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Injectable biomaterials for translational medicine

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Injectable therapeutics enabled by engineered biomaterials are becoming increasingly popular, transforming traditional clinical practice to become a minimally invasive and regenerative regime. Compared to preformed biomaterials, injectable biomaterials allow for more precise implantation into deeply enclosed anatomical locations and for the repair of irregularly shaped lesions, demonstrating great translational potential. Continuously emerging clinical needs and advances in materials science have driven an evolution in injectable biomaterials from structural fillers to multifunctional platforms. Integrating disparate functions to design injectable biomaterials for clinical translation remains a considerable challenge, as does the selection of the appropriate design considerations for specific applications. This article aims to review the design and fabrication considerations of injectable biomaterials in the context of medical translation, the engineering strategies used for new materials to meet the growing demands in regenerative and intelligent medicine, and the progress in their development for selected clinical applications. Specifically, three exemplary areas, injectable bone cements, hydrogels, and electronics, all of which demonstrate significant promise in terms of translation and commercialization, are reviewed in detail. In addition, their translational status and future challenges are discussed. It is also envisioned that the mutual collaboration between researchers, clinicians, entrepreneurs, engineers, and patients will inspire and catalyze the innovation and translation of injectable biomaterials.

Keywords: Injectable; Clinical application; Bone cement; Hydrogel; Electronics

Abbreviations: BcP, block co-polymer; BMPs, bone morphogenetic proteins; BSA, bovine serum albumin; CaP, calcium phosphate; CaHA, calcium hydroxylapatite; CBs, cucurbiturils; CDs, cyclodextrins; CNTs, carbon nanotubes; ECM, extracellular matrix; FasL, Fas ligand; FGFs, fibroblast growth factors; HA, hyaluronic acid; I-Cat, radioisotope-labeled catalase; IPNs, interpenetrating polymer networks; MCPM, monocalcium phosphate monohydrate; MMP, metalloproteinase; MTA, mineral trioxide aggregate; NPCs, neural progenitor cells; OVCF, osteoporotic vertebral compression fractures; PDGFs, platelet-derived growth factors; PEG, poly(ethylene glycol); PET, polyethylene terephthalate; PKP, percutaneous kyphoplasty; PLGA, poly(lactic acid-co-glycolic acid); PMMA, polymethyl methacrylate; PNIPAAm, poly(N-isopropylacrylamide); PPG, poly(propylene glycol); PVP, percutaneous vertebroplasty; RGD, arginine–glycine–aspartic acid; rh-BMP2, recombinant human bone morphogenetic protein-2; rh-PDGF, recombinant human platelet-derived growth factor; TGFs, transforming growth factors; VEGFs, vascular endothelial growth factors; a-TCP, a-tricalcium phosphate; b-TCP, b-tricalcium phosphate; l-ILEDs, microscale inorganic light emitting diodes.

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Introduction

The local injection of drugs and biologics, whose importance and advantages are self-evident compared to other administration routes, has become an inextricable part of routine medical activities spanning from treatment and imaging to anesthesia. Recently, the unprecedent development of engineered materials has spurred a growing interest in extending injectable therapeutics to include biomaterials, which enables a number of traditional treatments to become less invasive and more regenerative (Fig. 1). Injectable biomaterials are therefore considered to be the next wave of injectable therapeutics, following injectable drugs and biologics, which have experienced enormous success in the clinic [1]. Compared to previous injectables, injectable biomaterials offer several advantages, including a structural and physical framework or stroma, the controllable release of therapeutic agents, a functionalized micro-environment for tissue regeneration, and the possibility of monitoring or stimulating biological events. Injectable biomaterials combined with a wide array of cells, genes and growth factors can also generate better outcomes as the cargos can be better encapsulated, transported, retained, and delivered to the site of the injection [2–4] and the biomaterials, solely or in combination with the therapeutics, can also play a critical role in tissue regeneration and repair [5]. It is hoped that such advantages will continue to provide revolutionary and reliable concepts, tools, and methods to resolve medical and surgical problems. For example, from the history of percutaneous vertebroplasty and kyphoplasty since the 1980s, it is evident that advances in spinal fracture management are closely associated with the development of acrylic bone cement [6]. Likewise, root canal therapy was advanced since mineral trioxide aggregate (MTA) received the approval by FDA in 1998, which now remains as a key injectable cement for endodontic practice [7]. In addition, recent clinical trials of newly developed therapeutics for myocardial infarction and peripheral artery disease using catheters rely on a wide array of natural and synthetic injectable hydrogels [8].

Paraffin was first injected into the human body for soft-tissue contouring in the late 1890s [9], and since then injectable biomaterials have been used as fillers or binders to structurally occupy tissue cavities in plastic, orthopedic, and orthodontic surgery as the material can be injected into small, irregularly shaped, and deeply buried defects or cavities. Over the past few decades, the arena of injectable biomaterials research has expanded to wide spectra of material formulations and forms, with various functionalities, to meet the multiple treatment needs in the human body (Table 1). In general, the current injectable bioma-

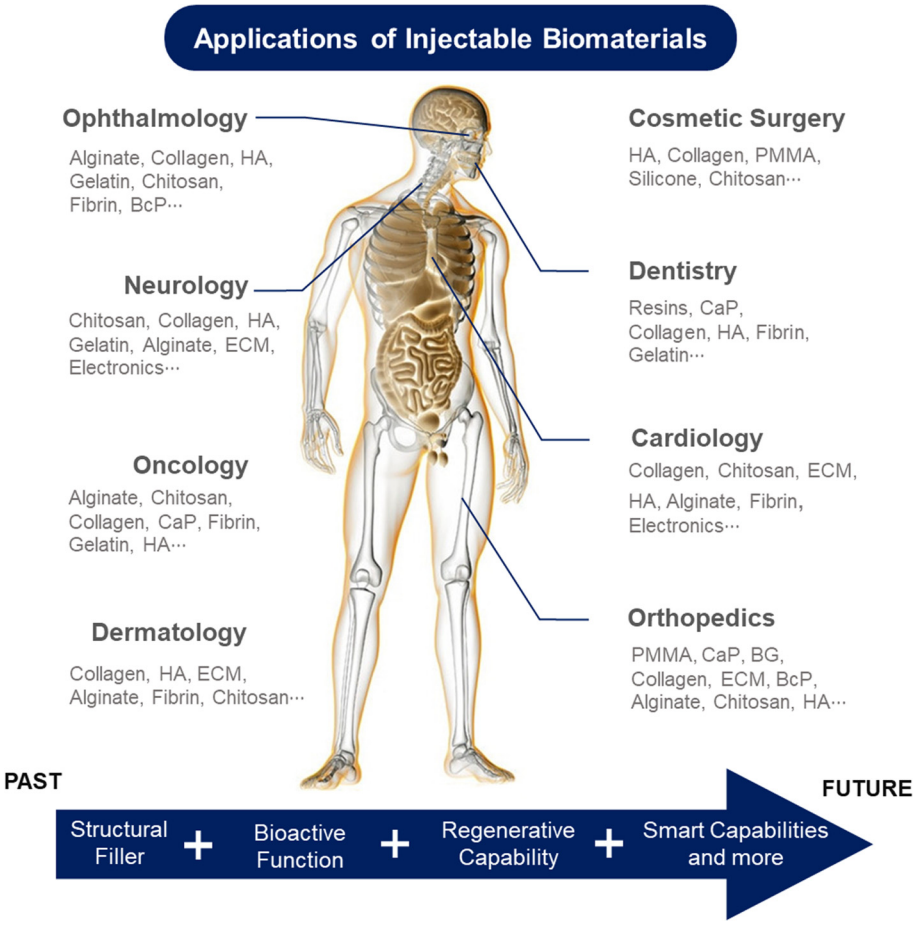

FIGURE 1

Applications of injectable biomaterials in medicine. BcP = block co-polymer; CaP = calcium phosphate; ECM = extracellular matrix; HA = hyaluronic acid; PMMA = polymethyl methacrylate.

TABLE 1

Selected examples of injectable biomaterials in translational medicine research.

Material	Material Form	Purpose or Function	Reference
Alginate	Hydrogel	Cell/drug delivery; Soft tissue regeneration; Cosmetic filler; Bone defects filler and bone regeneration	[10–12]
	Microspheres	Cell/drug delivery	[13]
Alloy (Nitinol)	Shape-memory Clip	Suture for wound closure	[14,15]
Bioactive glass	Cement	Bone defects filler and bone regeneration; Drug delivery	[16,17]
Block copolymers	Hydrogel	Cell/drug/vaccine delivery	[18,19]
Calcium phosphates	Cement; Paste	Bone defects filler; Root canal filler; Hard tissue regeneration; Cell/drug delivery; Thermotherapy for cancer treatment	[20–24]
Chitosan	Hydrogel	Cell/drug delivery; Soft tissue regeneration; Bone regeneration	[25–27]
	Microsphere	Drug delivery	[28]
	Nanoparticle	Drug delivery	[29]
Collagen	Hydrogel	Cosmetic filler; Soft tissue regeneration; Hard tissue regeneration; Cell/drug delivery; Thermotherapy	[30–33]
	Microsphere	Cell/drug delivery	[34,35]
Dextran	Viscous liquid complex	Iron-deficiency anemia treatment	[36]
	Hydrogel	Soft tissue regeneration	[37]
ECM	Hydrogel	Drug delivery; Soft tissue regeneration; Cosmetic filler	[38–40]
Electronics	Micromodule; Film; Mesh	Monitoring; Stimulating	[41–43]
Fibrin	Hydrogel	Soft tissue regeneration; Bone regeneration; Cell/drug delivery	[44–46]
Gelatin	Hydrogel	Soft tissue regeneration; Bone regeneration; Cell/drug delivery	[47–49]
	Microsphere	Drug delivery	[50]
Hyaluronic acid	Hydrogel	Cosmetic filler; Soft tissue regeneration; Bone regeneration; Cell/drug delivery; Tissue adhesion barrier	[51–54]
MTA	Cement	Endodontic applications	[55]
PEG and derivatives	Hydrogel	Bone tissue regeneration; Soft tissue regeneration; Cell/drug delivery; Biosensor; Radiotherapy spacer	[56–61]
Peptide and derivatives	Hydrogel	Soft tissue regeneration; Bone regeneration; Cell/drug delivery; Anti-bacterial applications	[62–66]
PNIPAAm and derivatives	Hydrogel	Soft tissue regeneration; Cell/drug delivery	[67–69]
PMMA	Cement	Bone filler	[70]
	Microsphere	Cosmetic filler	[71]
PLGA copolymers	Hydrogel	Cell/drug delivery	[72]
Polypeptide	Hydrogel	Soft tissue regeneration; Drug delivery	[73,74]
Silicone	Hydrogel	Cosmetic filler	[75]

terials can be used to repair both hard and soft tissues and demonstrate versatility in their structural, mechanical, functional, or physiological restoration of faulty or injured tissues.

More recently, the development of novel injectable biomaterials reveals there is a clear trend, driven by clinical needs, for better therapies and advanced medical diagnostics [76]. From the perspective of translational medicine, which centers on accelerating the process of biomedical research outcomes to benefit patients and clinicians, injectable biomaterials represent a potentially highly promising approach compared with other implantable materials for localized treatment and combinatorial therapeutics. There are an increasing number of forces from clinicians, patients, and researchers that are driving the development and improvement of new injectable biomaterials: (1) both patient and doctor choices of needing a minimally invasive intervention [77], such as the use of percutaneous catheters and small tubes to reach the lesion and deliver reparative agents; (2) the promises of regenerative approaches, including cell therapy and drug delivery, that require appropriate scaffolds or matrices for treatment purposes, such as recruiting endogenous cells, retaining cell activity, and the controlled release of materials [78,79]; (3) large variations in the size, shape, and location of lesions or defects, as well as the structural and mechanical requirements of reparation that pre-formed biomaterials are unable to adequately deal with [1]; and (4) the necessity for sensing, imaging, and detection in the vicinity of the lesion, especially in soft tissues, such as the retina [80], brain [81], spinal cord [82], and the myocardium [83]. These forces have driven an evolution in injectable biomaterials from structural fillers to multifunctional platforms. Yet integrating disparate functions to design injectable biomaterials for clinical translation remains a considerable challenge, as does the selection of the appropriate design considerations for specific applications.

The objective of this article is to review the design and fabrication considerations for injectable materials in the context of translational medicine; the engineering strategies for new injectable materials to meet growing demands, such as regenerative and intelligent medicine; and the progress in their development for selected clinical applications. This article is not intended to be a comprehensive review of the literature on injectable biomaterials but as a survey of selected injectable materials revealing the evolutionary path of this translational field. The primary focuses

here are injectable bone cements, hydrogels, and electronics, which reflect the progress of the field from a structural filler toward bioactive, regenerative, and intelligent formulations (Fig. 1). Outside this scope, there are recent reviews on injectable particulate systems [84,85], injectable biomaterials for plastic surgery [86], and dental and craniofacial regeneration [78,87].

Injectable bone cements

Bone cements are injectable and self-setting materials that are widely used in hard-tissue repair, such as in orthopedics, orthodontics, and plastic surgery. This category of injectable biomaterials usually undergoes a transition from the fluid or viscous state that can be readily injected through needles or a cannula to the solidified state that possesses a fixed geometry with increased mechanical strength and stability. Currently, acrylic cements, such as polymethyl methacrylate (PMMA), have become clinically standard augmentation materials for hard tissues and are injected into patients in millions of procedures conducted worldwide every year.

PMMA-based cements

The commercially available PMMA formula is mainly composed of a liquid methyl methacrylate monomer and a powdered MMA-styrene co-polymer sometimes accompanied by a radiopacifier ($BaSO_4$ or ZrO_2). Once mixed, the liquid monomer polymerizes around the pre-polymerized powder particles, initiating an exothermal setting process of approximately 10–30 minutes to form hardened, non-degradable PMMA (compressive strength $\geq$ 70 MPa, elastic modulus $\geq$ 1800 MPa) for hard tissue augmentation [88]. Due to these unique features, PMMA cements have become the only FDA-approved injectable materials used for percutaneous vertebroplasty (PVP) and kyphoplasty (PKP), which are the mainstream minimally invasive surgical treatments for osteoporotic vertebral compression fractures (OVCF) [89,90]. Injectable PMMA cements are also widely used in the augmentation of osteoporotic bone disorders using cannulated bone screws [91].

Despite clinical success in minimally invasive surgery, long-term clinical reviews have revealed that there are complications associated with PMMA cements, including secondary fractures of adjacent vertebrae or bone resorption due to the stress-shielding effect of stiff PMMA, loosening or displacement of the cement, poor osseointegration due to its bioinert and non-degradable nature, a high risk of cement leakage during injection, and tissue necrosis due to the exothermic polymerization reaction [92]. To resolve these risks, reformulation of the PMMA cement has become imperative but remains a major challenge since these risks originate from the intrinsic material properties of the PMMA itself. The only effective modification that has been achieved to date is the blending of PMMA with bioresorbable or biological components, which in comparison are softer and more bioactive. This modification imparts a substantially reduced elastic modulus, a lower setting temperature, improved osteogenesis and osseointegration, and increased porosity for bone in-growth [93–95]. A key consideration of this strategy is to carefully select components that tailor the mechanical properties of the PMMA cement while preserving its other desirable properties. For example, the modification of PMMA cement using natural hydroxyapatite and chitosan has been attempted, resulting in improvements in porosity and osteoconductivity, a reduction in the setting temperature, and a reduced elastic modulus [95]. A PMMA cement combined with mineralized collagen has been formulated to possess an elastic modulus similar to that of cancellous bone, a benign setting temperature, improved osteoconductivity, while retaining the appropriate compressive strength [70]. Besides, the improvement of osteoconductivity can be even more significant if highly bioactive components, such as bioactive glass [96] and silicate ceramics [97], are used as the fillers in PMMA. From a translational medicine perspective, modified PMMA formulations are worthy of further pursuit for translation and commercialization as many regulatory risks may be mitigated by the successful prior clinical record of PMMA cements.

Bioceramic-based cements

Since the clinical risks of PMMA cements are associated with their intrinsic material properties, researchers have sought alternative materials. Ideally, for use in hard tissue repair, injectable cement needs to be bioactive or bioresorbable and have adequate mechanical properties. More importantly, the degradation rate should match the bone in-growth rate to preserve the mechanical stability of the defect site [98]. Injectable bioceramics, first invented in the 1950s, represent an important class of potential materials that fulfill many of the above criteria, and their clinical uses include, but are not limited to, the treatment of bone, maxillofacial and oral defects, and the substitute for autografts.

The primary setting mechanism of bioceramic cements, unlike acrylic cements, is a dissolution–precipitation reaction of powdered precursors in aqueous medium and the interlocking of the precipitated crystals to form a rigid matrix. Depending on the precursor, hardened cements are commonly comprised of calcium phosphate [99], calcium silicate [100], calcium sulfate [101], magnesium phosphate [102], bioactive glasses [93], or their combinations [103,104]. Most of these injectable compositions demonstrate osteoconductivity or osteoinductivity and biodegradability.

The injectable bioceramics currently used in the clinic also have intrinsic drawbacks, most notably inferior mechanical strengths or brittleness (compressive strength usually from a few to less than 15 MPa) and inconsistent handling properties (e.g., setting time, injectability, and stability in body fluids). From both translational and regulatory perspectives, when used for hard-tissue repair or treatment, especially for repairing load-bearing bones, these drawbacks pose inevitable risks to patients.

Bioceramic-based cements with improved mechanical and handling properties

To date, there is no bioceramic cement product on the market that can be used for treating OVCF or as a substitute for load-bearing bones, due to their intrinsic low strength and high brittleness [105]. Although theoretically this weakness of bioceramic could be counterbalanced by new bone formation to replace the degraded ceramic, given the fact that this process could last from months to years, it is desirable that the injected cement have adequate mechanical strength, at least during the early stabilization

stage of bone fractures [106]. For load-bearing bone treatments, such as PVP or PKP, improved, mechanical properties of bioceramic that are comparable to that of PMMA cements are translational and regulatory requirements.

The current reinforcing strategy of injectable bioceramics mainly relies on the addition of a secondary phase (e.g., toughening fibers, whiskers, and particles) to the bioceramic cement, which, to some extent, sacrifices injectability but greatly increases strength and resistance to fracture through various toughening mechanisms, such as stress transfer, crack deflection, frictional sliding, and porosity reduction [107–110]. Two important factors in this strategy are the optimization of the adhesion between the additive and the cement particles and the homogeneous dispersion of the additive within the cement. As an example of this approach, Wang et al. combined carbon nanotubes (CNTs) or bio-mineralized CNTs with a calcium phosphate cement, increasing the compressive strengths by 24% and 120%, respectively [111]. Liu et al. incorporated a magnesium phosphate xerogel-like filler uniformly into the microporous structure of a calcium phosphate cement, achieving a compressive strength comparable to PMMA (~91 MPa) as a result of a reduction in microporosity [103]. Alternatively, macromolecular matrices can be used as a "glue" to form a crosslinked or physically entangled matrix with the bioceramic particles, creating a toughened composite [112,113]. In a recent study, a gelatinized starch-reinforced calcium phosphate cement was shown to have a strength similar to a clinically used PMMA cement after injection into osteoporotic sheep cadaveric vertebrae and rat femur defect *in vivo* [114]. This reinforcement is attributed to the depletion of intermolecular water from the gelatinized starch, resulting in the formation of a tough matrix, and an inter-particle interlocking effect between the cement particles and the matrix. Additionally, in a cannulated pedicle-screw fixation study, this formula exhibited a better anti-pullout property compared to a clinically used PMMA [115]. It is worth mentioning that these modifications usually do not alter the biocompatibility and bioactivity of the injectable bioceramics, some of which can even enhance biological responses *in vivo*. Also, these studies, though still at the laboratory stage, possibly pave the way for developing commercial bioceramic cements with comparable mechanics that could replace PMMA cements.

Injectable bioceramics, due to the nature of their complex compositions and setting mechanism, often suffer from inconsistent handling properties (including injectability and anti-washout properties in body fluids) that vary with external conditions (e.g., temperature, humidity, body fluids). These inconsistencies largely originate from the varying processing parameters, such as the particle properties of the bioceramic powder (e.g., size, surface charge, morphology etc.) and the powder-to-liquid ratio. Poor injectability primarily arises from the separation of the liquid and solid components during injection, which can be attributed to the filtration in the barrel or the suction and filtration in the needle [116]. Recent studies have suggested that optimizing injectability, in addition to experimental trials, can be achieved through both theoretical and computational analyses. Bohner and Baroud concluded, using both theoretical and experimental analyses, that the injectability of a calcium phosphate cement paste can be improved by (1) decreasing the average precursor particle size and particle–particle interactions, (2) increasing the viscosity of the mixing liquid or the liquid/powder ratio, and (3) using round deagglomerated particles and a broad particle size distribution [117]. Their conclusion is, however, not comprehensive due to the fact that there are diverse bioceramic cement formulae and a lack of standardized protocols for measuring the permeability and rheological properties of cement. The anti-washout property is defined as the ability of a cement to harden in a dynamic aqueous environment without disintegrating into small particles [118]. Various strategies, including strengthening the calcium phosphate particle interactions and increasing the setting liquid viscosity, have been explored to improve anti-washout property [119].

More recently, ready-to-use injectable bioceramics have been developed by stabilizing the cement precursors in the presence of a retarding agent (e.g., a solution containing divalent ions, such as Mg^{2+}, Ba^{2+}, Ni^{2+}, or Sr^{2+}) [120], a non-aqueous water-miscible fluid (such as glycerol and poly(ethylene glycol) (PEG)) [121,122], or a water-immiscible fluid (such as triglyceride with saturated fatty acids) [123,124] (Fig. 2). This new category of injectable bioceramics that eliminates the mixing step is of great interest to surgeons due to the ease of operation, low risk of contamination, and a reproducible cement performance [124]. There are only a few cement formulae in this category and they have compromised anti-washout and setting behavior compared to regular ceramic cements. Nevertheless, ready-to-use injectable bioceramics demonstrate high translational potential as they would simplify the surgery and lower the surgical risks.

Biological function of injectable bioceramic cements

With the tremendous progress in regenerative medicine, there is an increasing need to develop the next generation of bone cements that can provide a biomimetic micro-environment to better support tissue growth, regulate or enhance tissue regeneration at the cellular and molecular levels, and actively respond to the biological or mechanical environment. The biological function of injectable bioceramics is at the center of this translational medicine research.

Injectable bioceramic cements that can release bioactive ions. There have been numerous studies that can attest to the better clinical performance that can be achieved with a pre-designed bone-mimicking architecture releasing bioactive ions with optimal kinetics [125]. Bioactive ions (e.g., Ca^{2+}, Mg^{2+}, and PO_4^{3-}) released from calcium phosphate or magnesium phosphate bioceramics, via solution- or cell-mediated processes *in vivo*, play essential roles in regulating the migration, proliferation, and differentiation of bone cells, as well as in angiogenesis and collagen mineralization during bone formation [126–128]. In addition, Si-containing ions dissolved from silicate bioceramics can activate osteoblastic cells, endothelial cells, and human mesenchymal stem cells by regulating cellular activities and interactions, as well as the expression of numerous osteogenic and angiogenic marker genes [125,129]. In addition, trace elemental ions, such as Zn, Sr, Se, Mn, and Cu derived from injectable bioceramics, have also been reported to affect bone regeneration by activating osteogenesis and angiogenesis in tissues, at least to some extent [130]. These findings provide a valuable insight for regulating

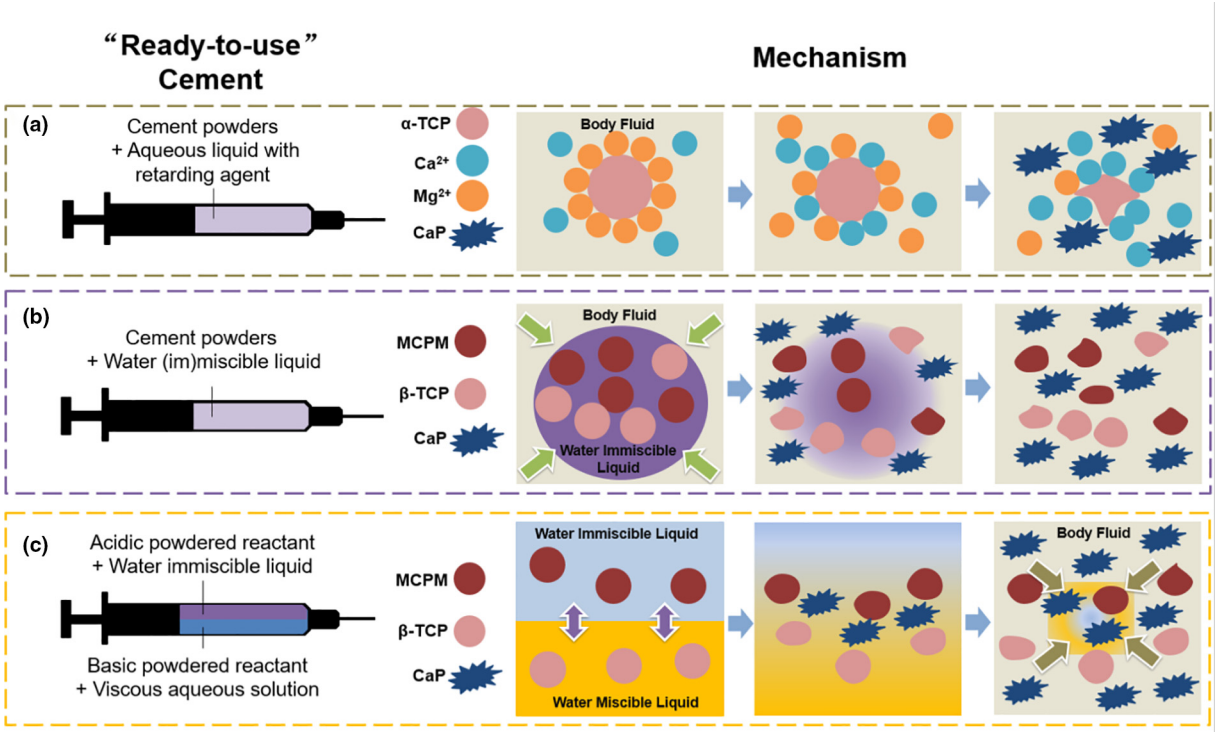

FIGURE 2

Three types of "Ready-to-use" injectable bioceramic cements and their working mechanisms. (a) Type A uses aqueous liquid with ionic retarding agent like $MgCl_2$. Mg^{2+} ions adsorb on the surface of α-TCP cement powders to suspend setting reaction and their replacement by Ca^{2+} can initiate α-TCP setting reaction. (b) Type B uses a nonaqueous liquid (water-miscible fluid or water-immiscible fluid) as retarding agent and setting reaction is facilitated by the diffusion of aqueous body fluid. (c) Type C consists of an acidic powdered cement reactant based on water-immiscible fluid with surfactants and the basic powdered cement reactant paste based on a viscous aqueous solution. α- or β-TCP: α- or β-tricalcium phosphate; CaP: transformed calcium phosphate; MCPM: monocalcium phosphate monohydrate.

the bone regeneration process through the design of a release profile for selective ions from injectable bioceramics. Moreover, the tailored release of multiple ions can synergistically activate multiple types of cells in sequence to maximize their regenerative potential [93,131].

Injectable bioceramic cements with porous structure. The biomimicry of natural bone, which encompasses the interconnected macroporous and microporous structures that allow cell spreading and the transport of nutrients, oxygen, blood, waste, and growth factors, is a key strategy for creating biologically functioning bone substitutes.

Generally, the self-setting reaction of injectable bioceramics comprises of dissolution and the subsequent reprecipitation of ceramic crystals, forming a microporous structure (pore size <100 μm) that increases the surface area in the hardened bioceramic scaffolds. Such structures can enhance cell–scaffold interactions and control drug delivery at the cost of a partial reduction in mechanical strength [132]. The main challenge is to create interconnected macropores (pore size >100 μm) in the cement, without losing its self-setting ability, injectability, and mechanical strength [133]. Commonly, degradable or soluble fillers that are added to injectable ceramics can act as porogens and reinforcing components [134,135]. For example, water-soluble mannitol can be used as a porogen and when mixed with a calcium phosphate cement can produce an injectable matrix with approximately 50% macroporosity, as has been reported by Xu et al. [136]. On the other hand, gas-foaming agents, such as hydrogen peroxide [137], magnesium granules [138], and carbon dioxide [139], as well as surface active foaming agents, such as albumin [140] and the surfactant sodium dodecyl sulfate [141], have also been used for a similar purpose. In addition, injectable foams can be prepared by syringe-foaming using a hydrophilic viscous polymeric solution [142].

Apart from *in situ* formation of porous matrix, injectable bioceramics have recently been used in the additive manufacturing to construct patient-specific porous scaffolds [143,144]. For this purpose, curing time of injectable bioceramics, which is associated with the densification and decrease in the porosity due to the development of microstructure during setting, becomes crucial. For instance, Chang et al. developed a calcium silicate cement possessing slow setting process at room temperature to print scaffold, resulting in a smooth printing process with controllable pore morphology, pore size, and porosity and a higher mechanical strength compared to 3D-printed calcium phosphate cement scaffold [145].

Injectable bioceramic cements that can deliver therapeutic molecules and cells. Selective combinations of therapeutic molecules with biomaterial matrices have been reported as another efficient way to stimulate cellular activities and enhance tissue repair and reconstruction. These therapeutic molecules include, but are not limited to, VEGFs, bone morphogenetic proteins (BMPs),

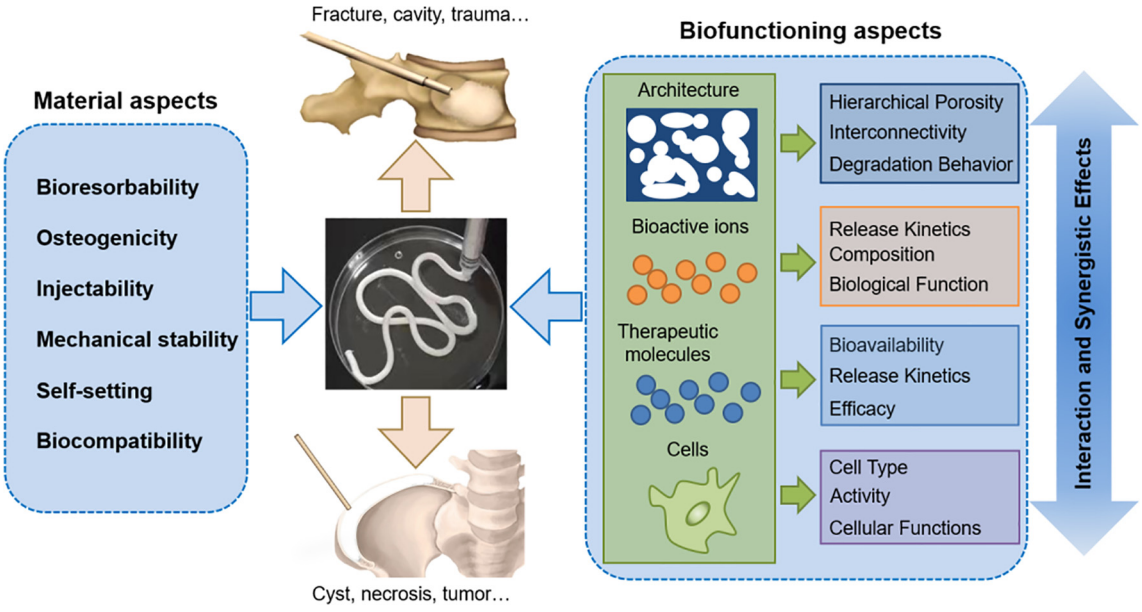

FIGURE 3

Considerations in translational research of injectable bioceramic cements for clinical uses.

transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and a number of peptides and drugs, such as gentamicin and bisphosphonates [146]. It is desirable that the supporting reservoir (i.e., the matrix loaded with the therapeutic molecules) should effectively deliver the molecules in a controlled and sustained manner, while maintaining their biological activities and therapeutic effects. Generally, these molecules can be blended with injectable bioceramic cements alone or in combination with the embedded carriers (e.g., mesoporous bioactive glass, CNTs, or biopolymers). The latter route provides better release control and preserves biological activity [147–149]. Additives, such as heparin, that tether therapeutic molecules to bioceramics can also be incorporated to allow for controlled release [150]. In combination of hierarchical porous structures, these molecule-loaded bioceramic scaffolds can provide a platform capable of supporting cell activities (e.g., migration, infiltration, and growth) and the simultaneous control of molecule dose and release kinetics [151,152]. Besides, injectable bioceramics with benign setting temperature can be used to co-print with biological components in the additive manufacture of porous scaffolds. For example, a ready-to-use calcium phosphate paste combined with chitosan/dextran sulfate microparticles that encapsulate either bovine serum albumin (BSA) or vascular endothelial growth factors (VEGFs) were used as a novel 3D printing ink [153].

Instead of therapeutic molecules, cells can also be delivered through injectable scaffolds. This cell delivery approach originates from the success of stem cell-seeded, pre-formed, bioceramic scaffolds for bone regeneration and repair [154,155], and the injectable cement enables cells to be seeded deep into the scaffold and be delivered by a minimally invasive route [156]. For this purpose, hydrogel microbeads are commonly added to the injectable bioceramics to preserve cell viability during the cement setting reaction without a significant impairment in cement injectability [157,158]. In addition, a gas-foaming porogen, such as CO_2, can be used to create macropores, resulting in an increase in cell viability by 37% following the addition of 15% porogen [139].

An important design criterion for injectable bioceramics with the capacity to deliver therapeutic molecules/cells is how to preserve their activity during the injection and setting processes, as well as guaranteeing the fate of the cargo after exposure to the complicated body environment. The interactions and synergistic effects of bioactive ions, porosity, therapeutic agents, and cells in bioceramics need to be systematically studied in the future. From a clinical translation perspective, the biological function of injectable bioceramic cements should not significantly compromise the material properties, such as mechanical stability, injectability, and setting behavior (Fig. 3).

Translational considerations for designing injectable bone cements

A balance of safety and efficacy is an important consideration for both researchers and regulators to allow for the translation of biomaterials to medical devices and has been systematically reviewed by others [159,160]. Besides this, the optimization of material design is an iterative loop that includes feedback from the end users. Two translational aspects specifically associated with injectable bone cements are discussed here.

For end-users like surgeons, the ease of injectability is reflected by the force required to inject a cement paste through delivery tools, such as a syringe or a cannula [161]. An injection force of 150 N is usually considered to be the upper-limit for a surgeon [162]. There are several material factors that influence the injectability of cement paste and its injection force, including viscosity, the maximum packing fraction of the powder, the liquid-to-powder ratio, and particle-particle interactions [116]. These factors should be studied and adjusted without significantly sacrificing the mechanical and setting properties of cement. In addition to material modification, re-design of the

delivery tools, such as increasing the cannula/syringe diameter and developing rotatory/hydraulic/automatic injection devices, have been attempted to enhance injection capability.

In clinical practice, injectable bone cements constantly face the risk of leakage to unwanted locations, causing complications. For example, the incidence of leakage in PKP and PVP into the pulmonary artery or the spinal canal can induce severe pulmonary emboli and paraplegia [163,164]. Currently, high-viscosity PMMA cement has been adopted to reduce the risk of leakage, at the cost of losing injectability and resulting in poor void filling and an increase in the extravasation of bone marrow into the cardiovascular system [162,165]. In recent studies of new injectable bone cements, leakage risk has not been symmetrically evaluated and is often overlooked in research. From a translational perspective, the thorough evaluation of leakage issue, both theoretically and experimentally, is necessary before the launch of new products.

Injectable hydrogels

Recently hydrogels have emerged as one of the most promising injectable biomaterials that can cater to diverse clinical demands, such as the delivery of drugs/cells, supporting tissue repair/regeneration, bio-adhesion, and immune-regulation [166]. Hydrogels are highly hydrated three dimensional (3D) polymeric networks that are stabilized by crosslinks of various natures [167]. The hydrated volume of hydrogels can easily enable the encapsulation of water-soluble drugs, especially macromolecular proteinaceous drugs, without adversely compromising the therapeutic efficacy of the drugs [168,169]. Furthermore, therapeutic cells, such as stem cells, can also be encapsulated in hydrogels, and the highly permeable 3D matrix of hydrogels supports the efficient diffusion of nutrients and metabolic wastes, which is essential for cell survival and function [166,170]. Therapeutic agents secreted by encapsulated cells, such as growth factors and exosomes, can also diffuse through the highly porous network of a hydrogel to reach surrounding cells and tissues. Their polymeric nature also makes hydrogels a highly customizable "blank slate" that can allow for further chemical modification and biofunctionalization to support more sophisticated functions. For example, decoration of the hydrogel with cell adhesive ligands can effectively promote cell attachment and subsequent cellular development in the hydrogel scaffold. The conjugation of tissue-adhesive chemical groups, such as catechol, can generate highly bio-adhesive hydrogels for wound closure. These hydrogel capabilities cannot be achieved in non-hydrated inorganic injectable biomaterials.

Because hydrogels are typically prepared by inducing a "sol–gel" transition of liquid precursor solutions via crosslinking, they can be designed to function as injectable biomaterials without compromising their aforementioned capabilities. Injectable hydrogels are highly advantageous for the precise implantation at deep and enclosed anatomical locations using specialized delivery devices. The fluid nature of the hydrogel precursor solutions results in the efficient filling of the target sites with irregular geometries and a seamless integration with surrounding tissues upon gelation. Injectable hydrogels enable minimally invasive procedures that can expedite healing after surgery.

Strategy for preparing injectable hydrogels

In situ gelation

The *in situ* gelation of injectable hydrogels generally relies on an extended crosslinking process to induce the gradual "sol–gel" transition of precursor liquids at the injection sites. A wide variety of chemical and physical crosslinking mechanisms have been used to develop *in situ* gelation hydrogels, such as click chemistry [171–174], enzymatic crosslinking [37,175,176], dynamic covalent bonds [177–183], and stereo-complexation [184]. For example, for cartilage repair Jin et al. developed an injectable HA/PEG hydrogel crosslinked by a Michael addition reaction [172]. Yesilyurt et al. synthesized injectable hydrogels by capitalizing on the dynamic covalent bonds formed between boronic acid derivatives and diols [179]. Lou et al. ingeniously incorporated a catalyst to accelerate the formation and exchange of hydrazone bonds, thereby enhancing injectability; the stability of the injected hydrogels can be maintained after diffusional loss of the catalyst [180].

The injectable hydrogels can also be prepared via the *in situ* gelation of the injected precursor solutions in response to specific stimuli present at the injection site, such as temperature [185,186], pH [187], and different ions [188]. For example, the conjugation or copolymerization of thermo-responsive synthetic polymers, such as poly(N-isopropylacrylamide) (PNIPAm), using hydrophilic polymers as the thermo-gelators, enables the reverse sol–gel transition of the injected precursor solution at target sites due to the hydrophobic aggregation of the PNIPAm components [185,186,189]. In addition, amphiphilic block copolymers, such as PEG-polyester, PEG-PPG(poly(propylene glycol)), and PEG-peptide, also exhibit excellent reverse thermo-gelation and can be fine-tuned by tailoring factors including block molecular weight, concentration, and block composition [190–193]. For example, Yu and Ding have systemically studied copolymers, such as PLGA-PEG-PLGA (poly(lactic acid-co-glycolic acid)), for preparing injectable thermo-gelation hydrogels for biomedical applications [194,195]. Liu et al. have developed highly robust supramolecular hydrogels based on hydrogen bonds, which can be injected upon softening at elevated temperatures [196,197].

A superior *in situ* gelation injectable hydrogel requires optimization of the gelation time of the precursor solutions to allow sufficient time for pre-injection mixing, subsequent injection, and complete filling of the target sites, while the gelation needs to occur in a timely fashion following these preceding events to minimize the loss of injectants to "off-target" locations. This optimization often requires lengthy repetitive design and trial processes and can be challenging to achieve.

Macroporous hydrogels

Macroporous hydrogels containing a large volume of interconnected pores can be injected in the "gel" form due to their ability to undergo large volume compaction [198,199]. A wide array of methods, including porogen templating [200], cryogelation [198,201], mechanical forces [202], and gas foaming [203], have been used to fabricate macroporous hydrogels. These hydrogels can be compressed down to below 10% of their resting volume when being injected and can rapidly recover their original volume and shape upon exiting the injection devices. Injectable

macroporous hydrogels can be prefabricated with pre-designed dimensions, geometry, physical and chemical properties, and pre-loaded with cargos, such as drugs or cells, before injection [198,204]. Bencherif et al. showed that an injectable cryogel can be used to localize tumor cells and deliver immunoregulatory agents for cancer vaccination [205]. Newland et al. developed a shape-memory injectable cryogel as a carrier of cells and growth factors for neural regeneration [204]. Compared with *in situ* gelation hydrogels, injectable macroporous hydrogels have some unique advantages, including shape memory, ease of use, and better-controlled physical and chemical properties. Nevertheless, it should be noted that cells carried by the macroporous hydrogels are seeded in the macropores of the hydrogel rather than encapsulated by the 3D hydrogel matrix, and this may lead to a different mechanosensing microenvironment of the cells.

Shear-thinning hydrogels

Distinct from *in situ* gelation hydrogels, shear-thinning hydrogels exhibit a unique shear-dependent and reversible "gel–sol" transition due to the reversible nature of their physical crosslinks [206]. Therefore, shear-thinning hydrogels can be injected in a pre-fabricated "gel" form via the "gel–sol" transition under the high shear stress formed during injection and immediately switch back to the "gel" form upon exiting the needle tip in the absence of the high shear stress. Previous work has shown that shear-thinning hydrogels are based on a number of physical interactions, including charge interactions [207,208], hydrogen bonding [209–212], biomacromolecules (e.g., peptide, proteins) interactions [213–217], dynamic covalent bonds [218,219], ionic interactions [220], host–guest complexations [221–227], polymer–nanoparticle interactions [199,228], hydrophobic interactions [184], and ligand-ion coordination [229,230] (Fig. 4). For example, Burdick et al. developed a series of injectable hydrogels based on host–guest complexation for various applications including 3D bioprinting [221,225,226]. Heilshorn et al. fabricated a family of shear-thinning hydrogels stabilized by peptide–protein interactions which can be further stabilized by conjugated thermo-responsive PNIPAm chains post injection [213,216,217]. Avery et al. formulated a shear-thinning gelatin and a silicate nanoplatelet hydrogel for endovascular embolization [199].

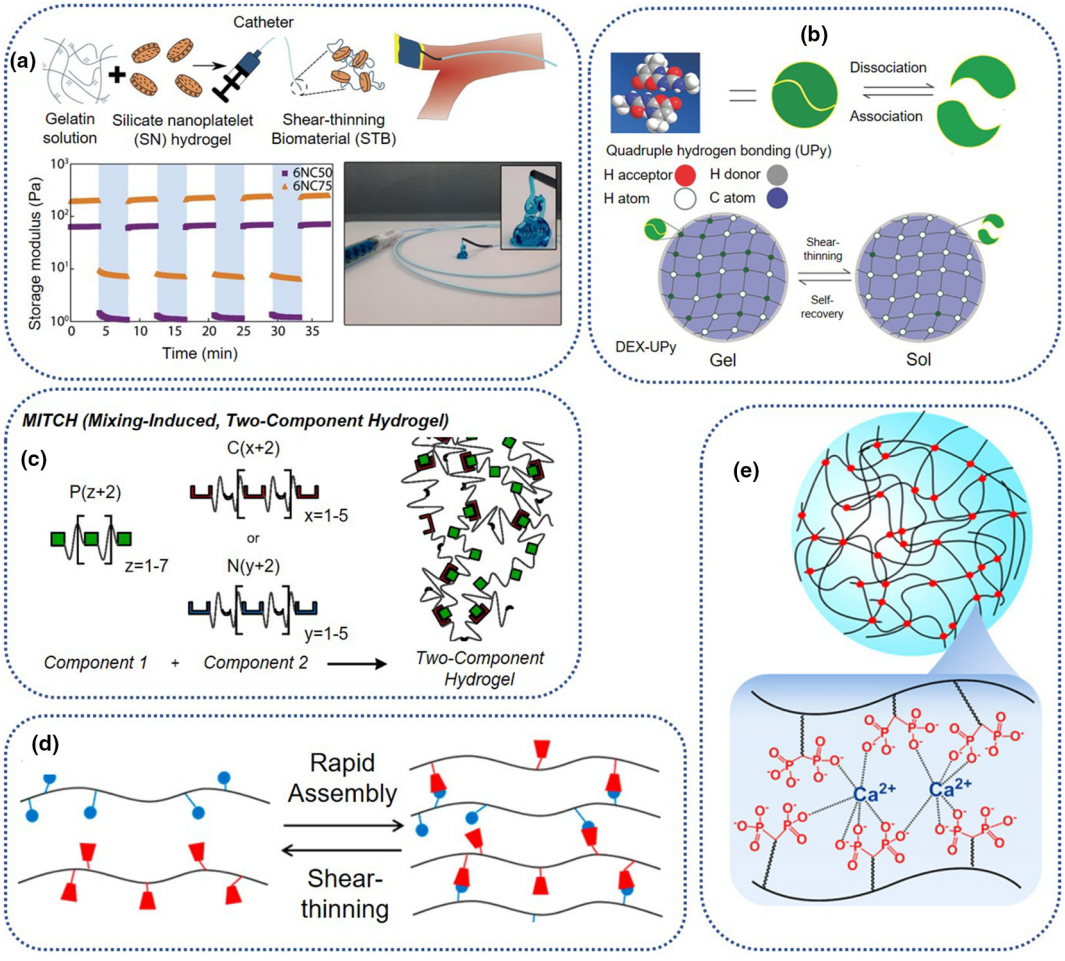

FIGURE 4

(a) An injectable shear-thinning hydrogel based on polymer–silicate nanoplatelet interactions for endovascular embolization [199]; (b) an injectable hydrogel crosslinked by multivalent hydrogen bonds [209]; (c) dual-component protein-engineered physical hydrogels stabilized by protein–protein interactions [213]; (d) the reversible crosslinks based on host–guest complexations enable rapid assembly and shear-thinning of the hydrogels [221]; e) the dynamic ligand–cation coordination bonds function as the dynamic crosslinks in the injectable hydrogels [229].

The "gelation first and injection later" feature of shear-thinning hydrogels offers several major advantages including: (1) ease of use at the point-of-care; (2) circumvention of the optimization of gelation kinetics; (3) minimization of leakage due to the instantaneous "sol–gel" transition after exiting the injection device; (4) efficient adaptation of the injected hydrogels to the complex geometries of the injection site due to the dynamic nature of the physical crosslinks; (5) the ability to preload drugs and cells in the hydrogel; and (6) a dynamic and permissive 3D matrix that can support biomimetic cell–hydrogel interactions and associated cellular behaviors.

Biomedical applications of hydrogels and design considerations for translation

Injectable hydrogels for drug delivery

Hydrogel matrix properties that govern drug release. The highlyhydrated 3D polymeric matrix of hydrogels is ideal for accommodating water-soluble small molecule or macromolecular drugs. However, the highly permeable nature of the hydrogel matrix also allows for the free diffusion of drug molecules, leading to burst release within a short period of time. Drug release kinetics are dictated by hydrogel mesh size and hydrogel–drug interactions [228,231]. Hydrogel mesh size (i.e., the spacing between neighboring polymer chains in the hydrogel network) typically ranges from a few nanometers to a few hundreds of nanometers [232,233]. The relative dimensions of the cargo drug molecules compared to the hydrogel mesh size determine the mode of drug release from hydrogels. When the mesh size is significantly larger than the hydrodynamic diameters of the drug molecules (small molecules or low molecular weight proteins), the release of the drug molecules is a diffusion-controlled process, which often results in substantial burst release [169]. The hydrogel network only provides conspicuous steric restrictions on the passive release of drug molecules when the mesh size is comparable to the hydrodynamic size of the drug molecules. Furthermore, attractive forces between drug molecules and the hydrogel matrix can significantly slow the release kinetics of drugs from hydrogels.

Strategies to extend drug release from hydrogels. The hydrogel mesh size can be fine-tuned by optimizing the hydrogel network structure, such as by simply adjusting the polymer concentration and crosslinking density. However, the unique requirements for injection limits the use of more sophisticated strategies for adjusting hydrogel network structure, such as the use of interpenetrating polymer networks (IPNs) [234]. Hydrogels with IPNs are typically not amenable for injection because the formation of IPNs requires immersing a prefabricated hydrogel network in a solution of a second monomer and polymerizing agents [235]. Drug–hydrogel interactions are highly effective in extending the release of payload drugs from injectable hydrogels. For example, the incorporation of cationic or anionic moieties in the hydrogel network can effectively prolong the release of oppositelycharged cargo molecules. The anionic sulfate group, or sulfate-containing moieties, such as heparin, have been widely used to slow down the release of cationic proteinaceous growth factors, which typically are positively charged due to their basic isoelectric point [236,237]. In addition to charge interactions, other physical interactions, including hydrophobic interactions, hydrogen bonding, and supramolecular bonding, have been utilized to optimize drug release kinetics. For example, macrocyclic molecules, including cyclodextrins (CDs) and cucurbiturils (CBs), contain a nanometer-scale hydrophobic cavity, which can harbor hydrophobic small molecules with a good binding affinity [238,239]. Once decorated in the hydrogel network, these macrocycles are particularly useful for the efficient loading of hydrophobic small molecule drugs in the hydrophilic hydrogel network. Feng et al. showed that an injectable supramolecular hydrogel containing excess beta-CD can be loaded with copious amounts of hydrophobic dexamethasone and mediate its sustained release for up to 2 weeks [223]. Appel et al. showed that CB-based host guest hydrogels were capable of sustaining the long-term release of proteins [240].

The incorporation of drug-eluting polymeric or inorganic micro/nanostructures, such as polymeric nano/microparticles [241,242], liposomes [243], polymeric micelles [244], and inorganic mesoporous silica nanoparticles [245], which are preloaded with drugs, in the hydrogels can further enhance the capacity of the hydrogels for drug delivery without affecting their injectability. Zhang et al. demonstrated that *in situ* self-assembled nanoparticles, driven by bisphosphate–cation coordination, can store abundant bioactive cations (e.g., Mg^{2+} and Ca^{2+}) in the highly permeable hydrogel matrix for their subsequent long-term release [246].

"Smart" drug delivery by injectable hydrogels. In addition to optimizing drug release via the passive drug–hydrogel interactions and doping of nano/microstructures, one desirable design feature is the incorporation of an active triggering mechanism in the injectable hydrogel. Designing injectable hydrogels to control the release of their cargo drugs in response to specific physiologicallyrelevant stimuli, such as pH [247,248], temperature [248,249], redox chemicals [249–251], ions [252,253], and enzymes [254,255], will significantly improve the therapeutic outcome of drug delivery. Chao et al. recently demonstrated the efficient delivery of a radioisotope-labeled catalase (I-Cat) via the Ca^{2+}-induced *in situ* gelation of an intratumorally injected alginate precursor solution [188] (Fig. 5a). The long-term entrapment of I-Cat in the tumor by the alginate hydrogels enables the sustained oxygenation of tumor as a result of catalyzing the degradation of tumor endogenous hydrogen peroxide, thereby increasing the efficacy of radiotherapy [188]. Purcell et al. utilized an MMP (metalloproteinase)-sensitive injectable hydrogel to achieve the triggered delivery of MMP inhibitors to suppress the overexpression of MMPs following myocardial infarction, thereby attenuating adverse left ventricular remodeling [255]. Zhang et al. showed that a phosphate prodrug encapsulated in an injectable nanocomposite hydrogel via phosphate–cation interactions showed rapid release upon dephosphorylation by the alkaline phosphatase produced by the differentiating stem cells in a positive feedback manner [246]. The rich literature on nanomaterial-based stimuli responsive drug delivery systems can provide valuable guidance for the design of injectable hydrogels with smart drug delivery capabilities.

Recently, injectable hydrogels have been increasingly employed as the carrier for localized anti-cancer drug delivery

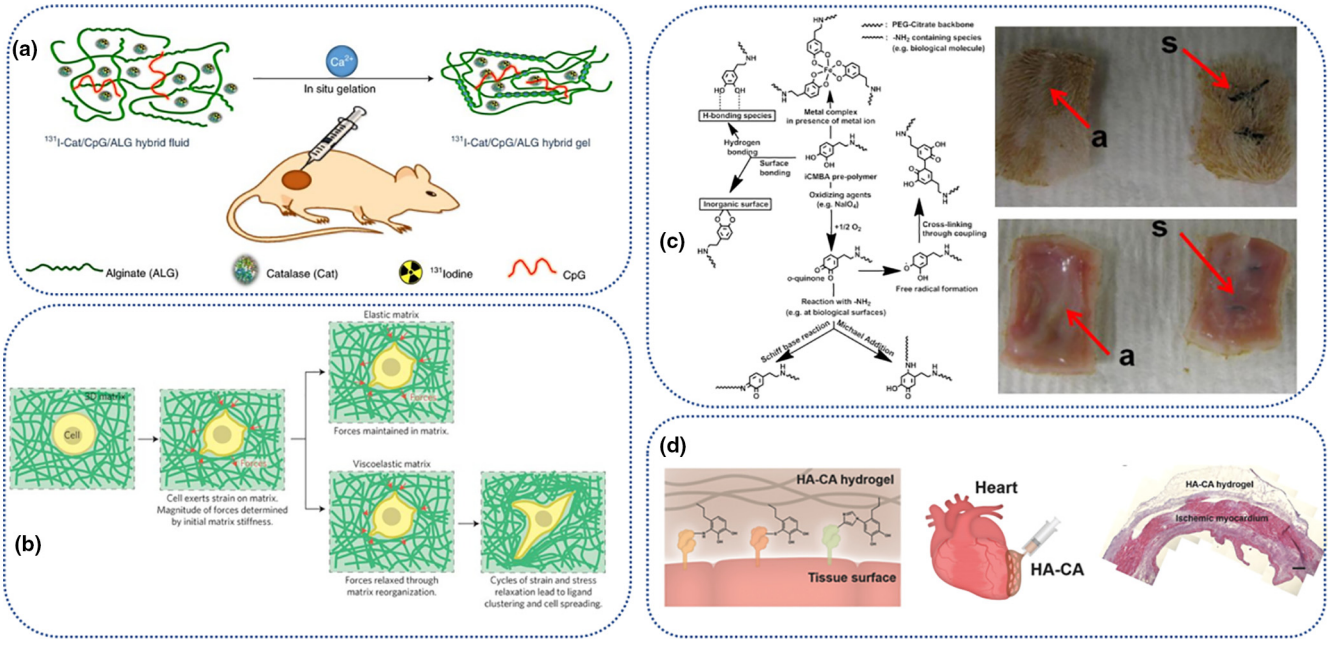

FIGURE 5

(a) *In situ* delivery of immunoregulatory agents and catalase via injectable hydrogels imparted potent anti-tumor responses [188]; (b) viscoelastic hydrogel matrix with fast stress relaxation provides permissive 3D microenvironment to facilitate cell–matrix interactions [272]; (c) mussel-inspired injectable bioadhesive hydrogels outperforming surgical suture in wound [285]; (d) injection of mussel-inspired bioadhesive hydrogels secured the transplanted cells on tissue surfaces and increased angiogenesis in vivo, leading to improved treatment of ischemic diseases [283].

as an alternative to common chemotherapy, displaying encouraging properties, such as off-target toxicity avoidance in normal tissues, sustained delivery of chemotherapeutic agents, tissue-mimicking mechanical properties, tunable degradation, and more efficient tumor growth inhibition [256–258]. To date, various injectable smart hydrogel-based drug delivery systems, including thermosensitive, pH-sensitive, photosensitive, dual-sensitive, and active targeting hydrogels, for the smart treatment of different types of cancer have also been developed [259].

Injectable hydrogels for cell-based therapies

Unique advantages and design considerations of injectable hydrogels for cell delivery. Therapies based on cells have attracted considerable research attention because of the unique capabilities of cells, including their ability to continuously synthesize a range of diverse therapeutic factors (e.g., growth factors and enzymes) and their ability to repair/regenerate injured tissues and organs. However, the direct administration of cells in a suspension solution often results in a limited therapeutic outcome due to lack of cell engraftment and significant cell death *in vivo*. Hydrogels not only isolate the delivered cells from immune responses but also provide a diffusion-friendly 3D environment to support various cellular activities including adhesion, proliferation, and differentiation. As described in the previous section, hydrogels can also be loaded with the necessary bioactive molecules to regulate cell behavior. Furthermore, injectable hydrogels can directly deliver and maintain a concentrated distribution of the cargo cells at the intended sites via the adhesion to target tissues and the physical protection from the cleansing by biofluids.

Biofunctionalization of injectable hydrogels. Injectable hydrogels based on natural ECMs or biomacromolecules possess intrinsic bioactivities that are essential for both the delivered cells and endogenous cells. ECM-based injectable hydrogels have been shown to promote the repair of injured organs/tissues, such as the myocardium [39]. On the other hand, injectable hydrogels, especially those prepared from synthetic polymers, typically lack sufficient bioactivity to support cellular activities and mediate immunoregulation. Emulating the biochemical complexity of the ECM has been a key theme in developing hydrogels for cell delivery. The classical arginine–glycine–aspartic acid (RGD) tripeptide derived from collagen and fibronectin is widely used in hydrogels to support cell adhesion, proliferation, and differentiation [260,261]. The incorporation of laminin into hydrogels has been shown to promote neurogenesis and soft tissue repair [262]. The capability of the implanted hydrogels to regulate the host immune responses is essential to the success of the cell delivery. A recent study showed that conjugation of the Fas ligand (FasL) to the injected microgels encapsulating allogenic pancreatic islet cells promoted graft acceptance for over 200 days by suppressing the host T lymphocytes [263]. In addition to the organic bioactive ligands, bioactive inorganic components, such as bioglass/bioceramics can also be used to biofunctionalize hydrogels. Chang et al. elegantly demonstrated that composite hydrogels encapsulating bioactive bioceramic particles promoted the regeneration of injured tissues, including bone, blood vessel, and skin, via the released bioactive ions [264,265]. These findings highlight the importance of biofunctionalization of the injectable hydrogels for highly efficient cell delivery.

Enhancing cell–hydrogel interactions in injectable hydrogels. A distinctive design consideration for hydrogel-based cell delivery, compared with that for drug delivery, is that the hydrogel matrix

needs to provide a permissive 3D microenvironment to allow the encapsulated cells to effectively interact and remodel [266]. It has recently been reported that cells encapsulated in stiff elastic hydrogels, but not in soft viscoelastic hydrogels, fail to complete mitosis due to the resistance of the hydrogel matrix to cellular expansion. Lutolf and Hubbell first developed cell-degradable synthetic hydrogels by incorporating crosslinker peptides that are sensitive to MMP and demonstrated the importance of cell-mediated hydrogel degradation to various cellular developmental events [267–269]. Khetan et al. further showed that MMP-induced hydrogel degradation promotes the mechanosensing-dependent osteogenesis of encapsulated stem cells [270]. Madl et al. also showed that neural progenitor cells (NPCs) cultured in degradable hydrogels exhibit enhanced stemness and neurogenic differentiation compared with cells cultured in non-degradable hydrogels [271]. A recent pioneering study by Chaudhuri et al. further revealed that in the absence of significant hydrogel degradation, the intrinsic fast relaxation kinetics of ionicallycrosslinked alginate hydrogel networks promoted the mechanosensing and osteogenesis of encapsulated stem cells [272] (Fig. 5b). Anseth et al. have shown that dynamic covalent crosslinks provide network adaptability for the encapsulated cells [273,274]. Together, these findings indicate that the capability of the injectable hydrogel networks to accommodate the constant cellular probing of their microenvironment is essential to the success of hydrogel-aided cell therapies, especially for cell-based regenerative medicine.

Injectable hydrogels for surgical assistance

Conventional cyanoacrylate-based adhesives (such as Super-Glue®) are cytotoxic and rigid, thereby limiting their widespread use [275]. Hydrogels have emerged as more cytocompatible and versatile tissue adhesives and have been used in many surgical applications. For example, COSEAL® (Baxter), DURASEAL® (Confluent Surgical) [276,277], and TISSEEL® (Baxter, fibrin glue) [278] are injectable hydrogel-based bio-adhesives used in various surgical procedures. A newly developed elastic human protein-based hydrogel sealant has shown improved performance for lung sealing [279]. However, these existing hydrogel-based bio-adhesives usually have limited adhesion strength due to poor hydrogel matrix cohesion and hydrogel–tissue interfacial adhesion, thereby yielding low levels of adhesion energy. Studies have reported non-injectable tough hydrogels, which exhibit enhanced adhesion energies due to the reinforced cohesion, interfacial adhesion, and energy dissipation in the hydrogel matrix [280–282]. However, these tough and adhesive hydrogels are typically not injectable and are also usually not amenable for encapsulation of cells due to the extensive crosslinking and toxic raw chemicals used in their fabrication. Nevertheless, these studies provide some valuable guidance on the design of injectable bioadhesive hydrogels. Despite remaining as a major technical challenge, developing injectable hydrogels that possess excellent wet adhesion toughness will be of high significance for a variety of clinical applications. The recent development of mussel adhesion-inspired and gel-point adhesive hydrogels shows a promising potential for achieving robust and cytocompatible wet adhesion [283–285] (Fig. 5c and d). For example, Yang et al. demonstrated an injectable starch-based gel-point adhesive hydrogel for treating myocardial infarction, which outperformed most existing acellular epicardial patches in reversing left ventricular remodelling and restoring heart function after both acute and subacute myocardial infarction in rats [284].

Injectable electronics

The future of injectable biomaterials includes the possibility of delivering much more complex materials or systems via cannulated channels or needles to the lesion, rendering more effective and intelligent diagnostics and therapeutics possible. A recent important direction with high clinical translational potential is the combination of electronics with *in vivo* sensing/monitoring/stimulating functions [286,287], with the capability of being injected. Injectable electronics are expected to detect and monitor biological interactions and physiological signals and also act as functional electrical stimulators or therapeutic devices [287,288]. The design considerations for rendering electronics injectable includes two strategies, in which electronics can be injected either in their entirety (integral strategy) or in parts followed by *in situ* assembly (separate strategy).

An early example of injectable integral electronics is a miniaturized cylindrical with a typical diameter of 2–3 mm and a length of 1–1.5 cm, capable of passing through a hyperdermic needle [289]. With the progress of fabrication technologies, the size of such electronics is continuing to decrease [290]. This miniaturized electronic device is typically used as a neurostimulator (Fig. 6a), consisting of an electrode for stimulation, an optional antenna for power and data telemetry, an outer layer as both the electronics–tissue interface and the protective layer shielding the electronics from the body and vice versa, several electronics to generate and control the electrical pulses, as well as optional sensors to detect and monitor a number of parameters (e.g., neural signals, temperature, or pressure at the tissue-stimulator interface, etc.) [287,290]. After over 20 years of technical development and clinical trials, injectable neurostimulator is progressing toward the first clinical trials but still faces challenges, such as wireless rechargeable microbatteries, bendability, and long-term reliability [291].

As an alternative to miniaturized electronics, recent advances in flexible electronic materials and fabrication techniques have enabled the creation of injectable bioelectronic unit with a large specific surface area, high electronic transport capability, and mechanical flexibility or stretchability [292,293], and a stable intimate interface for the target organs or tissues [294]. For example, a team led by Rogers and Bruchas exploited ultrathin, flexible PET substrates populated with microscale inorganic light-emitting diodes (μ-ILEDs) together with electrophysiological and temperature sensors, all mounted on removable plastic needles that facilitate insertion into the tissue (Fig. 6b) [43]. Detailed experimental and theoretical studies of the operation, ranging from heat flow aspects to inflammation assessments and a comparison to conventional devices, illustrate the promising potential of such injectable, flexible, and multifunctional electronics in the areas of diagnostics and therapeutics. As an alternative to flexible 2D substrate, a recent pioneering study by Lieber and his colleagues, used injectable, ultraflexible, and macroporous mesh electronics that can unroll to fill small cavities to

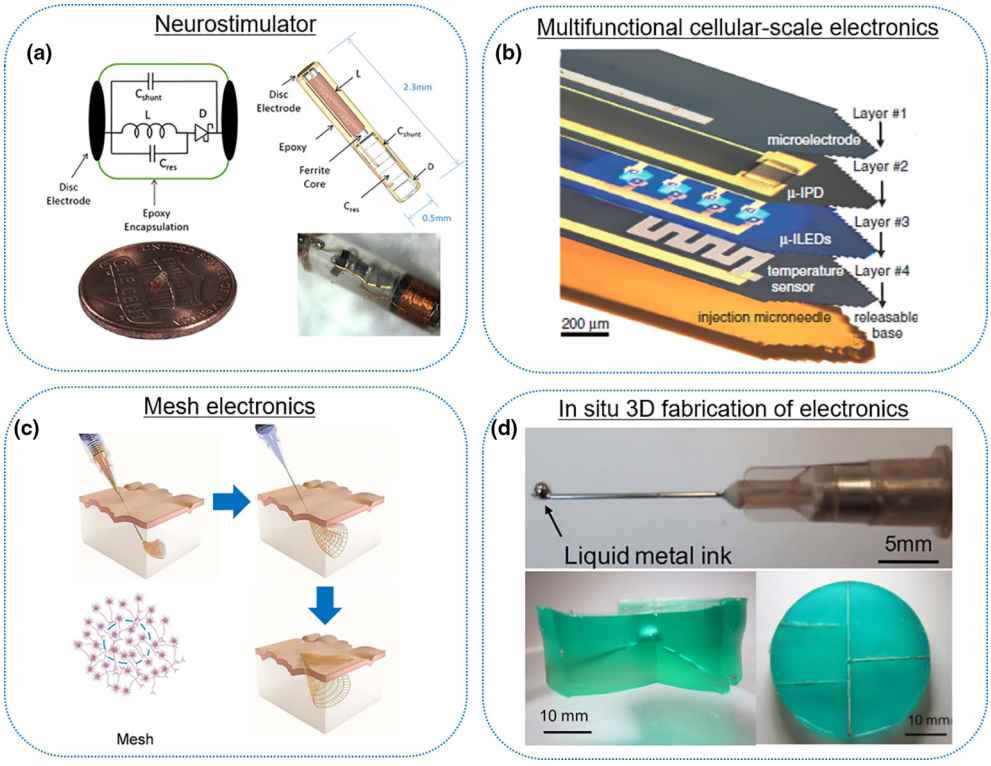

FIGURE 6

Selected examples of injectable electronics: (a) sub-millimeter, wireless neurostimulator: (Top) structure of assembled device, with a total volume of 0.45 mm^3. (Bottom) visual images of the device [290]; (b) an injectable, flexible, integrated multifunctional electronics, including layers for electrophysiological measurement (no. 1), optical measurement (no. 2), optical stimulation (no. 3), and temperature sensing (no. 4), all bonded to a releasable structural support for injection (microneedle) [43]; (c) schematic of injectable mesh bioelectronics can be injected through needles with a diameter as small as 100 micrometers, which is capable of interfacing with the brain tissue [42,296]; (d) *in situ* 3D fabrication of electronics of different layout by liquid metal (Top) injection into the packaging domains (Bottom) [297].

directly record changes in the electrical signals in the brain, down to the level of a single brain cell (Fig. 6c) [42,295]. Such unique mesh electronics do not elicit inflammation or scarring, in contrast to more conventional electronics like flexible thin films. Moreover, neurons were found to penetrate through the electronics' open mesh structure, thus demonstrating an unprecedented level of integration and compatibility with the brain circuitry. It has been noted that syringe injection also provides the opportunity to co-inject multifunctional mesh electronics together with other materials/cells into the host systems to provide unique engineering and biomedical applications [296].

As a separate strategy, the assembly of electronics inside the body relies on the sequential injections of a liquid metal ink (e.g., GaInSn) and a biocompatible flexible packaging material, providing the possibility of creating a variety of electronics with different embedded structures in the target tissues (Fig. 6d) [297]. Liquid metals possess several unique benefits for the quick and economic fabrication of flexible electronics, including a low melting point, ease of printing, high conductivity, and high biocompatibility [298]. In addition, unlike conventional implanted electronics, patients bearing injectable electronics made of liquid metals probably have no limitations for MRI (magnetic resonance imaging) examination due to the non-magnetic properties of liquid metals. This separate approach for injectable electronics is, however, still in the early stage of research, currently only allowing for simple electronic devices with limited functions.

Although the advances in injectable electronics are still not parallel with other injectable biomaterials, the progress in flexible materials and electronics are creating more possibilities of developing injectable 3D scaffold engineered with electronics, cells, and therapeutic agents for the smart treatment integrating *in situ* diagnosis, intervention, and tissue regeneration.

Translational status and outlook

With the growing knowledge and technology in biomedical and materials sciences, the innovation and translation of injectable biomaterials to fulfill unmet clinical needs is expected to thrive in the near future. In a review of the injectable biomaterials that have progressed into clinical trials in the past 5 years (Table 2), almost all the products have multiple biofunctions and a regenerative potential in addition to their role as a structural filler. Currently, there are more hydrogel products than cement or paste products in clinical trials, revealing that in translational medicine, hydrogels are emerging as the new injectable biomaterial. Also, calcium phosphate-based products for bone repair and HA products for the treatment of knee osteoarthritis, as well as for cosmetic surgery, are now the major players in the categories of injectable cements and hydrogels, respectively. It is worth

TABLE 2

Examples of clinical trials for injectable biomaterials from 2013 to 2018 (extracted from ClinicalTrials.gov).

Product Name (Clinical Trail Identifier #)	Material	Form	Disease or Indication	Description	Time of trial
AUGMENT Injectable Bone Graft (NCT01305356)	Calcium Phosphate + Collagen + Recombinant human platelet-derived growth factor (rhPDGF)	Paste	Degenerative joint disease; Congenital deformity; Osteoarthritis; Rheumatoid arthritis	Facilitating fusion in conditions or injuries requiring bone graft	2011–2014
CERAMENT™ |BONE VOID FILLER (NCT02567084)	Calcium sulfate + Calcium phosphate	Cement	Bone Cyst	Providing bone generation and bone remodeling in patients with benign bone tumors	2011–2014
Norian Drillable Bone Void Filler (NCT01132508)	Calcium phosphate + Reinforcing fibers	Cement	Tibia Fractures	Bone void filler	2008–2013
Inject BMP (ExcelOS-inject containing rh-BMP2) (NCT02714829)	Calcium phosphate + Recombinant human bone morphogenetic protein-2 (rh-BMP2)	Paste	Alveolar bone preservation	Bone void filler that can stimulate the production of bone	2016–2018
Radiesse (NCT03282357)	Calcium Hydroxylapatite (CaHA) microspheres suspended in an aqueous gel	Hydrogel	Nasolabial Folds	Correction of nasolabial folds	2017–2018
Biodentine (NCT03418246 NCT03686475 NCT02201641)	Calcium Silicate	Cement	Dental diseases	Dental filler	2015–2015 2015–2017 2012–2017
KIO014 (NCT03679208)	Chitosan	Hydrogel	Knee Osteoarthritis	Relief of joint pain and symptoms in knee osteoarthritis	2018–2019
Injectable Collagen Scaffold™ (NCT02786017 NCT02767817 NCT02635464 NCT02644447 NCT02745808)	Collagen + Human Umbilical Cord-derived Mesenchymal Stem Cells	Hydrogel	Decompensated Cirrhosis; Brain Injury; Chronic Ischemic Cardiomyopathy; Premature Ovarian Failure; Erectile Dysfunction	Symptom relief	2016–2018 2016–2018 2015–2019 2015–2018 2015–2018
CartiZol (NCT02539095)	Collagen	Hydrogel	Chondromalacia; Osteoarthritis; Traumatic Arthritis	Stimulating the neovascularization and cell penetration and to assist the natural wound healing process of cartilage	2014–2015
VYC-12 Hyaluronic Acid Injectable Gel (NCT02877069)	Hyaluronic acid	Hydrogel	Skin Roughness	Improvements of skin texture and quality	2015–2016
SUPARTZ; Euflexxa (NCT02110238)	Hyaluronic Acid	Hydrogel	Knee Osteoarthritis	Relief of joint pain and symptoms in knee osteoarthritis with material of bacterial origin or avian origin	2014–2016
Restylane Silk (NCT03461198)	Hyaluronic Acid	Hydrogel	Wrinkles	Cosmetic filler	2018–2019
Hyaluronic Acid Filler (NCT02868749)	Hyaluronic acid	Hydrogel	Abdominoplasty or bilateral breast reduction with a Wise pattern skin excision	Cosmetic filler	2017–2018
PERIOSYAL FILL (NCT02391974)	Hyaluronic acid	Hydrogel	Chronic Periodontitis	Treatment of Periodontitis	2015–2017
CINGAL (NCT02381652)	Hyaluronic acid + Triamcinolone Hexacetonide	Hydrogel	Knee Osteoarthritis	Relief of joint pain and symptoms in knee osteoarthritis	2015–2015

noting that the clinical trial of an injectable collagen scaffold containing umbilical cord-derived mesenchymal stem cells for the treatment of multiple organs has been launched in 2018 (NCT02786017, 02767817, 02635464, 02644447, and 02745808). In addition, injectable hydrogels for new clinical purposes, such as a spacer between neighboring organs to shield an

organ from radiation during radiotherapy (e.g., TraceIT and SpaceOAR), have also emerged.

As the outcomes of research move toward translation and commercialization, it will also be important to elucidate the mechanisms of interactions between an injectable biomaterial and its surroundings, which will lead to improved material design and modification. On the one hand, the results of characterizing materials may not be entirely clinically applicable because the injection or setting process vary greatly depending on the injection site and the patient's conditions. On the other hand, the interaction between the host tissue and the injectable material is largely unknown due to the lack of feasible *in vivo* evaluation or observation methods. Also, the scarcity of standard evaluation tools for injectable biomaterials (e.g., material and mechanical characterization methods, *in vitro* and *in vivo* models, and degradation models, etc.) poses a difficulty in fully understanding the change and fate of materials after injection into the body. Especially for bioresorbable, injectable materials, this difficulty hinders their clinical use for broader indications and increases the risks for clinicians and patients. For example, there is no consistent evidence that for injectable bioceramics their bioresorption *in vivo* occurs at a rate that is synchronized with new bone in-growth, rendering their application in load-bearing bones questionable. These problems need better solutions from future translational research and development.

Patient-specific injectable biomaterials is an important yet challenging future direction. For example, in the bone of osteoporosis patients, the porosity and mechanical stability of the injection site would be different from that of ordinary patients. Non-specific injectable biomaterials are thus expected to encounter the variations in materials distribution, degradation and bone tissue stimulation behaviors at target sites. Other factors, including age, medical history, life style, and postoperative rehabilitation, should also be fully considered in the design of injectable products.

By harnessing the ability to engineer injectable biomaterials and employing its minimally invasive advantage, injectable biomaterial therapies are becoming popular in clinical practice. Accordingly, specialized clinical skill training and delivery devices are also deserved. In addition, the high costs associated with the development and manufacture of medical-grade injectable biomaterials (e.g., animal-sourced or derived materials, and new synthetic materials with low yield) or with the incorporation of therapeutic agents (e.g., drugs, factors, and cells) also needs to be overcome. It is wise to consider the cost factors at the front-end of translation, ideally at the material design stage. But other crucial factors, such as safety and stability (e.g., shelf life, especially for cell-contained materials), must be balanced. Innovation and translation of injectable biomaterials will not be possible in the absence of collaboration between researchers, clinicians, entrepreneurs, engineers, and patients. Such mutual efforts are expected to catalyze the field toward identifying new solutions for unmet clinical needs.

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Data availability

There are no linked research data sets for this submission. The following reason is given: No data were used for the research described in the article.

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