquired from in vitro experiments has only been sparsely translated into in vivo cell transplantation experiments. Scaffold stiffness for in vitro experiments has been tuned across a broad, 100-fold range[74], but biomaterials used in vivo are typically confined to the low kPa region owing to the requirement of injectability. Injectable hydrogels must be relatively soft to pass through a small-bore needle. However, the range of scaffold stiffness can be expanded by, for example, secondary crosslinking of injectable hydrogels by an external stimulus, such as light, pH

or temperature, to fine-tune the resulting mechanical properties[75–77]. For example, the initial dynamic covalent crosslinking of an injectable hydrogel can be combined with thermoresponsive physical crosslinking of engineered proteins, such as elastin-like proteins[78]. The final storage modulus of the hydrogel can be tuned between 1 kPa and 2.75 kPa through varying the amount of incorporated elastin-like proteins.

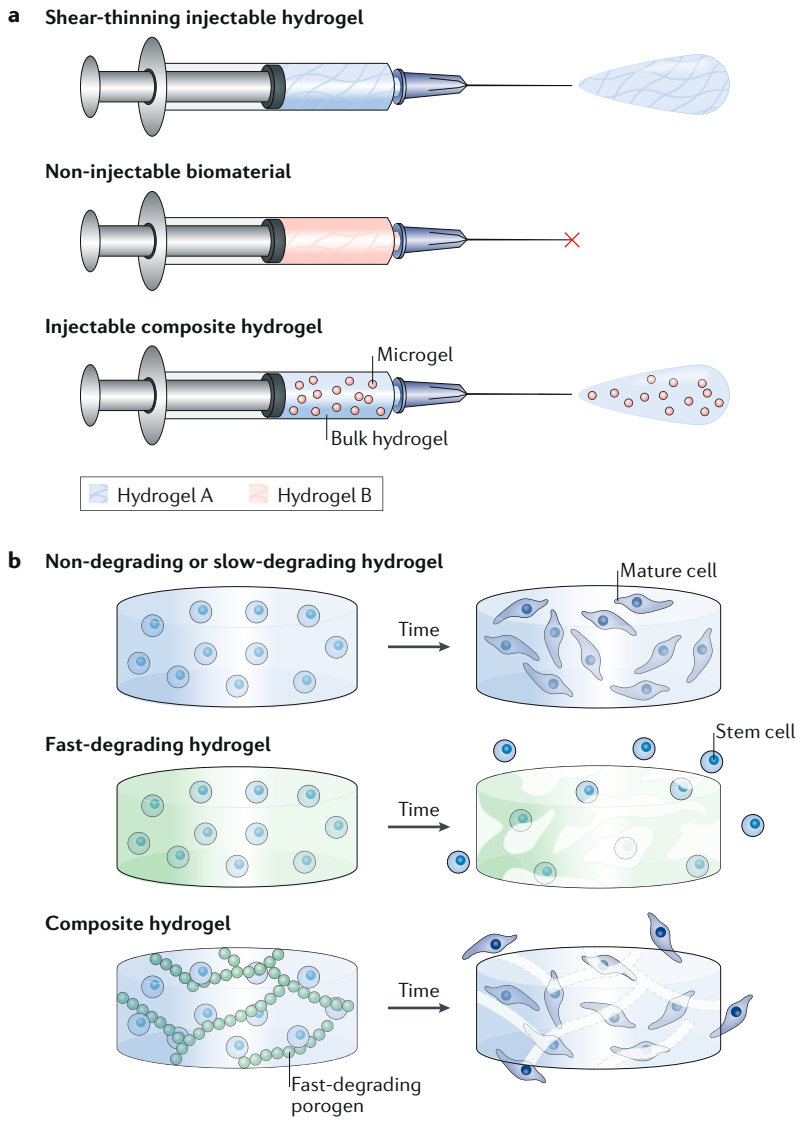


Fig. 2 | **Injectable biomaterials.** Composite hydrogels can be used as injectable biomaterials with multiple properties. **a** | Properties of an injectable biomaterial (top, hydrogel A) and a stiff non-injectable biomaterial (centre, hydrogel B) can be combined through the formation of a composite hydrogel. The initially non-injectable hydrogel can be formulated into micrometre-sized microgels and incorporated into the injectable bulk hydrogel, leading to the formation of a new injectable composite hydrogel (bottom). **b** | Slow-degrading hydrogels (blue) enable cell maturation but limit cell integration into the host tissue compared with fast-degrading hydrogels (green). Additionally, the mechanical properties of hydrogels provide stem and progenitor cells with important cues, which can induce their differentiation and maturation. However, owing to changes in the mechanical properties during the degradation process, fast-degrading hydrogels provide only limited control over cell fate. Composite hydrogels with fast-degrading porogens and a bulk hydrogel with defined and stable mechanical properties can be used to trigger in vivo cell differentiation and improve cell infiltration into the host tissue.

Prefabricated scaffolds can also be used for implantation[79–84]. Furthermore, composite hydrogels can be designed, in which preformed microgels are incorporated within a bulk hydrogel[85,86]. The mechanical, topological and chemical properties of the microgels can be tailored independently from the bulk hydrogel properties by combining multiple crosslink strategies (FIG. 2a). This approach enables the expansion of the versatility of injectable hydrogels. Microgel composition and chemistry can be freely chosen, and shear-thinning properties are controlled by the bulk hydrogel composition and crosslink method. Such a combinatorial approach can be applied to decouple the stiffness of the bulk hydrogel component from pore formation in vivo (FIG. 2b). For example, high molecular weight alginate bulk polymer can be combined with oxidized, hydrolytically labile alginate microbeads. In a rat cranial defect model, differentiation of transplanted MSCs can then be controlled by tuning the bulk alginate stiffness. The fast degradation of the microbeads also improves MSC migration and osteogenesis[87]. Therefore, separation of bulk and microscale properties allows for optimization of the scaffold design.

***Cytokine delivery.*** Differentiation of stem and progenitor cells in a dish is typically achieved by using an appropriate cytokine cocktail. However, this approach cannot be applied for the differentiation of transplanted cells in vivo, because injected cytokines would rapidly diffuse away from the transplantation area. Similar to growth factors, cytokines can also be incorporated in a scaffold to prolong their residence time at the transplantation site. For example, the transplantation of MSCs within an alginate hydrogel into the mouse subcutaneous space leads to substantial new bone formation only if the hydrogel also contains transforming growth factor-β3 (TGFβ3) and BMP2, which are both involved in normal bone development and repair[88]. Various criteria, such as the method of incorporation, release kinetics and concentration of cytokines, should be considered to control differentiation into specific cell types. For example, temporal control of certain cytokines is required for cell differentiation in early developmental stages[89]. In other cases, such as BMP induction of osteogenesis from MSCs[90], simply prolonging the treatment with a specific factor is advantageous in improving differentiation. The period of retention of a cytokine can be extended by different approaches. The molecule can be immobilized onto the main scaffold component through chemical bonds, protein–protein interactions or ionic interactions, or it can be encapsulated within microparticles or nanoparticles. For example, an extended release duration can be achieved by covalently immobilizing BMP2 to an injectable polyethylene glycol (PEG)-based hydrogel using azide/alkyne click chemistry[91]. Chemical immobilization enables quantitative BMP2 retention in the hydrogel for up to 21 days. By contrast, if BMP2 is simply adsorbed to the hydrogel, 47% of the protein is lost within two weeks. Prolonged BMP2 retention results in improved osteogenic differentiation of periodontal ligament stem cells after injection in the mouse

subcutaneous space compared with cells injected in a scaffold with adsorbed protein, whereas cell survival is seemingly unaffected. Similarly, prolonged, local release of dibutyryl cyclic-AMP from PLGA microspheres, distributed along the walls of chitosan channels, improves the differentiation of NSPCs within the channels towards the neuronal lineage if transplanted in the rat spinal cord after injury[92].

### *Improving transplanted cell engraftment*

The use of scaffolds for cell transplantation improves cell retention in the injection site. However, to promote recovery, cells need to eventually integrate into the host tissue, which requires migration of the transplanted and/or host cells. Biomaterial porosity and adhesion signals are both crucial for transplanted cell engraftment. For example, macroporous scaffolds lead to superior MSC engraftment, host cell infiltration and bone repair after transplantation in a mouse cranial defect compared with conventional nanoporous hydrogels with the same material backbone[93]. Similarly, the rate of void-forming porogen degradation in alginate gels correlates with the amount of MSC release in vitro and in vivo after transplantation in the mouse subcutaneous space[87]. Cell deployment is further substantially decreased in alginate gels without the void-forming component. Moreover, increasing the amount of RGD peptides within the alginate gels improves cell release from the material in vivo, presumably by providing more anchoring points for the generation of traction forces required for cell migration. Similarly, RGD functionalization of microporous alginate with shape-memory properties has been shown to be essential for cell infiltration in the host tissue and persistence in vivo[94].

In addition to biomaterial stiffness, viscoelasticity is also a crucial parameter for cell differentiation and proliferation in vitro[95–97] and for cell engraftment in vivo[98]. RGD-modified alginate hydrogels can be fabricated with different rates of stress relaxation, and MSC engraftment and new bone formation are substantially better in fast-relaxing hydrogels than in slow-relaxing gels, 3 months after transplantation of human MSCs in a rat cranial defect. Tissue remodelling and hydrogel degradation are further accelerated in the fast-relaxing gels, possibly owing to better host cell infiltration into the scaffold, demonstrated by a migration assay in vitro. Interestingly, empty (not cell-laden) fast-relaxing hydrogels induce similar levels of new bone growth to cell-laden fast-relaxing hydrogels, suggesting that the contribution of the transplanted MSCs to regeneration is minimal.

## Strategies influencing the host tissue niche

The tissue niche is an indispensable component of cell biology and function[99]. Under physiological conditions, niche cell types and molecules regulate stem cell quiescence, activation and differentiation[100]. However, cells are often transplanted into diseased or degenerated tissue, which is hostile and not conducive to regeneration. Biomaterials can be applied to modulate the host microenvironment and improve transplanted cell engraftment and tissue regeneration.

### *Mechanical stimulation*

The mechanical properties of biomaterials can be tailored to directly influence regeneration in the host tissue. This strategy has mainly been explored in musculoskeletal tissues. Beginning with seminal studies in the 1980s[101,102], it has since been demonstrated that controlled mechanical stimulation by cyclic and axial (that is, in the direction of the bone axis) movement can affect bone healing after fracture. Early timing and high strain rate of axial displacements, up to a maximum threshold, substantially improve bone healing[103–106]. The exact molecular mechanisms underlying this effect remain unclear, but integrin-mediated mechanosensation by bone cells is thought to be involved[107].

Instead of developing external actuation devices to deliver mechanical stimuli, biomaterials can be devised to provide biomechanical stimulation at the microscale. Incorporation of magnetizable components allows the use of external magnetic fields to modulate local biomechanical forces. For example, magnetic porous scaffolds can be fabricated by adding magnetite nanoparticles to the nucleation reaction of hydroxyapatite and collagen I[108]. The resulting composite material exhibits a homogeneous distribution of magnetite throughout its volume and is biocompatible, inducing no adverse reactions and substantially improving new bone growth in rabbits compared with non-magnetized hydroxyapatite–collagen I scaffolds[109]. Similarly, a magnetic nanofibrous scaffold can be created by electrospinning poly(D,L-lactic acid) (PLA) mixed with hydroxyapatite nanoparticles and iron oxide nanoparticles[110]. Implantation in a rabbit bone defect and application of an external magnetic field result in accelerated bone formation and increased deposition of osteocalcin and collagen compared with implantation alone. The resorption of the transplanted biomaterial is also faster in the presence of the magnetic field, providing evidence for tissue remodelling.

Interestingly, external magnetic fields have been shown to promote osteogenesis and to regulate the direction of bone growth on their own[111]. Therefore, it is unclear whether the beneficial effects of magnetic biomaterials are caused by biomechanical stimulation or by other confounding factors. Poly(caprolactone) (PCL) scaffolds can be impregnated with magnetite nanoparticles to assess the activation of mechanotransduction signalling pathways in primary osteoblasts in the presence of an external magnetic field[112]. In the cultured osteoblasts, Rho GTPase, focal adhesion kinase (FAK) and paxillin — all typical effectors of integrin-mediated biomechanical signalling — are synergistically activated by the presence of magnetite nanoparticles in the scaffold and the external magnetic field. Implanting the PCL composite with or without magnetite in a mouse bone defect model and applying an external magnetic field further demonstrate that the presence of magnetite in the material and the external magnetic field exhibit an additive effect with regards to new bone formation.

Mechanical stimulation can also be applied to muscle tissue to improve structural organization and force generation in vitro[113–116], and there is some evidence

of a potential effect on muscle recovery after injury in vivo[117,118]. Cyclic compressive stimulation promotes force recovery and decreases the expression of inflammatory markers in a rabbit exercise-induced muscle injury model[119]. This effect depends on the frequency and magnitude of the stimulation[120]. Initiating mechanical stimulation immediately after injury further improves force recovery compared with a delayed onset of stimulation[121]. 'Biphasic' porous, magnetic hydrogels can be synthesized by crosslinking alginate mixed with iron oxide microparticles[122] in the presence of a magnetic field, resulting in a concentration gradient of iron oxide within the hydrogel. This gel, implanted in a mouse model of muscle injury, can be externally stimulated with a magnet to induce cyclic mechanical compressions[123]. Interestingly, the mice treated with the gel and magnetic stimulation show increased muscle fibre size and decreased fibrosis compared with mice treated with the gel alone or untreated mice. This improved tissue phenotype is correlated with a significant increase in maximum contractile force.

Co-delivering mechanical stimulation with cells offers another avenue to improve cell transplantation. For example, magnetic nanoparticles can be immobilized onto MSCs by binding to either integrins or a mechanosensitive receptor[124]. Injection of the nanoparticle-modified MSCs into chick femur explants and application of an external magnetic field, inducing a mechanical stimulus, lead to a 31–34% increase in bone formation and significantly higher bone density than in saline-injected femurs. Bone formation in femurs injected with unmodified MSCs in the presence of a magnetic field or modified MSCs in the absence of a magnetic field is similar to in saline-injected femurs.

Therefore, the mechanical properties of biomaterials can induce regenerative responses in the host tissue. Progress in 'smart' polymers that respond to external cues, such as magnetic fields, may obviate the need for complex and cumbersome external actuation devices.

### *Inducing plasticity in the host tissue*

Transplanted cells need to survive and integrate into the host tissue to provide functional benefits in cell replacement approaches. Tissue degeneration is associated with fibrosis and scar tissue formation[125], which present a barrier to the integration of transplanted cells. In the CNS, for example, a glial scar forms after injury owing to the excessive proliferation of astrocytes and pericytes, resulting in hypertrophy and the secretion of ECM molecules. This glial scar is a physical and chemical barrier to regeneration[126]. The ECM molecules chondroitin sulfate proteoglycans (CSPGs) are abundant in the glial scar and play a pivotal role in inhibiting regeneration[127,128]. CSPGs are normally part of perineuronal nets, which are responsible for maintaining the maturity of adult neuronal connections and for limiting pathological plasticity[129]. However, the presence of perineuronal nets also prevents transplanted cells from establishing connections with host neurons. Treatment with chondroitinase ABC (ChABC), a bacteria-derived enzyme that degrades CSPGs, has shown promise in improving recovery after spinal cord injury (SCI)[130] and stroke[131,132]. Combined with cell transplantation, ChABC promotes the formation of graft–host connections and migration of transplanted cells in the spinal cord[133,134] and retina[135,136]. Notwithstanding these promising results, prolonged ChABC delivery is plagued by the invasiveness of catheter systems and their vulnerability to infection, as well as the low stability of the enzyme at 37 °C (REF.[137]). Material delivery systems can bypass these issues and achieve a minimally invasive, prolonged release of ChABC.

Affinity-based release strategies can be applied by taking advantage of the binding affinity of the Src homology domain 3 (SH3) to proline-rich peptides[138,139]. ChABC can be expressed as a fusion protein containing SH3, and SH3-binding peptides can be chemically immobilized on MC. By selecting SH3-binding peptides with different affinities for SH3, the ChABC release rate can be tuned. Importantly, hydrogel-bound ChABC does not lose its bioactivity over a 7-day release period. This delivery system can be used to decrease CSPG levels and induce behavioural recovery in a rat model of SCI[140]. Approaches for combining MC-ChABC with cell transplantation are still in their infancy, but initial studies show promise in improving tissue repair[141].

Incubation of ChABC with trehalose also preserves its conformational stability and maintains its bioactivity for up to 15 days, as compared with 3 days in the absence of trehalose[142]. ChABC and trehalose can be loaded into lipid microtubes, which can then be mixed with an agarose gel. Implanted in a rat SCI model, the biomaterial-delivered ChABC leads to a decrease in CSPG levels and perineuronal nets 2 weeks after injury, which cannot be achieved with saline-delivered ChABC. Interestingly, if the microtube delivery system is loaded with both ChABC and neurotrophin-3 (NT-3), some behavioural recovery can be observed.

In addition to removing the impediment to regeneration caused by inhibitory molecules such as CSPGs, material strategies can also be applied to deliver factors that directly promote plasticity and rewiring. A major pro-plasticity factor in the CNS is brain-derived neurotrophic factor (BDNF)[143]. Since its identification in the 1980s[144,145], a plethora of studies have demonstrated that BDNF is essential for the synaptic changes triggered by learning and memory[146–148], as well as for neural rewiring and recovery after injury[149,150]. Through inducing plasticity, BDNF treatment increases graft–host connections in peripheral nerve grafts and for embryonic neurons transplanted into the spinal cord after SCI[151,152]. Furthermore, BDNF treatment promotes visual recovery when combined with embryonic retinal grafts in a model of retinal degeneration[153]. However, BDNF treatment can also cause the formation of pathological connections, functional deterioration and spasticity[154,155]. Therefore, treatment with exogenous BDNF has to be precisely localized at the injury site and temporally regulated. However, BDNF does not cross the blood–brain barrier, and thus, lentiviral constructs are mostly used to induce its expression at the injury site, which limits the spatiotemporal control of BDNF expression. Incorporation of BDNF into biomaterial delivery systems can be applied to address these challenges.

For example, BDNF-loaded PLA scaffolds with oriented macropores can be applied to bridge a transection defect in a rat model of SCI[156]. The scaffolds can be implanted in fibrin gels containing acidic fibroblast growth factor and compared against PLA scaffolds without BDNF. In the BDNF-containing scaffolds, increased cell migration and laminin deposition are observed. Moreover, neuroprotection on the rostral side of the injury can be improved. However, the number of myelinated axons inside the scaffold is approximately an order of magnitude lower for both the BDNF and control PLA scaffolds compared with the fibrin gel alone, suggesting that PLA is not ideal for SCI transplants. Alternatively, BDNF added to agarose hydrogels substantially improves axon infiltration into the scaffold after implantation in rat models of SCI[157,158]. Similarly, poly(ethylene-co-vinyl acetate) (EVA) can be used to create tubular scaffolds for nerve guidance in a dorsal root axotomy model[159]. BDNF can be first incorporated in the EVA tubes and then released in a bioactive form for over 30 days. However, releasing BDNF from the EVA tubes leads only to a trend towards an increased number of myelinated axons in the scaffold in vivo, which is not statistically significant. Despite the promising results obtained with these materials, no functional recovery was observed in these studies. Tubular constructs are applicable for transection models of SCI because they can serve as bridges for repair; however, injectable materials are preferable for other applications because they limit host tissue damage.

Scaffolds composed of natural ECM, such as HA, are often thought of as biocompatible and limit adverse cell responses. For example, BDNF can be delivered to a stroke-injured mouse brain using a commercially available injectable hydrogel containing thiolated HA and collagen, crosslinked via PEG-diacrylate[160]. Interestingly, hydrogel-mediated BDNF delivery leads to behavioural recovery in two different tasks, which cannot be achieved when animals are treated with soluble BDNF. Hydrogel-mediated BDNF delivery triggers changes in neuronal connectivity, leading to a pronounced increase in axonal connections within the peri-infarct cortex and between the peri-infarct cortex and contralateral brain areas. Interestingly, the newly formed connections are restricted to brain areas that are already axonal targets of the peri-infarct cortex but do not occur in other brain areas, allaying concerns about pathological plasticity. In addition to potentiating connections of existing neurons, hydrogel-mediated BDNF delivery also stimulates the accumulation of newborn neurons in the peri-infarct cortex. Hydrogel-mediated BDNF delivery can also be employed in a non-human primate model of stroke, with detectable levels of BDNF reported in the peri-infarct area, validating the potential of this approach for clinical translation.

### *Inducing vascularization*

Regeneration processes are strongly dependent on the host vasculature for the supply of oxygen, nutrients and growth factors. Diffusion limits of oxygen, nutrients and metabolites result in a maximum distance of 150–200 μm between cells and blood vessels[161] to ensure adequate supply. Angiogenesis — the process of new blood vessel formation — is initiated by an angiogenic signal, which is often released by tissues in response to hypoxia[162]. This signal induces detachment of pericytes that surround the existing blood vessel, followed by disassembly of endothelial cell–cell junctions. Endothelial tip cells sprout and migrate towards the angiogenic stimulus, and stalk cells, located behind the tip cells, proliferate to generate the trunk of the newly formed vessel. ECM deposition and pericyte coverage ensure maturation and stabilization of the vessel.

Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor and has been the focus of many therapeutic approaches targeting the vasculature[163]. For example, VEGF can be immobilized on collagen sponges through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)–*N*-hydroxysuccinimide (NHS) chemistry. Transplanted into a rat model with a heart defect[164], such VEGF-containing scaffolds exhibit significantly higher blood vessel density and overall thickness and recruit more haematopoietic cells and myofibroblasts than scaffolds without VEGF. Importantly, transplantation of bone marrow cells within VEGF-containing collagen scaffolds greatly improves their survival. A longstanding caveat of VEGF therapeutics is the issue that soluble VEGF leads to the generation of leaky and unstable blood vessels, which has hindered its clinical application[165]. Incorporating VEGF in biomaterials not only provides spatiotemporal control over its release but can also alter its presentation to cells, resulting in increased potency[166]. Matrix-bound VEGF induces differential phosphorylation of the VEGF receptor, promotes receptor clustering and activates different intracellular pathways than soluble VEGF[167]. The association of the VEGF receptor with β1-integrin is essential for the differential activity of matrix-bound VEGF (FIG. 3a). Acrylate and acrylamide-based nanoparticles, the in vivo degradation rate of which can be controlled by the chirality of the peptide crosslinker[168], can be loaded with VEGF and encapsulated in HA hydrogels, crosslinked by Michael addition. Co-delivery of fibronectin fragments that bind α3β1 and α5β1 integrins to the mouse subcutaneous space or stroke-injured brain[169] leads to the generation of new blood vessels with a more mature morphology and with decreased leakiness and tortuosity, resembling wild-type blood vessels, compared with VEGF nanoparticles co-delivered with control fibronectin fragments that bind αvβ3 integrin.

A compelling alternative to using angiogenic factors is to modulate the physicochemical properties of the material to promote angiogenesis[170]. For example, thin membranes made of a variety of materials and with varying pore size have been used to demonstrate that pore size can greatly affect neovascularization upon implantation in the rat subcutaneous space[171] (FIG. 3b). Pore sizes of 0.8–8 μm lead to the formation of 80–100-fold more blood vessels than smaller pores, with the effect being consistent among multiple materials. Therefore, greater pore size leads to increased neovascularization of materials; however, the absolute pore size values of these scaffolds (as opposed to thin membranes) are typically an order of magnitude higher, between 50 and 300 μm (REFS[172,173]).

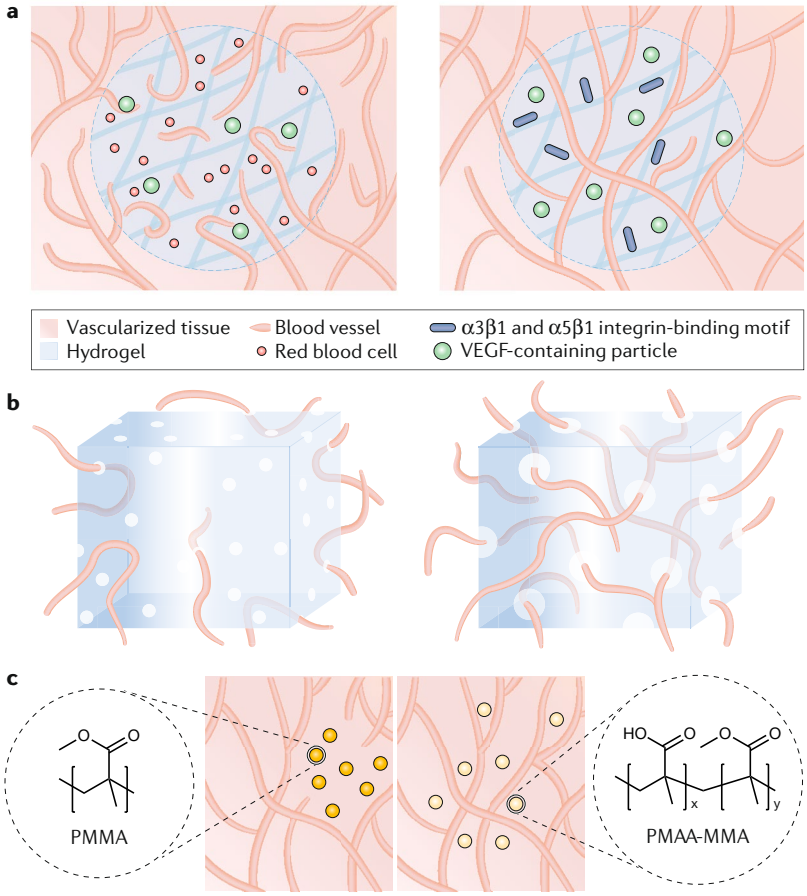


Fig. 3 | **Regulation of angiogenesis by biomaterials. a** | Treatment with soluble vascular endothelial growth factor (VEGF) induces tortuous and leaky blood vessel formation. Colocalization of VEGF and β1 integrin-binding motifs within a hydrogel leads to successful material vascularization, with mature blood vessels. **b** | Biomaterial pore size affects angiogenesis. Larger pores lead to improved angiogenesis. **c** | Poly(methacrylic acid-co-methyl methacrylate) (PMAA-MMA) beads, but not poly(methyl methacrylate) (PMMA) beads, induce angiogenesis after implantation in vivo.

In addition to pore size, the diameter of pore interconnections within a material affects vascularization[174]. TCP scaffolds with varying pore size and interconnection diameter were implanted in rabbits to assess neovascularization[175]. Pore sizes greater than 400 μm led to the formation of blood vessels of larger diameter, whereas greater interconnection diameters (ranging from 70 to 200 μm) led to both the formation of a higher number of blood vessels and larger blood vessel diameters. These findings suggest that, at least in the pore size range examined here (300–700 μm), pore interconnectivity is a more important factor for vascularization than pore size.

Interestingly, the choice of biomaterial backbone can influence angiogenesis irrespective of porosity or protein tethering. For example, poly(methacrylic acid-co-methyl methacrylate) (PMAA-MMA) beads, implanted in myocutaneous (skin and underlying muscle) rat grafts, increase blood vessel counts in the graft by more than 40% compared with poly(methyl methacrylate) (PMMA) beads or no bead treatment[176] (FIG. 3c). Similarly, in a mouse model of diabetes, PMAA-MMA beads improve neovascularization and promote wound closure in full-thickness wounds (skin and underlying muscle)[177]. However, PMAA-MMA beads are very brittle. Porous scaffolds constructed from poly(butyl methacrylate-co-MAA)[178] have tunable mechanical properties and mitigate the brittleness observed with porous PMAA-MMA scaffolds. Implanted in the mouse subcutaneous space, poly(butyl methacrylate-co-MAA) scaffolds lead to an approximately twofold increase in blood vessel formation compared with control poly(butyl methacrylate) scaffolds, owing to the upregulation of the sonic hedgehog (Shh) pathway, which is involved in angiogenesis[179,180]. Furthermore, PMAA influences the host immune response to the biomaterial; more neutrophils and macrophages are recruited to the PMAA-treated tissue than with PMMA controls, with the macrophages being polarized towards an anti-inflammatory state[180]. Moreover, after incubation in human serum, protein adsorption differs between PMAA and PMMA, causing a decrease in complement activation in PMAA scaffolds[181]. Although more studies need to be performed to decipher the exact mechanism of action of PMAA on angiogenesis, these data suggest that differences in initial protein adsorption to PMAA modulate immune cell recruitment, such as macrophage recruitment, and lead to the activation of the Shh pathway. This, in turn, promotes angiogenesis by affecting macrophages and endothelial cells. It remains elusive whether this pro-angiogenic behaviour is unique to PMAA or whether it can also be elicited by other materials.

A conceptually different strategy to promote new blood vessel formation is through regulation of vasculogenesis, the process by which endothelial progenitor cells (EPCs) give rise to new endothelial cells to form de novo blood vessels[182,183]. The number of circulating EPCs declines in vascular diseases, such as type 2 diabetes[184] and atherosclerosis[185], providing the impetus for the development of EPC transplantation approaches. Hydrogels, such as alginate, fibrin and HA, can be used as EPC delivery vehicles to improve cell retention and neovascularization at the host site[186,187]. For example, the delivery of human EPCs in an alginate hydrogel containing chemically immobilized RGD and physically mixed VEGF leads to marked improvements in blood flow, vessel density and tissue viability in a mouse model of hindlimb ischaemia[188]. Interestingly, the EPCs, the alginate scaffold and VEGF are all required to realize the full magnitude of this effect.

Biomaterials can also be applied to form primitive vascular networks from endothelial cells in vitro that can then be transplanted in vivo to achieve faster perfusion and anastomosis with the host vasculature[189] compared with biomaterial–endothelial cell constructs that are transplanted without being first cultured in vitro. The addition of pericytes[190,191] and the use of micropatterning techniques to control the geometry of the preformed vascular network[192] further improve blood perfusion and vasculature maturation in vivo. Such engineered vasculatures can also be used for therapeutic cell transplantation. For example, the addition of aligned endothelial cells to a fibrin–collagen I scaffold that contains primary

hepatocytes leads to significantly improved hepatocyte activity after transplantation in the mouse fat pad[192]. Importantly, this effect depends on blood perfusion of the transplanted endothelial cell vasculature. Similarly, adding endothelial cells to a collagen I module containing primary pancreatic islets results in glycaemic control after transplantation into the subcutaneous space of diabetic mice. The co-transplanted endothelial cells contribute to the formation of vasculature inside the transplanted module, which connects to the host blood supply[193].

Thus, biomaterials can provide a multipronged strategy to modulate vascularization. Protein loading, internal structure, backbone material selection and vascular cell delivery can all be exploited to fine-tune host blood vessel growth and optimize regenerative outcomes.

### *Tuning the immune response*

The immune system was long considered an impediment to the success of cell transplantation approaches, and research focused on inhibiting the immune response to transplanted cells. However, the immune system is being increasingly recognized as a major regulator of the balance between tissue regeneration and degeneration. T helper 1 ($T_H1$)-type immune responses are associated with inflammation and tissue damage, whereas $T_H2$-type immune responses are considered to mediate tissue healing and regeneration (BOX 1). This rule has been demonstrated to also be valid for biomaterial-mediated regeneration.

A biomaterial-driven $T_H2$ immune response is essential for implanted ECM-mediated regeneration[194]. For example, ECM from decellularized tissue promotes functional tissue recovery when implanted after muscle volumetric injury[195,196] in animal models and humans. Such natural biomaterials, including bone-derived ECM, cardiac muscle-derived ECM and collagen, recruit T cells and polarize them into a $T_H2$ phenotype. However, this regenerative response is lost in recombination activating gene 1 *(Rag1)*$^{-/-}$ mice, which lack mature B and T cells, and in *CD4*$^{-/-}$ mice, which lack $CD4^+$ T cells. Repopulation of *Rag1*$^{-/-}$ mice with wild-type T cells, but not with $T_H2$-deficient T cells (rapamycin-insensitive companion of mammalian target of rapamycin *(Rictor)*$^{-/-}$), rescues the regenerative response. Comparing immune responses elicited by 14 different US Food and Drug Administration (FDA)-approved, tissue-derived ECM products after implantation in an abdominal wall muscle defect in the rat[197] shows that there is a significant positive correlation between tissue recovery and both the number of M2 macrophages and the M2:M1 ratio recruited to the biomaterial (BOX 1).

Therefore, biomaterials approaches aim to tip the $T_H1/T_H2$ balance towards the latter, which can be achieved by tuning the material type, backbone composition, architecture, mechanical properties and functionalization.

***Biomaterial type.*** The type of biomaterial can exert a profound effect on the host immune response. For example, PLGA, which is often used for cell transplantation[198,199], acts as an adjuvant, inducing inflammatory $T_H1$-type immune responses[200,201]. Dendritic cell (DC) maturation and inflammatory cytokine secretion are early hallmarks of a $T_H1$-type response. Interestingly, PLGA, chitosan and alginate induce DC maturation and pro-inflammatory cytokine secretion, eliciting a $T_H1$-type response, whereas HA downregulates DC maturation and pro-inflammatory cytokine secretion, and agarose has no effect[202]. Exposure to biomaterials also affects the potential of DCs to induce T cell activation and polarization[203]. The response of the immune system to the biomaterial depends on receptor-mediated

**Box 1 | $T_H1$ versus $T_H2$ immune responses**

T cells are activated by antigen-presenting cells, such as dendritic cells, and are a key part of the body's adaptive immune response to specific antigens. T cells can be divided into $CD4^+$ T helper ($T_H$) cells, which secrete cytokines and coordinate the innate and adaptive arms of the immune response, and $CD8^+$ T-effector cells, which kill cells compromised by pathogens or cancer. Different types of pathogens induce differential polarization of $T_H$ cells: intracellular pathogens such as viruses and bacteria trigger the activation of $T_H1$ cells, whereas extracellular parasites, such as helminths, cause polarization into $T_H2$ cells[263,264]. The $T_H$ cell types are distinguished by their cytokine secretion profile, with $T_H1$ cells secreting interleukin (IL)-2, interferon-γ (IFNγ) and tumour necrosis factor (TNF)-α and $T_H2$ cells secreting IL-4, IL-5 and IL-13 (REF.[265]). The specific cytokine profiles cause $T_H1$ cells to stimulate strong $CD8^+$ T cell responses, which eliminate pathogen-infected host cells, and cause $T_H2$ cells to stimulate strong B cell responses for antibody production.

In addition to their roles in pathogen elimination, $T_H1$ and $T_H2$ responses are important modulators of tissue repair[266]. $T_H1$ responses are involved in tissue damage and inflammation, whereas $T_H2$ responses induce tissue remodelling and angiogenesis. Besides $T_H1$ and $T_H2$ phenotypes, other $CD4^+$ T cell subsets that also play a role in the immune response have been identified[267].

Macrophages are part of the innate immune response, which is triggered immediately upon exposure to a pathogen and is not antigen-specific. However, as an immune reaction proceeds and adaptive immunity is activated, blood-derived monocytes migrate into the target tissue and differentiate to create macrophages. Similar to the $T_H1$–$T_H2$ paradigm, macrophages can be broadly classified into M1 and M2 phenotypes, based on their marker expression, cytokine profile and functions[268,269]. M1 macrophages are activated by IFNγ and secrete TNFα and IL-12, which stimulate $T_H1$ cells and promote an inflammatory, microbicidal environment. M2 macrophages are activated by IL-4 and IL-13 and stimulate $T_H2$ cells, basophils and eosinophils to promote tissue regeneration. Therefore, T cells and macrophages operate within a mutually dependent network, which coordinates polarization into inflammatory or tissue healing phenotypes[270]. In this Review, the term $T_H1$-type response encompasses both $T_H1$ cells and M1 macrophages, and the term $T_H2$-type response includes both $T_H2$ cells and M2 macrophages. Of note, M1 and M2 phenotypes are extreme ends of a spectrum, and intermediate macrophage phenotypes are often encountered in vivo[271].

binding of immune cells to the biomaterial, for example, by integrins[204], Toll-like receptors (TLRs)[205,206] or CD44 (REF.[207]). Moreover, the physicochemical material properties, such as hydrophilicity[208,209] or surface roughness[210], have an impact on the immune response owing to differences in nonspecific protein adsorption on the material, which affects initial immune cell adhesion.

HA is one of the most compelling immune-regulating materials because its effects depend on the molecular weight of the polymer chains[211]. High molecular weight (HMW) HA is abundant in the ECM of multiple tissues and is indispensable for maintaining homeostasis through exerting an anti-inflammatory role[212], as exemplified by HA-synthesis knockouts[213,214]. HMW HA can directly affect macrophages[211,215,216], DCs[202,217] and T cells[218], thus preventing inflammation on multiple fronts. By contrast, HA fragments and low molecular weight (LMW) HA are generated during injury and promote $T_H1$-type inflammatory responses[219]. The effects of LMW HA are just as multifaceted. LMW HA induces maturation, cytokine secretion and $T_H1$-type polarization on macrophages[211,220] and DCs[221]. These seemingly contradictory effects of HA can be explained by differential binding to CD44 — the main HA receptor. Longer HA chains can simultaneously bind multiple CD44 molecules on the cell surface, altering their clustering and thus modifying downstream signalling pathways[222]. Owing to the multivalent nature of the HA–CD44 interaction, longer HA molecules bind more stably, and the amount of bound HA on the cell surface increases with molecular weight, which can also affect cell signalling[223,224]. In addition, LMW HA, but not HMW HA, can induce inflammatory signalling by binding to TLR4 and TLR2, which are normally employed for detecting molecular patterns in bacteria[225,226]. Thus, modulating the chain length of HA provides an opportunity to tailor the immune response elicited by the material.

***Material chemistry.*** The chemistry of polymers can be modified to modulate their interaction with the immune system. Generally, hydrophobic materials induce acute, inflammatory immune reactions[227]. The surface chemistry can be altered to include hydrophilic groups such as –COOH, –OH or $–NH_2$ to modulate protein adsorption, complement activation and immune cell adhesion on the material[228]. Hydrophilic PEG has been widely used to decorate biomaterial surfaces to decrease protein binding[229,230], even though its efficacy in vivo has been questioned[231–233]. Alternatively, zwitterionic polymers, which combine positive and negative charges, can be employed to mitigate immune cell recognition of the material and a fibrotic response in vivo[234,235] owing to their stronger electrostatic interactions with water molecules compared with surfaces that form hydrogen bonds with water, making water displacement for protein binding energetically inefficient[236,237]. Interestingly, surface chemistry can be used not only to modulate the amount of protein binding on the material but also to change the conformation of proteins. For example, the secondary structure[238] of fibrinogen and its binding to domain-specific antibodies[239] depend on the surface to which it is adsorbed. Depending on the fibrinogen protein domains that are exposed on the surface, differential downstream integrin activation, focal adhesion signalling and platelet binding are triggered[228,240]. It remains unclear how the modification of biomaterials with cell-adhesive peptides influences the inflammatory response. Both a greater number of adhering macrophages without activation[241,242] and peptide-dependent polarization[243] have been reported.

***Material architecture.*** The shape of a biomaterial can also influence the inflammatory reaction. Implantation of rod-shaped polymers with either a triangular, circular or pentagonal cross-sectional shape[244] in rat muscle has shown that the foreign body response is shape-dependent in this model. Triangular polymers elicit a stronger immune reaction than pentagonal polymers, followed by circular polymers. This shape effect is consistent among six different polymeric biomaterials. Similarly, poly(tetrafluoroethylene) (PTFE) discs with a smooth surface trigger only a moderate immune reaction in the rat subcutaneous space, compared with PTFE discs with conical projections on the surface[245], suggesting that materials with sharp features, such as angles, induce a more pronounced inflammatory response in the host than materials with a smooth surface. However, the exact mechanisms mediating this effect remain ill-defined thus far.

The size of spherical biomaterials also has an effect on the immune response[246]. Interestingly, large spheres 1.5–2.0 mm in diameter exhibit markedly decreased macrophage adhesion and immune activation compared with smaller spheres 0.3–0.5 mm in diameter after injection in the mouse intraperitoneal space and the monkey subcutaneous space. This effect is reproducible for a range of materials, including alginate, steel, glass, PCL and polystyrene. For example, alginate spheres with a diameter of 1.5 mm can deliver pancreatic islets in diabetic mice, achieving glycaemic control for 175 days in 30% of the mice, whereas spheres with a diameter of 0.5 mm fail after 30 days owing to excessive cellular deposits and fibrosis. Therefore, modifying the physical parameters of a biomaterial can be used to modulate the host immune response; however, altering only the shape is insufficient for consistent glycaemic control.

***Material functionalization.*** Biomaterials can be used to control the presentation of immune regulatory molecules at the microscale. Immune activation requires ligand–receptor binding with innate (for example, macrophages) or adaptive (for example, T cells) immune cells. By incorporating such ligands in scaffolds, the efficiency of their presentation to immune cells and their proximity to secondary signals modulating the specificity of the induced response can be controlled. This approach is being explored in the tumour immunotherapy field but is still in its infancy in regenerative medicine. For example, lipid nanodiscs decorated with peptide antigens and an immunostimulatory molecule induce a 47-fold increase in the number of antigen-specific cytotoxic T cells compared with

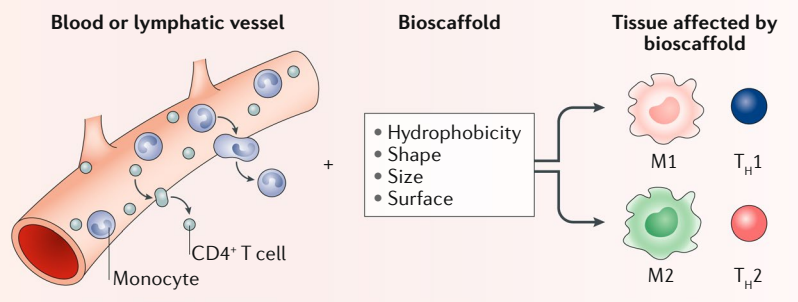


Fig. 4 | **Biomaterials impact host immune response.** Multiple biomaterial properties, including hydrophobicity, shape, size and surface roughness, can influence the local immune response, regulating $CD4^+$ T cell polarization into T helper 1 ($T_H1$) and $T_H2$ phenotypes and monocyte-derived macrophage polarization into inflammatory or pro-regenerative phenotypes (M1 and M2).

delivery of soluble molecules and antigens[247]. Similarly, a scaffold of mesoporous silica rods and liposomes with the incorporated T cell stimulatory signals anti-CD3, anti-CD28 and interleukin (IL)-2 can be used for T cell expansion ex vivo, with the resulting cell number depending on the relative density of stimulatory molecules on the polymer surface[248].

Inducing antigen-specific immune modulation by specific functionalization of materials to promote regeneration is a compelling new avenue of research. For example, IL-4, which is the main $T_H2$-polarizing cytokine, can be released from implanted biomaterials to increase M2 macrophage polarization, decrease fibrosis and improve tissue integration[249–251]. Materials can also be loaded with anti-inflammatory small molecules, such as dexamethasone, to decrease $T_H1$-type responses in vitro and in vivo[252].

Biomaterials provide a multifaceted tool to modulate the host immune response on multiple fronts (FIG. 4). Backbone selection, surface modification, physical properties and ligand immobilization can all be exploited to generate materials with immune-regulating properties.

## Conclusions and outlook

Biomaterials have come a long way in regenerative medicine and now constitute an indispensable component of a successful cell transplantation strategy, along with cell preparation and chemokine selection. Different downstream applications require diverse physicochemical properties and thus, careful biomaterial design.

Most strategies to date have focused on manipulating a single parameter of the donor cell–host response; however, combinatorial approaches that aim to improve donor cell survival and to simultaneously decrease inflammation and remove barriers to integration have to be developed. Manipulating one aspect of regeneration may affect another in unexpected ways. For example, inducing a $T_H2$ immune response not only decreases inflammation but also promotes angiogenesis, as seen in the case of MAA polymers.

Strategies have emerged to simultaneously modulate multiple facets of the regenerative response. For example, immune modulation by heparin nanoparticles can be combined with stimulation of angiogenesis by matrix-bound VEGF in an HA hydrogel[253]. Interestingly, tissue regeneration and behavioural recovery in stroke-injured mice is observed only with the combined treatment[254]. Combinatorial approaches will inarguably be the next frontier in biomaterials research for regeneration.

Multiple unexplored avenues for biomaterials in regenerative medicine remain. Often, biomaterials are designed first, and then applications are explored; however, by understanding the biological system into which biomaterials are implanted, design criteria can be specified and outcomes improved. For example, the interactions between stem and progenitor cells and their niche are complex and depend on a multitude of chemical and physical factors, many of which have not yet been elucidated. Instead of attempting to recreate this convoluted microenvironment within a material, it may be advantageous to manipulate the niche cells to alter their phenotype. Such 'activated' niche cells could then provide an array of stimuli to transplanted cells in physiologically relevant concentrations and time frames, thus potentially improving regeneration. Niche-targeted therapeutics are the subject of intensive research efforts in stem cell biology, particularly for the haematopoietic system[255,256], and might also be a promising research direction for the biomaterials community. For example, HSCs do not express the parathyroid hormone (PTH) receptor, whereas osteoblasts, which are an indispensable component of the HSC niche, express the PTH receptor[257]. PTH activates the Notch pathway in osteoblasts, which in turn promotes transplanted HSC engraftment in lethally irradiated mice[258]. Therefore, instead of directly targeting HSCs, osteoblasts in the niche can be activated by PTH to then induce the desired effect on HSCs. Similarly, the secretion of chemokines, for example, CXCL12, by endothelial and mesenchymal cells in the HSC niche is required for HSC survival, expansion and differentiation towards the lymphoid lineage[259]. Patients requiring HSC transplants are likely to have undergone chemotherapy or radiotherapy, which disrupts the HSC niche. Therefore, factors that induce remodelling of the HSC niche, for example, through promoting survival or proliferation of host cells, are actively being investigated.

This approach is not restricted to the stem cell niche. Ciliary neurotrophic factor (CNTF) promotes photoreceptor survival in animal models of retinal degeneration and in humans[260,261]. Interestingly, CNTF does not exert its effect by directly binding to photoreceptors but rather by stimulating neuroprotective signal secretion by the Muller glia[262]. Taking advantage of such signalling events and implementing them in biomaterials-mediated cell transplantation strategies could lead to desired donor cell–host interactions and regenerative responses through modulation of the host site and cells.

Biomaterials are an essential component of the regenerative medicine toolkit. To improve cell survival and integration, and to enable regeneration and clinical translation, the interactions between donor cells, material and host must be further elucidated and coordinated.

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**Competing interests**
The authors declare no competing interests but acknowledge a composition of matter patent on HAMC cell delivery.

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