Advancements in cell-delivering injectable hydrogels with biological and physicochemical functions

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Abstract

Injectable hydrogels that can be administered via syringes have enormous potential as cell-delivering carriers for cell transplantation therapy. Owing to their beneficial properties, including biocompatibility, biodegradability, tissue adhesive properties, and scaffold functions, injectable hydrogels can be used to improve the delivery efficacy and survival of transplanted cells post-transplantation. Moreover, this delivery method requires no culture or invasive surgical procedures, leading to reduced cost, process time, and patient burden. To develop injectable hydrogels for clinical translation, hydrogels have been functionalized using various biological and physicochemical engineering approaches for angiogenesis induction, suppression of immune rejection, viscoelasticity, and pore formation for cell infiltration. This focus review discussed the optimal design of injectable hydrogels for cell delivery. Moreover, this focus review summarized the different approaches available to improve the biological and physicochemical functions of hydrogels, listed their impacts on cellular functions, and highlighted their therapeutic efficacy.

Keywords: injectable gel, regenerative medicine, polymeric hydrogel, scaffold

Introduction

Regenerative medicine is an emerging therapy that aims to regenerate dysfunctional organs by transplanting stem, differentiated, and genetically engineered cells into the patients.[1] Regenerative medicine has gained much attention as an alternative to organ transplantation, which is affected by the chronic shortage of organ donors. To date, over 1000 clinical trials have been conducted,[2] and many regenerative medicine products for cell therapy, gene therapy, cord blood therapy, and tissue engineering have been approved worldwide. Cell-based therapies serve as versatile approaches to treat intractable diseases. Many factors, including the injection site, properties of the delivery device, and carrier materials/buffers, influence the efficacy of cell therapy. Although many products are administered via injection of a cell suspension into blood vessels and tissues using a syringe or catheter, efficient delivery to the target organs and tissues is difficult due to the rapid diffusion of cells. In a clinical study, intracoronary injection of bone marrow stem cells resulted in only 1.3–2.6% of the cells remaining at the site of administration after approximately 1 h, whereas most cells were observed in the liver and spleen.[3] Intravascularly injected mesenchymal stem cells (MSCs) mostly failed to reach the target tissues and get trapped in the lungs or liver, affecting their efficacy.[4] Post-transplantation cell survival is a key factor affecting therapeutic efficacy. Approximately 90% of

dopaminergic neurones died during the first four days post-implantation due to apoptosis.[5] Transplantation-associated cell death is caused by various factors, including damage by mechanical forces during injection and unfavourable host microenvironment for cell-adhesive scaffolds and growth factors. Despite many clinical trials, successful cell-based therapy still faces many challenges.

Biomaterials are powerful tools to overcome these challenges and improve the rates of cell engraftment and survival.[6] Tissue engineering-based approaches facilitate the reconstruction of tissue-like structures of skin, cartilage, and heart tissues in the form of cellular aggregates/sheets or cells with polymeric scaffolds that are marketed as regenerative medicine products. Another method for cell delivery is the use of injectable hydrogels. An injectable hydrogel is a promising soft material composed of polymeric network structures with abundant water that can be injected into the body using a syringe and catheter for biomedical applications, such as wound healing, haemostasis, cell transplantation, and drug delivery.[7] Pre-gel solution containing cells are injected into the body and rapidly form hydrogels in tissues. Injectable hydrogel-based cell delivery has many advantages: (i) retention of transplanted cells due to tissue adhesion, (ii) support of cell functions as a scaffold, (iii) availability for immediate use of isolated cells, and (iv) minimally invasive procedure with syringe injection (Figure 1). Local administration of

injectable hydrogels to the tissues enhances the delivery efficiency and retention of cells and avoids the risk of thrombosis observed with the intravascular administration of cells.[8] Although 49% of registered MSC clinical trials used the local administration of cell suspension to the tissues in 2008, injection of cell suspension results in insufficient cell retention and survival at the site of administration.[9] Cell encapsulation in injectable hydrogels enhances the localization to target tissues by covering the tissue surfaces without affecting the geometry of the tissue surfaces and anchoring them without suture.[10] Injectable hydrogels not only deliver and retain cells as a reservoir but also provide a cell adhesive matrix as a scaffold, preventing anchorage-dependent cell death (anoikis) and supporting fundamental cell functions, such as cell survival, proliferation, and differentiation.[11] As this approach can be used without cell culture (cells are injected directly after collection from the body), long-term cell culture is not required, enabling low-cost regenerative treatment. Furthermore, injectable hydrogels do not require invasive surgical procedures, thereby reducing the patient burden.

This focus review discusses the fundamental properties of injectable hydrogels for cell delivery and material design, lists their biological and physicochemical functions, and summarizes their effects on transplanted cells and therapeutic efficacy. The clinical aspects of cell therapy, including cell source and manufacturing, have been previously

reviewed.[1,2] Therefore, this article focuses on the design strategies for cell-delivering injectable hydrogels.

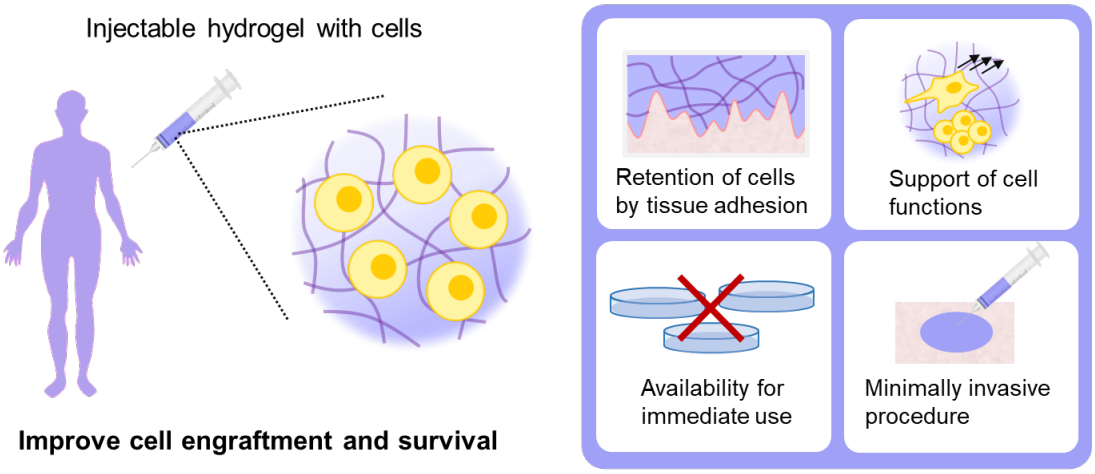

Figure 1. Schematic of injectable hydrogels for cell therapy. Injectable hydrogels improve the retention and survival of transplanted cells by facilitating robust tissue adhesion and scaffold functions.

Design of injectable hydrogels for cell delivery

Injectable hydrogels have been widely employed as medical tissue adhesives to prevent postoperative complications, such as air leak, bleeding, abdominal adhesion, and infection.[12] To design injectable hydrogels for cell delivery, various requirements in material design need to be satisfied. Cells are sensitive to external stimuli and should be kept under physiological conditions (e.g. temperature, pH, and ionic strength). In particular, the gelation process is crucial, which often causes cell damage due to chemical and physical reactions, including the reaction of amino groups on cell surfaces with *N*-hydroxy succinimide or electrostatic interactions between negatively charged cell membranes and positively charged polymers.[13] Ideal design of functional injectable hydrogels is considered to be easy to handle, biocompatible components, mild crosslinking reactions, and cell-adhesive scaffolds. Many studies have reported the development of various types of polymer-based injectable hydrogels, including covalent crosslinks,[14] cell-polymer hybrids,[15] protein hydrogels,[16] and granular hydrogels,[17] for cell delivery applications. Fibrin glue is a clinically available injectable hydrogel that can be used as a cell-delivery carrier because of its high biocompatibility and cell-adhesive properties. Pre-gel solutions composed of fibrinogen and thrombin were mixed with allogeneic MSCs and delivered to improve the recovery of cardiac function in a porcine

infarction model.[18]

Two types of delivery systems are available for cell delivery: double- and single-syringe systems. Double-syringe system-based injectable hydrogels, such as fibrin glue,[19] albumin-glutaraldehyde adhesives,[20] and poly(ethylene glycol) (PEG) adhesives,[21,22] have been used for strong tissue adhesion via covalent bonding to extracellular matrix (ECM) proteins in the tissues and long retention of hydrogels.[7] Most double-syringe systems are based on the chemical crosslinking of two components to form hydrogels such as thiol-acrylate reaction,[23] amine-aldehyde reaction,[24] amine-activated ester reaction,[25] and catechol-based adhesives,[26] which undergo rapid gelation. Non-specific chemical reactions can lead to cytotoxicity in transplanted cells and inflammatory responses in host tissues.[27] Therefore, more biocompatible injectable hydrogels are needed to use the double-syringe systems for cell delivery. Moreover, a spray device that enables the complete mixing of the two components without damaging the cell is required for cell delivery. In contrast, a single-syringe system provides a simple and easy delivery agent. The single-syringe system requires no mixing devices for injection.[28] Injection of pre-gel or gel of stimuli-responsive polymers to the tissues forms hydrogels via external stimuli. For example, pre-formed hydrogels can be injected by shear force owing to their shear thinning property.[29] Physically crosslinked hydrogels, including hydrogen bonded

gels,[30] inclusion complexes,[31] and polymer–clay nanocomposites,[32] often possess shear thinning properties that are suitable for cell delivery using a single-syringe system. Moreover, a pre-gel solution of temperature-responsive polymers[33-35] and photo-crosslinking polymers[36] causes gelation after injection into the body in response to temperature changes and light exposure. Although a single-syringe system can be used for clinical translation, it is required that tissue adhesiveness of physically crosslinked hydrogels to target organs is sufficient and the use of a photoinitiator and light exposure do not cause serious damage to the tissues.

Biological functions

In addition to material design for the injection and gelation processes, the biological and physicochemical functions of injectable hydrogels must be considered to develop efficient cell delivery carriers. Unlike general pharmaceuticals, living cells must survive under physiological conditions and integrate into the host tissues after delivery. However, diseased sites are exposed to harsh conditions, such as the lack of nutrients and oxygen, immune responses to eliminate transplanted cells, and reduced cell infiltration into hydrogels, leading to poor integration into the host. To solve these problems, several approaches have been proposed to promote the biological and physicochemical functions of injectable hydrogels (Figure 2).

Biological functions of injectable hydrogels can be tailored using engineering approaches, including modification with synthetic biomimetic ligands, use of natural ECM, and incorporation of signal molecules. Cell adhesion is mediated via binding between cell surface receptors (e.g. integrin) and ECM components (e.g. fibronectin).[37] Through the activation of focal adhesin kinase, mechano-transduction pathway is initiated, facilitating cell survival.[38] Introduction of integrin-binding ligands (e.g. RGD, YIGSR, and IKVAV sequence-containing peptides) has been widely studied to enhance cell adhesion in hydrogels,[39] which may reduce post-transplantation anoikis and determine

their cell fate. To reconstruct natural ECM microenvironments, decellularised ECM (dECM) would be powerful as a naturally derived biocompatible, organ-specific biomaterials.[40] dECM can be obtained from biological tissues via decellularization processes, such as detergent treatment[41], freeze-thawing,[42] and hydrodynamic pressure treatment.[43] Owing to the preservation of cell-binding ligands and physical micro/nanostructures similar to natural ECM, dECM functions as a favourable microenvironment for cells. Several studies on tissue engineering approaches using dECM (recellularization, culture, and transplantation) have demonstrated the successful generation of organ-like structures and their integration into the host.[44] Therefore, dECM-based injectable hydrogels can be used as biologically functional carriers for cell delivery.[45]

Preventing the lack of oxygen and nutrients in the cellular microenvironment is the main challenge post-transplantation. In the body, blood capillaries exist in a range of 150–200 μm around cells to supply oxygen and nutrients except some tissues, such as cartilage.[46] Thus, thick hydrogels over diffusion limit (> 200 μm) may cause hypoxia and necrosis in encapsulated cells.[47] Oxygen-producing cryogel prevented the necrosis of islets and facilitates their survival and fucntion.[48] Moreover, several studies reported that incorporation of vasculogenic factors (e.g. vascular endothelial growth factor, VEGF)

drastically enhanced angiogenesis around hydrogels and improved the survival of transplanted cells.[49,50] Much efforts are focused on controlling release profile of growth factors to prolong cell survival and reduce side effects in the host.[51] Incorporation of attractive interaction between polymer chains in hydrogels and growth factors enables to avoid the burst release and control the local concentration.[50] As an alternative to post-angiogenic approach, transplantation of pre-vascularised tissues would serve for rapid vascularisation as tissue engineering-based approach,[52,53] although these are not often injectable materials.

To further improve graft survival in terms of biological function, the suppression of immune rejection is a key issue. Especially, when allogenic cells are used for the purpose of reducing time and cost of the therapy, the difference in major histocompatibility causes severe immune rejection, such as inflammatory cytokine production and phagocytosis by immune cells, resulting in damages in the transplanted cells.[54] For example, in islet transplantation, 70% of the transplanted islets were detached within a few hours to a few days due to the rejection.[55] Although a combination of several immunosuppressive agents, such as steroids, calcineurin inhibitors, mTOR inhibitors, and antibody drugs, are used to suppress immune rejection, their long-term systemic administration increases the risk of various complications, such as opportunistic

infections, hypertension, diabetes, and cancer.[56] To reduce systemic complications, several biomaterials, such as drug-encapsulating microparticles,[57] microencapsulation agents,[58] and cell coatings,[59] have been developed. Injectable polymeric microparticles immobilized with Fas ligands (apoptosis-inducing factor) were used to improve islet transplantation efficiency by local immunomodulation of regulatory T cells.[60] Furthermore, transplantation of antigen-specific regulatory T cells with biomaterials induced immune tolerance in the patient.[61] Biomaterials-based local immunomodulation can suppress effector T cell functions at the site of transplantation and induce immune tolerance, thereby increasing engraftment rates.

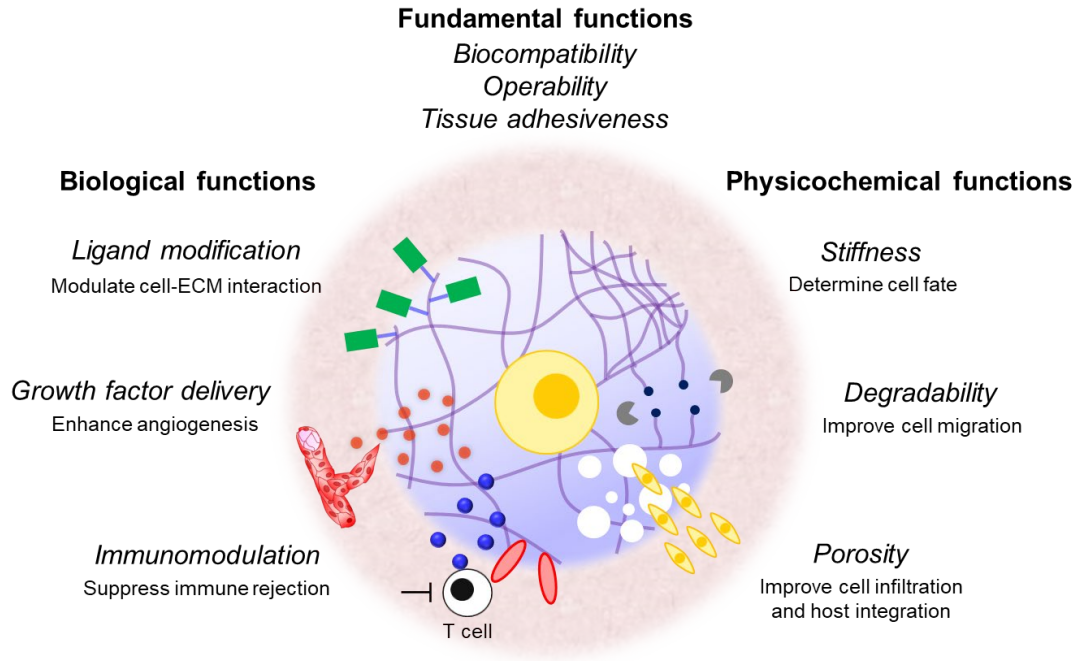

Figure 2. Fundamental, biological, and physicochemical functions of the injectable hydrogels necessary for cell engraftment and survival after cell delivery.

Physicochemical functions

As cells sense their three-dimensional (3D) microenvironments to perform fundamental functions, including adhesion, proliferation, and differentiation,[37] the physicochemical characteristics of scaffolds are crucial for determining the fate of transplanted cells. Among numerous studies on the 3D-culture of cells using hydrogels, many have investigated the mechanisms by which the mechanical properties and stimuli affect the cellular functions.[62] In the body, shear stress by blood flow is applied to endothelial cells, and cartilage is exposed to high compressive stress, facilitating cellular orientation, differentiation, and tissue development.[63] Notably, 3D-cultured cells behave differently in vitro depending on the substrate stiffness, thus affecting the differentiation of stem cells[64] and secretion of proangiogenic signals.[65] Hydrogel stiffness has critical impact on in vivo bone formation after MSC transplantion.[66] In addition to their stiffness, the viscoelasticity of hydrogels also affects the cell behaviors.[67] Although elastic material retains the applied force over time, viscoelastic material shows time-dependent stress relaxation under applied constant load via rearrangement of the molecular network structures. Viscoelastic properties are seen in native ECM, such as the collagen matrix, and play crucial roles in cell functions; therefore, viscoelastic hydrogels exhibit potential as biomimetic scaffolds. It has been reported that viscoelastic hydrogels enhanced cellular

spreading and differentiation[68] and control tissue organization.[69] This concept has been extended to cell delivery applications, and viscoelastic injectable hydrogels have been developed for cartilage regeneration.[70]

Degradability of a hydrogel is an important parameter in material design for cell delivery. Prolonged degradation of hydrogels improves cell retention in the body.[10] On the other hand, when the degradation is too slow in highly crosslinked hydrogels, spreading and migration of transplanted cells or cellular infiltration to hydrogels are limited and inflammatory responses against hydrogels occur, which results in poor integration to the host. Porosity of injectable hydrogels is another important physicochemical function affecting cell survival after transplantation. Because of the diffusion limits of oxygen and nutrients, cells require a pathway to obtain them from host tissues. In contrast, injectable hydrogels typically encapsulate cells in densely crosslinked polymer networks (e.g. approximately 20 nm pores in PEG gels[71]), which lack macro/microporous structures to exchange biological signals and cells with host tissues. Thus, the introduction of interconnected macro/microporous structures into injectable hydrogels would improve the survival of transplanted cells and the cellular infiltration necessary for rapid integration.[72] To date, several approaches using particle porogens,[73] microgels,[74,75] and peptide nanofibers[76] have been reported to engineer pore-forming or

porous injectable hydrogels. Both pore size and interconnectivity are key parameters for effective cell infiltration.[6,77] For example, Pore sizes of 0.8–8 μm showed 100-fold increase of blood vessel formation than that of smaller pores.[78]

A bioinspired approach using liquid–liquid phase separation (LLPS) has been developed to design injectable hydrogels with controlled and interconnected porous structures and improve host integration for cell delivery (Figure 3a).[79] LLPS occurs due to the interaction between one or more polymers in the solution. LLPS has gained increasing attention in biology as it compartmentalises as a non-membrane organelle in cells and plays key roles in biomolecular condensation and signal transduction. Based on the dynamic LLPS processes initiated by the post-translational modification of intrinsically disordered regions of proteins, LLPS has been artificially induced via the chemical modification of proteins with patchy self-associating units to control the internal structure of injectable hydrogels (Figure 3b). Gelatin (G) has been modified with a hydrogen-bonding functional group, the 2-ureido-4[1H]-pyrimidinone (UPy) unit, to form G modified with the UPy unit (GUPy). When GUPy solution was mixed with non-modified G solution, micro-sized fibrous phase-separated structures, called microcapillary networks (μCNs), were observed via confocal laser scanning microscopy (CLSM) (Figure 3c). Although both the main polymers are transparent, GUPy formed

LLPS structures owing to the self-associating ability of UPy units via quadruple hydrogel bonding. By changing the mixing ratio of G and GUPy, not only μCN but also gyroid-like and droplet structures can be formed in the hydrogels. Although the structures formed by LLPS were often characterised as droplets and bicontinuous structures,[80] a few micrometres of fibrous structures, such as the blood capillary networks, can be formed in the LLPS hydrogel. Blocking the formation of hydrogen bonds using urea prevented LLPS formation, highlighting the importance of multivalent hydrogen bonding among the UPy units for LLPS.

As biocompatible crosslinking, thiol-ene reaction was used to form hydrogels. LLPS structures were formed by mixing thiolated gelatin (GTH) and vinyl sulfonated gelatin (GVS) with GUPy, and gelation was simultaneously initiated by crosslinking GTH and GVS after LLPS formation. In the LLPS hydrogel, the LLPS-induced fibre structure of GUPy was not chemically cross-linked and eluted as a porogen when injected into tissues to form porous structures. The permeability of proteins was higher in LLPS hydrogels than in non-porous hydrogels owing to the micrometre-scale pores, suggesting the effective delivery of oxygen and nutrients to the encapsulated transplanted cells. Adhesion and spreading of MSCs encapsulated in μCN hydrogels were substantially enhanced compared to that of non-porous hydrogels (Figure 3d). CLSM observations

revealed that the porous structures not only enhanced mass transport but also functioned as voids for cell infiltration. To confirm the therapeutic efficacy of this method, MSC-delivering injectable hydrogels were administered in a mouse model of hindlimb ischaemia. Compared to the administration of MSC suspension and MSC encapsulated in non-porous hydrogels, μCN improved blood flow of hindlimb after the ischaemia and suppressed the necrosis due to efficient delivery of MSCs and vasculogenic signals via the μCN structure (Figure 3e). These pore-forming or porous injectable hydrogels with various physicochemical functions hold immense promise for enhancing the therapeutic efficacy of cell transplantation.

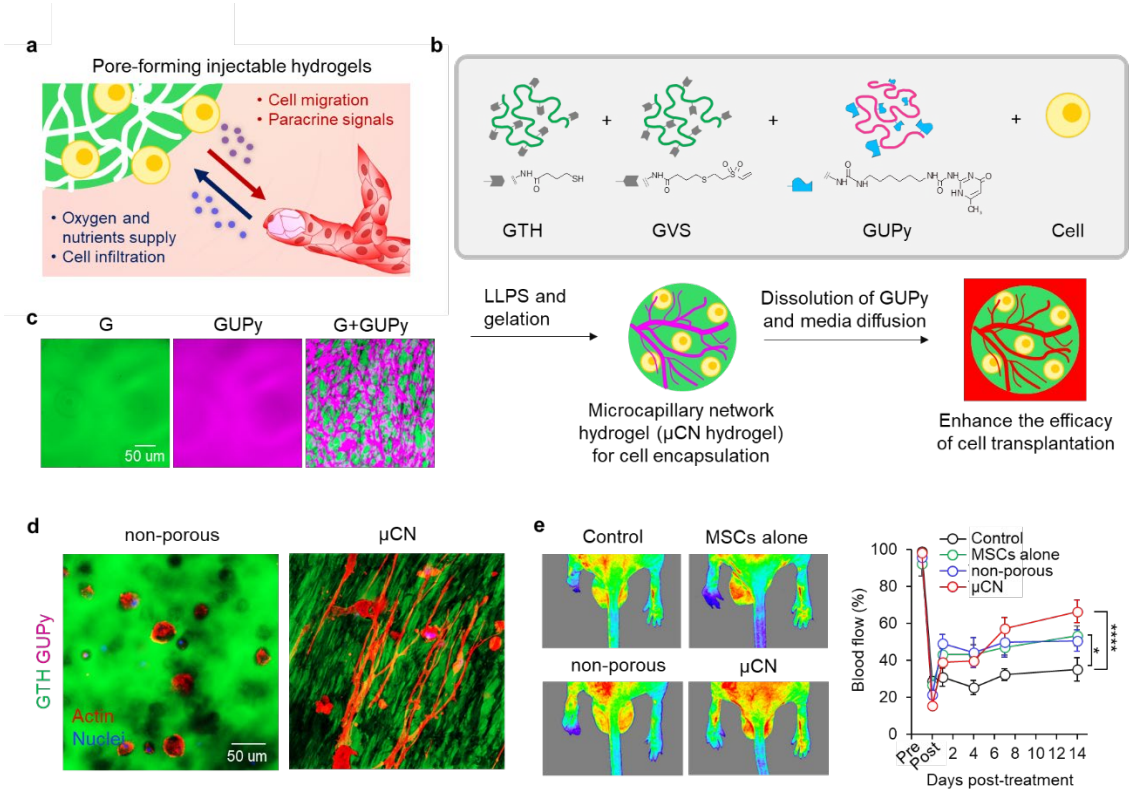

Figure 3. (a) Schematic of the enhanced host integration of transplanted cells using pore-forming injectable hydrogels. (b) Preparation of microcapillary network (μCN) hydrogels via liquid–liquid phase separation (LLPS). μCN hydrogels were formed via LLPS of gelatin (G) modified with the 2-ureido-4[1H]-pyrimidinone unit (GUPy) and thiol-ene covalent crosslinking. GUPy was dissolved under physiological conditions, and the medium diffused into the μCN. (c) LLPS after mixing of the G and GUPy solutions. (d) Cell migration was increased in μCN hydrogels compared to that in non-porous hydrogels. (e) Hindlimb blood flow monitored using a laser Doppler imaging system. Reproduced from ref.[79] with permission from Elsevier.

Conclusions and future perspectives

This focus review discussed the use of polymer-based injectable hydrogels as delivery carriers for cell transplantation. As the microenvironment of damaged tissues is unsuitable for cell delivery owing to excess inflammation and irregular ECM structures, the functions of transplanted cells are hindered. Therefore, novel design concepts for biologically and physicochemically functional injectable hydrogels are needed to further improve the efficacy of cell therapy. As various events are involved in tissue regeneration, combinatorial approaches can be used to modulate multiple factors. Material properties for cell therapy are based on the specific application, with different requirements for cardiovascular, cartilage, and nerve regeneration therapy. Therefore, materials should be tuneable and specific to the target tissue.[81] Further material and biological analyses will enhance our understanding of the complex interactions among materials, donor cells, and host tissues. Overall, cell-delivering injectable hydrogels can overcome the clinical translational barriers and increase the efficacy of cell therapy.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

I would like to thank Dr. Tetsushi Taguchi (National Institute for Materials Science) and my group members for assistance with this work. This work was partly supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (grant nos. 22H03962 and 23K25216), Uehara Memorial Foundation, Medical Technology from the Japan Agency for Medical Research and Development, and Inamori Research Grants from the Inamori Foundation.

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Graphical abstract

Injectable hydrogels hold promise as cell-delivering carriers for cell transplantation therapy in regenerative medicine. Injectable hydrogels possess various benefits including biocompatibility, biodegradability, tissue adhesive properties, scaffold functions, and minimal invasiveness. To overcome the barrier for clinical translation, biological and physicochemical functionalization is desirable, which improve delivery efficacy to targets and graft survival post-transplantation. This revies discussed the design strategy of injectable hydrogels for cell delivery and summarized the approaches available to improve the biological and physicochemical functions of hydrogels.