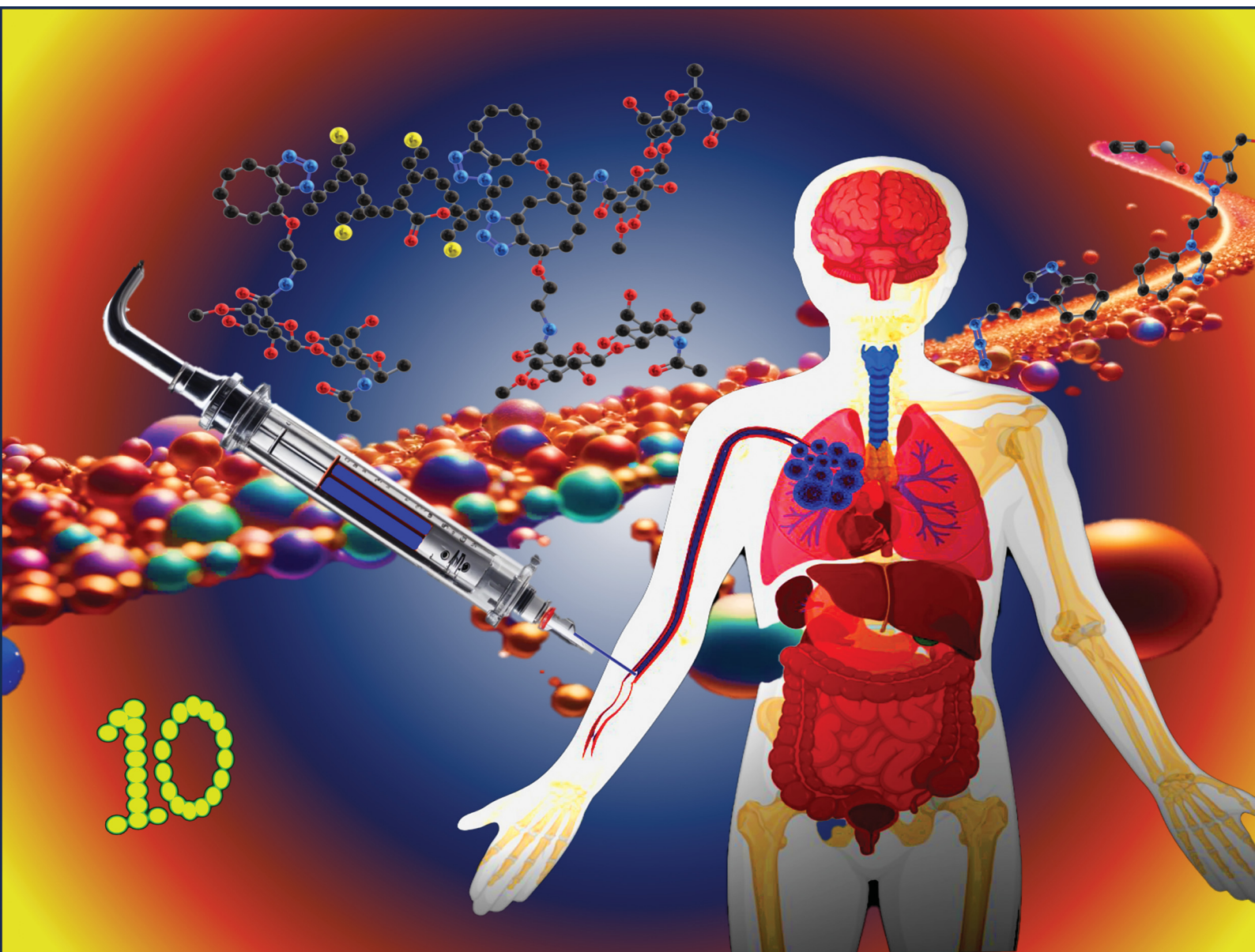


# Journal of Materials Chemistry B

Materials for biology and medicine

rsc.li/materials-b



ISSN 2050-750X

**PERSPECTIVE**

Monalisa Mukherjee *et al.*  
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Cite this: *J. Mater. Chem. B*, 2023,  
11, 10761

## Injectable organo-hydrogels influenced by click chemistry as a paramount stratagem in the conveyor belt of pharmaceutical revolution

Abhyavartin Selvam,<sup>ab</sup> Misba Majood,<sup>id</sup> †<sup>a</sup> Radhika Chaurasia, †<sup>a</sup> Rupesh,<sup>a</sup>  
Akanksha Singh,<sup>a</sup> Tapan Dey,<sup>a</sup> Omnarayan Agrawal,<sup>a</sup> Yogesh Kumar Verma<sup>c</sup> and  
Monalisa Mukherjee<sup>id</sup> \*<sup>a</sup>

The field of injectable hydrogels has demonstrated a paramount headway in the myriad of biomedical applications and paved a path toward clinical advancements. The innate superiority of hydrogels emerging from organic constitution has exhibited dominance in overcoming the bottlenecks associated with inorganic-based hydrogels in the biological milieu. Inorganic hydrogels demonstrate various disadvantages, including limited biocompatibility, degradability, a cumbersome synthesis process, high cost, and ecotoxicity. The excellent biocompatibility, eco-friendliness, and manufacturing convenience of organo-hydrogels have demonstrated to be promising in therapeutizing biomedical complexities with low toxicity and augmented bioavailability. This report manifests the realization of biomimetic organo-hydrogels with the development of bioresponsive and self-healing injectable organo-hydrogels in the emerging pharmaceutical revolution. Furthermore, the influence of click chemistry in this regime as a backbone in the pharmaceutical conveyor belt has been suggested to scale up production. Moreover, we propose an avant-garde design stratagem of developing a hyaluronic acid (HA)-based injectable organo-hydrogel *via* click chemistry to be realized for its pharmaceutical edge. Ultimately, injectable organo-hydrogels that materialize from academia or industry are required to follow the standard set of rules established by global governing bodies, which has been delineated to comprehend their marketability. Thence, this perspective narrates the development of injectable organo-hydrogels *via* click chemistry as a prospective elixir to have in the arsenal of pharmaceuticals.

Received 25th July 2023,  
Accepted 22nd September 2023

DOI: 10.1039/d3tb01674a

rsc.li/materials-b

### 1. Introduction

The development of injectable organo-hydrogels has marked an important stride in the clinical advancement of the field of materiobiology to act as an attribute in other domains such as regenerative medicine, therapeutics, and vehicles of therapies. Organo-hydrogels are unique biomimetic materials designed by either chemically or physically crosslinking polymeric frameworks derived from natural as well as synthetic sources. These hydrogels possess a highly hydrophilic nature due to their functional moieties leading to extensive water abundance, which complements their biomedical applicability along with

other paramount characteristics, *viz.*, porosity, softness, biocompatibility, flexibility, and permeability as well as absorptive activities.<sup>1,2</sup> These attributes contribute to their biomimeticity, which broadens the horizon of the pharmaceutical industry. Consequently, the strategy of hydrogel preparation modifies the innate characteristics of hydrogels, which thereby influences their clinical applicability in drug delivery, bioimaging, tissue engineering, cancer therapy, gene delivery, wound dressing/healing and so on.<sup>3–22</sup> Along these lines, physically engineered organo-hydrogels are developed by reversible physical interactions and their solubility can be tuned according to ambient conditions.<sup>4</sup> Contrastingly, chemically prepared gels are more stable in physiological environments due to covalent bonds formed by means of chemical reactions, such as Schiff base reactions, click chemistry, enzyme-mediation, Diels–Alder reactions, thiol exchange/disulfide crosslinking, and Michael-type addition.<sup>23,24</sup> Importantly, there are high chances of lixiviation of unreacted residual monomers post synthesis, and hence a further purification stage needs to be carried out.<sup>25</sup>

<sup>a</sup> Amity Institute of Click Chemistry Research and Studies, Amity University  
Uttar Pradesh, Noida, 201313, India. E-mail: mmukherjee@amity.edu<sup>b</sup> Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida,  
201313, India<sup>c</sup> Stem Cell & Tissue Engineering Research Group, Institute of Nuclear Medicine and  
Allied Sciences, Defence Research and Development Organisation, New Delhi,  
110054, India

† These authors have contributed equally.

Contemporarily, injectable hydrogels have demonstrated to be a promising strategy in lieu of pre-developed hydrogels as they not only possess the characteristics of archetypal hydrogels but can also be introduced into human beings with nominal invasiveness to target sites of treatment, even those of irregular morphologies.<sup>26</sup> Organo-hydrogels originating from organic constituents, *viz.*, cellulose, agar, chitosan, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyacrylamide (PAAm), *etc.*, and classified as natural or synthetic polymers are a highly biocompatible class of injectable hydrogels. Injectable organo-hydrogels can be conceptualized as simple liquid injections that gelate *in situ* to form a solid hydrogel when introduced into the human system.<sup>27</sup> As a result, they have been integrated in the delivery of small and bioactive molecules to administer inflammations, cancers, and other infection-prone conditions as well as scaffolds for reparation and regeneration of hard and soft tissues.<sup>28</sup> Recently, new additions have been introduced to the field of injectable hydrogels possessing self-healing, bio-responsive and shear-thinning features, which can be directly administered to patients in their gel phase.<sup>26,29–31</sup> Injectable organo-hydrogels should possess certain hallmarks, which include the capacity to undergo gelation quickly, allowing for easy transportation of the pre-gel or sol to ensure the appropriate injectability at the administration site. Additionally, the hydrogel should be able to fill voids or vacancies effectively and maintain stability.<sup>23</sup> Recently click-chemistry-based hydrogels have been gaining considerable interest due to their designability as per desire, versatility and simplicity of synthesis. Click chemistry refers to reactions that are selective and occur spontaneously with appreciably fast rates and high yields. Click-chemistry-based hydrogels are formed by spontaneous mixing of required compounds and can offer numerous functionalities to be used for biomedical as well as other applications. Such hydrogels can also encapsulate live cells and exhibit long-term viability.<sup>32</sup> Click-chemistry-based hydrogels have also demonstrated 3D printing properties and can produce 3D tissue constructs.<sup>33</sup> Thus, hydrogels bear tremendous potential to be used in regenerative medicine.

In a pool of contemporarily fascinating biomaterials, the rationale of this perspective manifests the clinical translation of biomimetic injectable organo-hydrogels with the development of bioresponsive and self-healing injectable organo-hydrogels in the emerging pharmaceutical revolution. In order to satisfy the ameliorating demand for novel drugs, we suggest click chemistry complimented by bio-orthogonal chemistry to lucratively influence upscaling of the production of clinically viable injectable organo-hydrogels. Likewise, we conceptualize an innovative design technique for developing a hyaluronic acid (HA)-based injectable hydrogel to be soon realised for its pharmaceutical precedence. Correspondingly, the marketability of injectable organo-hydrogels has been explained from the regulatory implications established by governing bodies such as the Food and Drug Administration (FDA) and European Medical Agency (EMA). Eventually, we summarize the potential outcomes of injectable organo-hydrogels as a pharmaceutical saviour in a cornucopia of medical events.

## 2. Clinical promise of injectable organo-hydrogels

The scope of clinical translation of injectable organo-hydrogels has attracted the attention of various researchers in academia and industry with a focus on pharmaceuticals and materio-biology. Thenceforth, exploration of this avenue has splurged over the years to improve the current strategies of treatments using advancing techniques of implementing hydrogels of organic composition in tissue regeneration, cancer therapy, and dental *vis-à-vis* maxillofacial remedies. Table 1 summarizes the benefits of implementing injectable organo-hydrogels in various biomedical applications.

## 3. Neoteric breakthroughs of organo-hydrogels having biomedical implications

In this section, nascent advances in injectable organo-hydrogels, which have provided new horizons in solving biomedical predicaments, have been highlighted to display the salience and pharmaceutical promise of this biomimetic system from the pool of archetypal pharmaceutical agents and biomaterials. Herein, we aim to promote the notion of bioresponsive and self-healing injectable organo-hydrogels targeted for drug delivery, wound healing, and other means of tissue engineering and regenerative medicine to be predominantly employed in the coming pharmaceutical revolution.

### 3.1 Bioresponsive organo-hydrogels

Bioresponsive organo-hydrogels or synonymously referred to as stimuli-responsive organo-hydrogels are one such kind of new-age injectable gels predominantly explored in delivery systems of small and bioactive molecules. The benefit of implementing bioresponsive organo-hydrogels is the effective drug loading and controlled release of imperative compounds to the human systems without delay. In this section, we highlight the remarkable results of injectable bioresponsive organo-hydrogels as delivery vehicles and their application in regenerative medicine.

**3.1.1 Delivery vehicles.** Injectable organo-hydrogels are being exploited as delivery vehicles for targeted delivery of drugs, proteins, genes, and other bioactive compounds and have been strongly established at the lab-scale. Extensive *in vivo* investigations validate the immaculate delivery performance of injectable organo-hydrogels derived from synthetic as well as natural constituents. Sustained drug delivery is of immense importance, especially in cases where a longer retention time of drugs is required, which in turn reduces the incidence of administering multiple maintenance doses of the same drug.<sup>60</sup> Continuing on the same path, a biodegradable and thermally-responsive lysozyme-loaded hydrogel composed of *N*-isopropylacrylamide (NIPAAm), methacrylate-poly(lactide) (MAPLA), and 2-hydroxyethyl methacrylate (HEMA) was fabricated as a means for protein delivery. When injected into the back of

Table 1 Clinical progress of injectable organo-hydrogels for various indications

Organo-hydrogel	Origin	Composition	Preparation	Mode of Injection	Indication	Clinical Status ( <a href="https://clinicaltrials.gov">https://clinicaltrials.gov</a> )	Ref.
EUFLExxA <sup>®</sup> (Ferring Pharmaceuticals Inc.)	Natural	Hyaluronic acid (HA)	Physical interaction	Intra-articular	OA	FDA (2003)	34
INFUSE <sup>®</sup> bone graft (Medtronic Sofamor Danek USA, Inc.)	Natural	Collagen and recombinant human bone morphogenetic protein-2	Physical interaction	Spinal	Spinal fusion, orthopaedic trauma along with spine and oral-maxillofacial surgeries	FDA (2002) for spinal fusion	35
Osteogenic protein 1 (OP-1 <sup>®</sup> ) implant, OP-1 <sup>®</sup> Putty (Stryker Biotech)	Natural	Collagen, carboxymethylcellulose, and recombinant OP-1	Physical interaction	Spinal	Posterolateral lumbar spinal fusion	FDA (2001)	36
TraceIT <sup>®</sup> Hydrogel Tissue Marker (Augmenix, Inc.)	Synthetic	Polyethylene glycol (PEG)	Chemical reaction	Percutaneous	To radiographically label soft tissue during or for subsequent surgical operations	FDA (2013)	37
Bulkamid <sup>®</sup> hydrogel (Searchlight Pharma)	Synthetic	Polyacrylamide	Chemical reaction	Transurethral	Stress urinary incontinence in females	FDA (2006)	38 and 39
Coaptite <sup>®</sup> (BioForm Medical, Inc.)	Synthetic	Calcium hydroxylapatite, sodium carboxymethylcellulose, glycerin	Physical interaction	Submucosal	Female stress urinary incontinence	EMA (2003)	40
Algisyl-LVR <sup>®</sup> Hydrogel Implant (LoneStar Heart, Inc.)	Natural	Alginate	Physical interaction	Percutaneous	Advanced heart failure	FDA (2005)	41–45
Aquamid (Henning Bliddal)	Synthetic	Polyacrylamide	Chemical reaction	Intra-articular	Knee OA	EMA (2001)	
PAAG-OA (Contura)	Synthetic	Polyacrylamide	Chemical reaction	Intra-articular	Knee OA	Clinical trials	NCT03060421 (NA)
Aquamid (A2 Reumatologi Og Idrettsmedicin)	Synthetic	Polyacrylamide	Chemical reaction	Intra-articular	Knee OA	Clinical trials	NCT04045431 (NA)
Hymovis Viscoelastic Hydrogel (Fidia Farmaceutici s.p.a.)	Natural	High molecular weight hyaluronan	Physical interaction	Intra-articular	Knee OA	Clinical trials	NCT03067090 (NA)
HYADD <sup>®</sup> 4 Hydrogel (Fidia Farmaceutici s.p.a.)	Natural	Non-crosslinked hyaluronic acid alkylamide	Physical interaction	Intra-articular	OA	Clinical trials (Phase III)	NCT01372475 (NA)
Promedon	Natural	Hydroxyethyl cellulose	Physical interaction	Knee	OA	Clinical trials	NCT02187549 (NA)
Naofumi Takehara	Natural	Gelatin with basic fibroblast growth factor	n/d	Intra-myocardial	Ischemic cardiomyopathy	Clinical trials	NCT04061733 (NA)
VentriGel (Ventrix, Inc.)	Natural	Native myocardial extracellular matrix	Physical interaction	Trans-endocardially	Myocardial infarction	Clinical trials (phase I)	NCT00981006 (NA)
Neo-kidney augment (inRegen)	Natural	Gelatin with selected renal cells	Chemical reaction	Kidneys	Type 2 diabetes and chronic kidney disease	Clinical trials	NCT02525263 (NA)
Renal autologous cell therapy (inRegen)	Natural	Gelatin with renal autologous cells	Chemical reaction	Renal cortex	Chronic kidney disease from congenital anomalies of the kidneys and urinary tract	Clinical trials (phase I)	NCT04115345 (NA)
GelStix <sup>®</sup> Nucleus augmentation device (Dr med. Paolo Maino Viceprimario Anestesiologia)	Synthetic	Polyacrylonitrile	Chemical reaction	Intra-discal	DDD	Clinical trials	NCT02763956 (NA)
The Second Affiliated Hospital of Chongqing Medical University	Natural	Unknown/human amniotic epithelial cells	n/d	Uterine cavity	Asherman's syndrome	Clinical trials (phase I)	NCT03223454 (NA)
Vantas <sup>®</sup> (Endo Pharmaceuticals)	Synthetic	Histrelin acetate, poly(2-hydroxyethyl methacrylate), poly(2-hydroxypropyl methacrylate) and gonadotropin releasing hormone	Chemical reaction	Subcutaneous	Palliative treatment of prostate cancer	FDA (2004)	46

Table 1 (continued)

Organo-hydrogel	Origin	Composition	Preparation	Mode of Injection	Indication	Clinical Status ( <a href="https://clinicaltrials.gov">https://clinicaltrials.gov</a> )	Ref.
EMA (2005) SpaceOAR <sup>®</sup> Hydrogel (Augmenix, Inc.)	Synthetic	PEG	Chemical reaction	Percutaneous	To shield exposed tissues during prostate cancer radiotherapy	FDA (2015) EMA (2010)	47 and 48
Memorial Sloan Kettering Cancer Center	Synthetic	PEG	Chemical reaction	Visceral pleura	Lung biopsy	Clinical trials (phase III)	NCT02224924
Augmenix, Inc.	Synthetic	PEG/SpaceOAR <sup>®</sup>	Chemical reaction	Between the rectum and prostate	Spacing in radiation therapy for prostate cancer	Clinical trials (phase III)	NCT01538628
University of Washington	Synthetic	PEG/TraceIT <sup>®</sup>	Chemical reaction	Near the circumference of tumour bed	Imaging of bladder carcinoma	Clinical trials	NCT03125226
Absorbable Radiopaque Hydrogel Spacer (Thomas Zilli, University Hospital, Geneva)	Synthetic	PEG/TraceIT <sup>®</sup>	Chemical reaction	Between the prostate/vagina and the rectum	Spacing in radiation therapy for rectal cancer	Clinical trials	NCT03258541
Royal North Shore Hospital	Synthetic	PEG/SpaceOAR <sup>®</sup>	Chemical reaction	Between the rectum and prostate	Spacing in radiation therapy for prostate cancer	Clinical trials	NCT02212548
Absorbable Radiopaque Tissue Marker (Washington University School of Medicine)	Synthetic	PEG/TraceIT <sup>®</sup>	Chemical reaction	Resection bed	Imaging of oropharyngeal cancer	Clinical trials (phase I)	NCT03713021
Absorbable Radiopaque Tissue Marker (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins)	Synthetic	PEG/TraceIT <sup>®</sup>	Chemical reaction	Between the pancreas and duodenum	Imaging of pancreatic adenocarcinoma	Clinical trials	NCT03307564
Gut Guarding Gel (National Cheng-Kung University Hospital)	Natural	Sodium alginate/calcium lactate	Physical interaction	Submucosal	Gastroenterological tumor and polyps	Clinical trials	NCT03321396
Radiesse <sup>®</sup> (Bioform Medical, Inc.)	Synthetic	Hydroxylapatite, carboxymethylcellulose	Physical interaction	Dermis	Abet with the correction of facial folds and wrinkles	FDA (2006) EMA (2004)	49
Radiesse <sup>®</sup> (+) (Merz Pharmaceuticals)	Synthetic	Hydroxylapatite, carboxymethylcellulose with lidocaine	Physical interaction	Dermis	For the purpose of stimulating natural collagen production along with treating wrinkles and facial folds	FDA (2015)	50
BellaFill <sup>®</sup> (Suneva Medical, Inc.)	Synthetic	Polymethylmethacrylate beads, collagen and lidocaine	Physical interaction	Dermis	Facial wrinkles and folds	FDA (2006)	51
Sculptra <sup>®</sup> (Sanoofi Aventis U.S.)	Synthetic	Poly-L-lactic acid	Physical interaction	Dermis	For the purpose of correcting facial fat and shallow to deep nasolabial contour deficiencies in addition to treating wrinkles	FDA (2004) EMA (2000)	52
Fibrel <sup>®</sup> (Serono Laboratories)	Natural	Collagen	Physical interaction	Dermis	Correction of depressed cutaneous scars	FDA (1988)	53
Zyplast(R) <sup>®</sup> and Zyderm(R) <sup>®</sup> (Inamed Corporation/Allergan, Inc.)	Natural	Bovine collagen	Chemical reaction	Dermis	Correction of contour deficiencies	FDA & EMA (1981)	54
Restylane <sup>®</sup> Lyft, Restylane <sup>®</sup> Refyne, Restylane <sup>®</sup> Defyne (Galderma Laboratories, L.P.)	Natural	Hyaluronic acid with lidocaine	Chemical reaction	Subcutaneous, dermis, lips	For treating conditions such as volume deficit, facial folds, wrinkles, midface contour deficiencies and perioral rhytids	FDA (2012) EMA (2010)	55

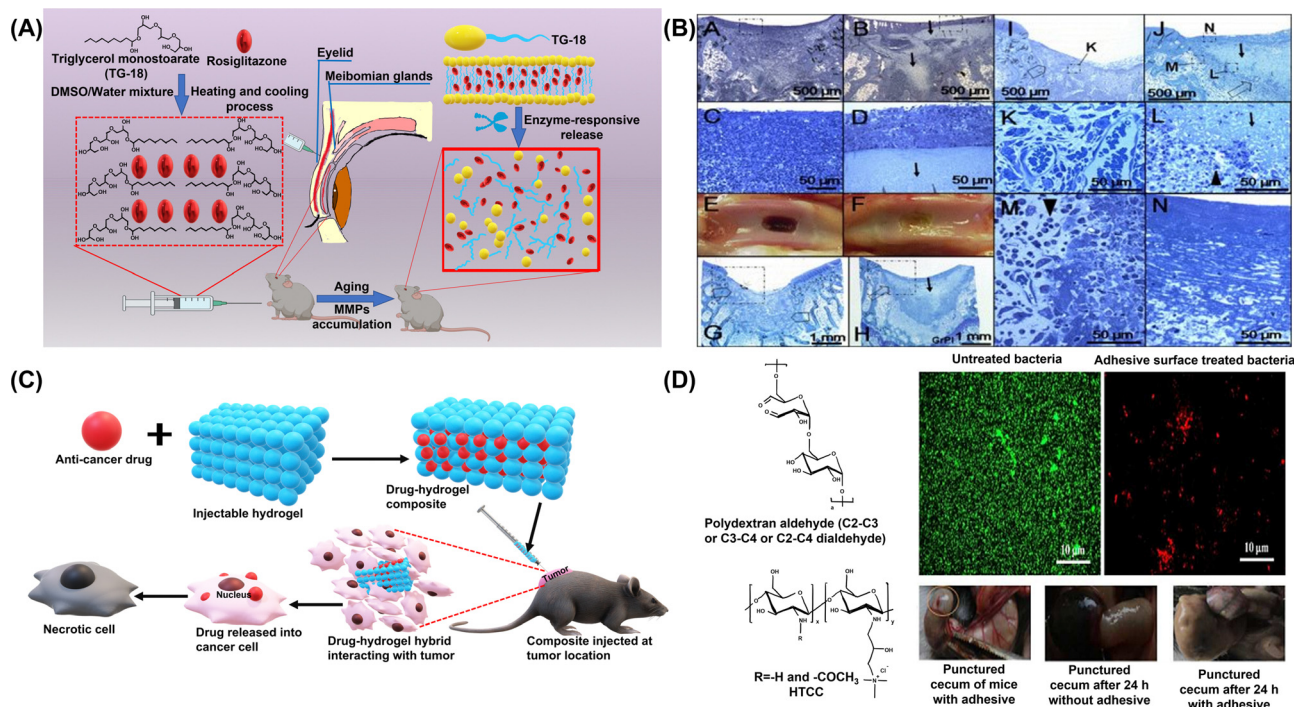
Table 1 (continued)

Organo-hydrogel	Origin	Composition	Preparation	Mode of Injection	Indication	Clinical Status ( <a href="https://clinicaltrials.gov">https://clinicaltrials.gov</a> )	Ref.
Belotero balance <sup>®</sup> (+) Lidocaine (Merz Pharmaceuticals) Revanesse <sup>®</sup> Versa+	Natural	Hyaluronic acid with lidocaine	Chemical reaction	Dermis	Moderate to severe facial wrinkles and folds	FDA (2019)	56
Hylaform <sup>®</sup> (Hylan B gel), Captique Injectable Gel, Prevelle Silk (Genzyme Biosurgery)	Natural	Hyaluronic acid with lidocaine	Chemical reaction	Dermis	Moderate to severe facial wrinkles and creases	FDA (2018)	57
Teosyal <sup>®</sup> RHA (Teoxane SA)	Natural	Modified hyaluronic acid derived from a bird (avian) source	Chemical reaction	Dermis	Correction of moderate to severe facial wrinkles and folds	FDA (2004) EMA (1995)	58
n/d – not defined.	Natural	Hyaluronic acid	Chemical reaction	Dermis	Facial wrinkles and folds	FDA (2017) EMA (2015)	59

Sprague-Dawley rats, these systems underwent sol-to-gel transition, following which they released lysozyme in a sustained manner, thus avoiding the initial burst effect, which is quite common among conventional drug delivery systems. Moreover, these systems were also found to be non-toxic to fibroblasts, even at higher concentrations.<sup>61</sup> Furthermore, to expedite the rate of diabetic wound healing, Lee *et al.* synthesised a hydrogel through the crosslinking of PEF-PLGA-PEG which was further utilised for the delivery of TGF- $\beta$  1 plasmid. In addition to providing sustained delivery of the plasmid, the hydrogel scaffold also exhibited the ability to accelerate the process of re-epithelialization, promote more organised extracellular matrix deposition and cell proliferation, and stimulate collagen production in a diabetic mouse model.<sup>62</sup> A silk-elastin like protein polymer (SELP) hydrogel was synthesised as a carrier to deliver plasmid gene and the pRL-CMV vector released the plasmid effectively in a size dependent manner. In comparison to naked DNA, delivery of pRL-CMV from a SELP hydrogel significantly ameliorated transfection in a murine model of human breast cancer by 1–3 orders of magnitude. The concentration of the polymer in the hydrogel was found to be correlated with the release of a bioactive adenoviral vector.<sup>63</sup>

An intricately engineered bioresponsive hydrogel scaffold composing triglycerol monostearate was loaded with Rosiglitazone (Rosi-hydrogel), as shown in Fig. 1(A).<sup>64</sup> It was observed that this hydrogel system could treat age-related meibomian gland dysfunction (ARGMD) in a mouse model, thus proving as an effective treatment to alleviate inflammation of the delicate eyelids, thus indicating its potential as a controlled release drug delivery system. Lee *et al.* synthesized a thermosensitive elastin-based polypeptide (EBP) and ligand-responsive calmodulin (CaM) injectable hydrogel as EBP-CaM-EBP (ECE) triblock copolypeptides for the purpose of controlled drug delivery. In similar circumstances, concentrated ECE triblock copolypeptides showed thermally induced gelation, leading to physically crosslinked hydrogels. Depending on the conformational change of the CaM middle block brought about by binding either Ca<sup>2+</sup> or Ca<sup>2+</sup> and trifluoperazines (TFPs) as ligands, they displayed controlled rheological and mechanical properties.<sup>65</sup> Turabee *et al.* aimed at delivering protein molecules without deteriorating their inherent bioactivity through fabrication of a temperature- and pH-responsive bioresorbable injectable hydrogel system composed of polypeptide block copolymers, namely polyethylene glycol (PEG), temperature-responsive poly( $\gamma$ -benzyl-L-glutamate) (PBLG), and pH-responsive oligo(sulfamethazine) (OSM), for the sustained delivery of proteins *in vivo*. The anionic properties of the scaffold allowed for the efficient loading of the model protein-lysozyme and when injected into the Sprague-Dawley rats subcutaneously, it was observed that the lysozyme loaded block copolymer formed a viscoelastic gel *in vivo* which could deliver the model protein in a sustained manner for at least a week.<sup>66</sup>

As a long-term controlled release system, injectable hydrogels exhibit exceptional capacity to encapsulate drugs and biomolecules. For instance, injectable dopamine-based polydopamine hydrogels were fabricated through the crosslinking



**Fig. 1** (A) Schematic representation of hydrogel formation and Rosi release for treating ARMGD. Reproduced from ref. 64. Copyright 2021, Elsevier. (B) *In vivo* delivery, retention of chitosan gel, and inflammatory response in example ~3 mm deep osteochondral defects in 4-month-old NZW rabbits. Osteochondral holes 3 mm in diameter and ~3 mm deep were drilled into the femoral-patellar groove (A–N) with one control defect allowed to bleed (A, C, E, G, I, K) and the other defect loaded with chitosan gel (B, D, F, H, J, L–N). After 1 day (A–D) or 8 days (E–N) of normal load-bearing, the joints were recovered and processed for histology. Reproduced from ref. 69. Copyright 2005, Elsevier. (C) Application of injectable organo-hydrogels in delivery of anti-cancer drugs. (D) Schematic illustration and *in vivo* wound healing activity of the injectable HTCC organo-hydrogel. Reproduced with permission.<sup>70</sup> Copyright 2017, American Chemical Society.

of a mixture of quaternised chitosan, gelatin and dopamine. These hydrogels have enabled sustained and effective treatment for Parkinson's disease. The release profiles of the drug loaded hydrogel scaffolds indicated their suitability as potential drug carriers for the enduring management of Parkinson's disease.<sup>67</sup> Zubik *et al.* focussed on assembling thermo-responsive hydrogels composed of poly(*N*-isopropylacrylamide) (PNIPAAm) braced up with cellulose nanocrystals through covalent or non-covalent linkages. To test their application as drug-delivery vehicles, metronidazole, a drug used in wound dressing, was loaded into the system and it could be corroborated that the hydrogel scaffold had efficient drug-delivering capability as it exhibited an initial burst release of the drug at the beginning, following which the drug release occurred in a gradual and sustained manner at 37 °C.<sup>68</sup> Furthermore, the loading and targeted *in vivo* delivery of anti-cancer compounds is schematically described in Fig. 1(C).

**3.1.2 Regenerative medicine.** In order to overcome palliative measures, materiobiologists have found it imperative to develop novel strategies for managing organ damage with successful transplantation or replacement. Many issues like those of graft rejection make it quite difficult for such surgeries to become successful and thus more efficient substitutes are of pressing need. For instance, an injectable temperature-responsive chitosan-β-glycerophosphate-hydroxyethyl cellulose (CH-GP-HEC) hydrogel was synthesized which was further encapsulated

with mesenchymal cells (MSCs) to resolve defects related to cartilage tissue with minimal pain and invasion. When investigated for regenerative potential, it was found that the hydrogel scaffolds provided a suitable and optimum environment for human MSCs to differentiate.<sup>71</sup> In another study, cartilage tissue engineering was effectuated using hydrogels such as PuraMatrix, alginate or atelopeptide collagen embedded with human auricular chondrocytes. When investigated for regenerative medicine, it was found that the hydrogel scaffold system could deliver the human auricular chondrocytes quite effectively.<sup>72</sup> Moving further, an experimental design focussed on the fabrication of a chitosan-based hydrogel for the purpose of cartilage formation *in vivo* as well as *in vitro*. For the same, the newly synthesized hydrogel system was loaded with primary articular chondrocytes. It was found that, in such a manner, the hydrogel scaffold could treat chondral defects effectively, when tested in a rabbit chondral defect model while also leading to the accumulation of the cartilage matrix *via* chondrocytes, as presented in Fig. 1(B).<sup>69</sup> Another experimental design focussed on hyaline cartilage repair in ovine microfracture defects by assembling and injecting chitosan-glycerol/blood implants. Upon histological examination of sheep, it was found that at 6 months the best hyaline tissue repair was associated with the implant while also showing aggravated levels of collagen and cells in the defects treated with our scaffold.<sup>73</sup> Furthermore, an injectable hydrogel system was fabricated

using an antibacterial polymer, *N*-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC), and a bioadhesive polymer, polydextran aldehyde, as shown in Fig. 1(D). When tested for its wound healing activity in the treatment group of Wistar rats, the adhesive hydrogel could treat the wounds effectively, especially after days 15 and 20.<sup>70</sup>

### 3.2 Self-healing organo-hydrogels

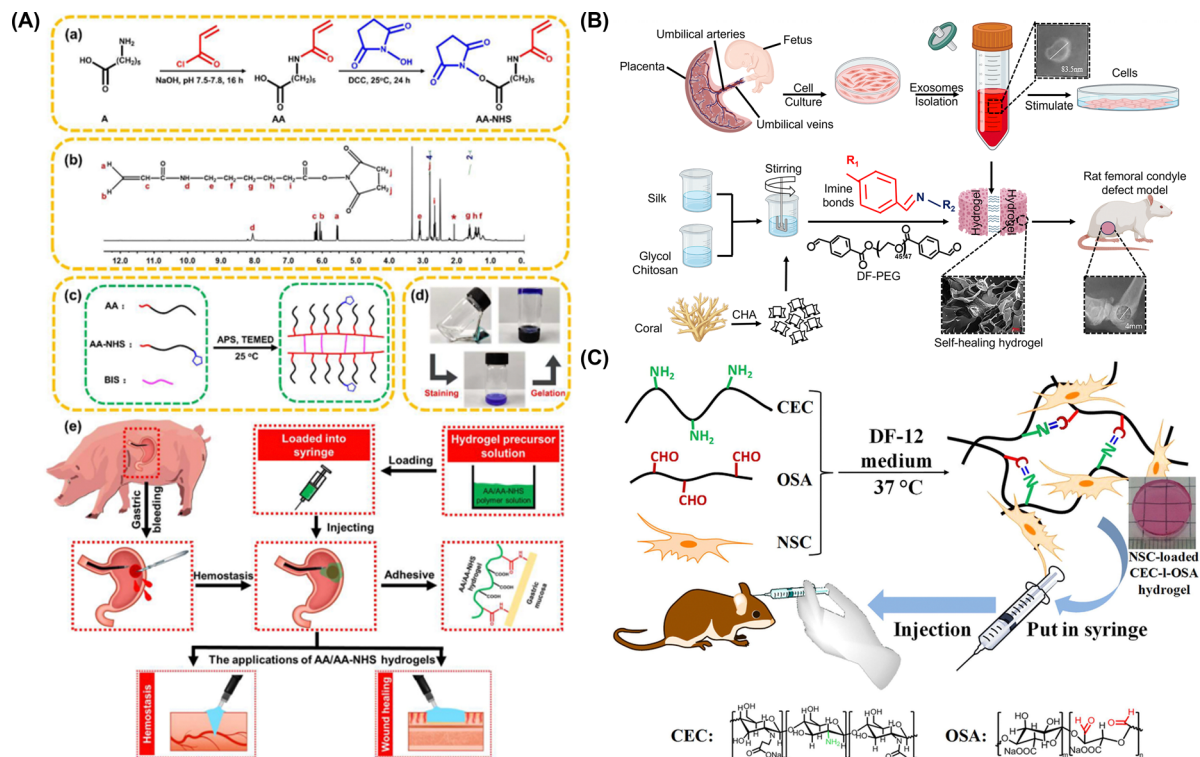
Interestingly, in the wide arena of hydrogels, self-healing hydrogel systems emerged as scaffolds, capable of restoring the morphological traits and mechanical traits following recurrent damage.<sup>74</sup> The superior durability and stability of self-healing hydrogels allow them to reform their shape, injectability, and stretchability, thus acceding them to repair themselves autonomously when ruptured or traumatised.<sup>75</sup> Self-healing hydrogels regenerate spontaneously after mechanical damage *via* dynamic covalent cross-linkages and covalent and non-covalent interactions.<sup>76</sup> The hydrogel heals damages and reforms shapes due to the dynamic equilibrium between dissociation and recombination of various interactions. Dynamic covalent bonds typically have stable and slow dynamic equilibria, whereas non-covalent interactions have fragile and fast dynamic equilibria.<sup>77</sup> In recent years, the dynamic properties of injectable organo-hydrogel systems have intrigued researchers to broaden their horizons in terms of establishing these scaffolds as those with superior and more effective biomedical applications such as wound healing,<sup>78</sup> tissue repair and engineering,<sup>30</sup> 3D printing,<sup>79</sup> and as drug delivery vehicles.<sup>80</sup>

**3.2.1 Self-healing hydrogels for wound management.** The scope of injectable organo-hydrogels has been established to treat various kinds of wounds and multiple studies have shown how these systems can serve as better alternatives to conventional wound-healing treatments. For instance, an injectable, self-healing and antibacterial polypeptide-based FHE hydrogel (F127/OHA-EPL) was fabricated to release exosomes derived from adipose-derived mesenchymal stem cells (AMSCs-exo) upon responding to stimuli so as to facilitate synergistic healing of chronic wounds and skin regeneration. In *in vivo* culture, it was found that the multifunctional organo-hydrogel so formed effectuated faster wound closure rates, re-epithelialisation and angiogenesis along with redeposition of collagen on the site of diabetic wounds, as compared to the FHE hydrogel and exosomes alone.<sup>84</sup> H. Xuan in another study synthesised an avant-garde injectable hydrogel system which was forged using Schiff's base reaction to link carboxymethyl chitosan and aldehyde functionalised sodium alginate along with the introduction of short nanofibers of carboxymethyl-functionalized polymethyl methacrylate (PMAA). The excellent self-healing activity of the system was attributed to the nanofibers. Further, when tested in rats, the synthesised hydrogel scaffolds displayed a faster rate of wound closure along with effective re-epithelialization and angiogenesis was observed which facilitated in accelerated wound closure rates.<sup>85</sup> Furthermore, another study involved the fabrication of adhesive haemostatic antioxidant conductive photothermal antibacterial injectable nanocomposite hydrogel composed of a graft of hyaluronic acid and dopamine reduced graphene oxide for the purpose of

wound dressing. The results showed that the synthesised hydrogel system surpassed the human skin in terms of mechanical properties while also augmenting the vascularization and collagen deposition, and improving the granulation tissue thickness, all of which contributed to the escalated rates of wound closure in rats. Further, their adhesive nature also contributed to a longer stay time over the wounds.<sup>86</sup> Another study involved the modelling of injectable self-healing hydrogels for maintaining gastric homeostasis and promotion of wound healing. This hydrogel system was endowed with adhesive and pH-responsive qualities too. The hydrogel was composed of acryloyl-6-aminocaproic acid (AA) and AA-*g*-*N*-hydroxysuccinimide (AA-NHS) which enhanced the adhesive properties of the hydrogel, which is presented in Fig. 2(A). In addition, this hydrogel exhibited excellent self-healing and homeostatic properties, while from the results obtained in a gastric wound model, it could be concluded that this hydrogel scaffold effectuated enhanced gastric wound healing properties through deposition of type 1 collagen, revascularization and angiogenesis as well, which are crucial for proper wound closure.<sup>81</sup> Interestingly, another self-healing, injectable, platelet-rich plasma hydrogel was assembled based on chitosan, silk fibroin, and PRP (CBPGCTS-SF@PRP) for the purpose of treating diabetic wounds. The hydrogel showed promising results as it successfully led to the chemotactic migration of mesenchymal cells, protected PRP from rigorous enzyme hydrolysis and promoted cell proliferation, and when tested in a type 2 diabetic rat model, it effectuated heightened levels of collagen deposition, nerve repair and augmented rates of angiogenesis, thus proving to be a promising candidate in the nexus of wound healing.<sup>87</sup>

**3.2.2 Self-healing hydrogels for tissue regeneration.** Tissue regeneration is a complex process that involves re-epithelialization, cell proliferation and extensive tissue repair. One of the most persistent problems regarding conventional regenerative medicine is immune rejection.<sup>88</sup> To tackle this issue and other therapy related side effects as well as serious repercussions, injectable organo-hydrogels can be considered as a promising avenue for successful regeneration of the tissue. Various studies have been conducted to establish these scaffolds as successful candidates for tissue regeneration. For instance, to aid effective bone regeneration, a self-healing hydrogel composed of a human umbilical cord mesenchymal stem cell (hucMSC)-derived exosome carrier was fabricated, as depicted in Fig. 2(B). The results indicated that these scaffolds effectuated differentiation while also promoting the proliferation and migration of human umbilical vein endothelial cells. These results could be confirmed after the hydrogel system could cure a bone defect in a rat model while also exhibiting good biocompatibility.<sup>82</sup> In another study, injectable conductive self-healing hydrogels were assembled by carrying out a Schiff's base reaction between dextran-*graft*-aniline tetramer-*graft*-4-formylbenzoic acid and *N*-carboxyethyl chitosan as carriers for myoblast cells, which were further used for muscle regeneration. The results indicated that the synthesised hydrogel scaffolds had good biocompatibility while also leading to muscle regeneration by effectuating successful release of myoblasts from the hydrogel





**Fig. 2** (A) Synthesis of AA and AA-NHS ester copolymers (a),  $^1\text{H}$  NMR spectrum of AA-NHS (b), preparation and network of the AA/AA-NHS hydrogel (c), optical images of AA/AA-NHS hydrogel polymer solution pre- and post-crosslinking and subsequent staining of solution with crystal violet (d), and gastric haemostasis and wound healing activities of AA/AA-NHS hydrogels (e). Reproduced from ref. 81. Copyright 2020, Springer Nature. (B) Schematic depiction of isolation and characterization of hucMSC-derived exosomes and creation of the CHA/SF/GCS/DF-PEG organo-hydrogel to be injected in Sprague-Dawley (SD) rats with induced femoral condyle defects. Reproduced from ref. 82. Copyright 2020, Frontiers. (C) Synthesis and transplantation strategy of injectable NSC-loaded, self-healing CEC-I-OSA hydrogels. Reproduced from ref. 83. Copyright 2016, Springer Nature.

system into the tissue microenvironment.<sup>89</sup> In another study, self-healing hydrogels were synthesised using adamantine- and cyclodextrin-modified HA, encapsulated with endothelial progenitor cells (EPCs) which was then injected into the ischemic myocardium. The results were observed in a rodent model of acute myocardial infarction and it was found that an increase in vasculogenesis was observed compared to treatment with EPCs or the hydrogel alone.<sup>90,91</sup>

**3.2.3 Delivery vehicles.** Since conventional systems of drug delivery have a myriad of drawbacks and repercussions associated with them, the organ-hydrogel scaffold system can be considered and established as a substitute to deliver drugs and biomolecules more effectively inside the body. For instance, chitosan-PEG based hydrogels were synthesized with the aim of making them anti-inflammatory, anti-bacterial and applicable in drug delivery and wound healing of ulcers.<sup>92</sup> An experimental study reported the fabrication of an injectable self-healing hydrogel composed of *N*-carboxyethyl chitosan and oxidised sodium alginate for the delivery of neural stem cells, as illustrated in Fig. 2(C). With a marked expression of the neuronal markers nestin and b-III tubulin but not the glial marker GFAP, the hydrogel supported the homing, proliferation (up to 5 days), and differentiation of the encapsulated NSCs, suggesting the promotion of NSC differentiation into neurons rather than glial cells. One week after injection,

NSC-loaded hydrogels completely filled the lesion site with cells evenly distributed throughout the hydrogel in mice with focal cerebral ischemia.<sup>83</sup> Similarly, the delivery of optogenetics (genetically targeted neurons expressing light-responsive opsin molecules) was realised by co-injecting bacteriorhodopsin (HEBR) plasmid in conjugation with a chitosan-based self-healing hydrogel with strong shear-thinning properties as a potential temporal-spatial remedy against neurodegeneration.<sup>93</sup> Furthermore, an organo-hydrogel based on a 4-arm star PEG functionalized with vinyl sulfone and short dithiol crosslinkers was created to resemble cartilage. Chondrocytes were introduced into osteochondral defects in immunodeficient mice using the hydrogel. Following 12 weeks, there was an aggravation in proliferation, chondrogenic differentiation, and ECM deposition. The coalescence of hyaline cartilage with the surrounding cartilage allowed for cartilage repair.<sup>94</sup>

## 4. Pharmaceutical expansion of injectable organo-hydrogels complimented by click chemistry

Click chemistry is a novel approach gaining interest owing to its benefits including one-step reactions that are rapid, simple to perform, regiospecific involving benign solvents.<sup>95</sup> These reactions

are biorthogonal, as the azide moiety is absent in nature, and hence do not react with natural biomolecules.<sup>96</sup> This section conceptualizes click chemistry to act as a backbone in the conveyor belt in mass production of injectable organo-hydrogels for the new-age pharmaceutical revolution. Along these lines, the current development of bio-orthogonal chemistry in this regime has also been discussed. Ultimately, we propose an avant-garde design stratagem to create a hyaluronic acid (HA)-based injectable hydrogel *via* click chemistry to be exploited for its biomedical advantage.

#### 4.1 Biomedical applications of the organo-hydrogel developed *via* click chemistry

Click chemistry provides the much-required multifunctional hydrogel materials, which are interesting biomaterials for tissue engineering and bioprinting ink applications owing to their excellent ability to form hydrogels with printability instantly and to retain live cells in their 3D network without losing the mechanical integrity even when swollen. Cu(I)-catalyzed processes for hydrogel synthesis were quite popular and are popularly referred to as click chemistry. This was the case for all the hydrogels that were investigated.<sup>97</sup> SP-AAC click chemistry was used to create a hydrogel that was generated from dextran that had been modified with azide and cyclooctyne components. To evaluate the efficacy of this injectable hydrogel for cartilage tissue engineering, chondrocyte cells were included both singly and in the form of spheroids. Spheroids, on the other hand, led to a greater formation of chondrocyte extracellular matrices than did individual cells.<sup>98</sup> A biocompatible, *in situ* cross-linking HA-based injectable hydrogel was developed by Han *et al.* for cartilage regeneration. The gel was formed by modifying HA with 4-arm PEG azide and dibenzyl cyclooctyne independently to produce the desired level of high cross-linking, which was accomplished using the SP-AAC mechanism. The gel was produced because of the reaction that occurred between the azide and dibenzyl cyclooctyne groups during the copper-free click reaction. *In vitro* testing was conducted using a variety of different compositions to evaluate the varied necessary qualities of the injectable hydrogel. They administered the hydrogel subcutaneously to mice and monitored the change in the volume of the gel across a variety of time intervals. After performing *in vivo* tests on mice to regenerate cartilage, they observed the removal of white solid tissue-like hydrogels from the animals. This hydrogel technology has potential for the regeneration of cartilage *in vivo*, showing positive outcomes.<sup>99</sup> Ethylenediaminetetraacetic acid (EDTA) is the chemical used to remove copper ions from hydrogels. However, it may be challenging to employ the same procedure in the context of injectable hydrogels for regenerative medicine or targeted drug administration.<sup>100</sup> Even though scaffold-free approaches are being researched for tissue regeneration, injectable *in situ* hydrogels, with or without live cells, growth factors, biomolecules, nanoparticles, or microspheres, are promising candidates for tissue engineering applications with improved mechanical properties and biocompatibility.<sup>101</sup> In general, click-chemistry-based hydrogels for tissue engineering

applications are promising and have been extensively explored all over the world in recent years for a variety of biomedical applications. These applications include 3D bioprinting, drug delivery, diagnosis, tissue engineering, and regenerative medicine, among other things. Experiments should be designed to create innovative biomaterials that are suitable for certain applications, taking into consideration the numerous benefits and drawbacks associated with the many different synthesis pathways. For instance, it has been shown that thiol-based hydrogels with residual initiators might generate a slight immunogenic response at the cellular level. For this reason, extreme caution should be exercised during the hydrogel synthesis process to ensure that any potentially harmful catalysts or initiators are eliminated. Alternatively, time- and cost-saving purification methods should be utilised to obtain higher-quality products by means of click chemistry reactions. Combining the benefits obtained from various click chemistry methods or its main biomaterials to generate multifunctional and biocompatible biomaterials is essential for the development of such hydrogels with superior qualities. This is one of the most critical criteria. At the same time, the reactions should not disrupt or interfere with other processes, and it should not have any unintended side effects or cross reactions with other unanticipated reaction groups. Recently, new technologies have been proposed, such as the surface modification of living cells with ligands for crosslinking with biocompatible hydrogels. This is just one example of the tremendous expansion that is occurring in this field. Fig. 3 describes the various approaches of click reactions in the preparation of injectable organo-hydrogels and their corresponding biomedical applications.

#### 4.2 Biomedical applications of bio-orthogonally derived injectable organo-hydrogels

In order to characterise the reactions that occur in the presence of macromolecules such as proteins or living cells, *etc.*, many words have been utilised. Some of these phrases are bio-orthogonal and bio-click. The fact that bio-orthogonal approaches often do not interfere with other regular biochemical processes is the primary benefit of utilising these approaches.<sup>105</sup> Using bio-orthogonal click cross-linking processes of azide-modified mammalian cells with alkyne-modified biocompatible polymers, another group generated cell cross-linked live bulk hydrogels (bAlg-DBCO). The purpose of the research was to provide evidence that the covalent coupling of live cells, which acted as active cross-linking sites, paves the way to produce multifunctional hydrogels that possess one-of-a-kind functions that are derived from the cells themselves, as presented in Fig. 4(D). To show the usefulness of their methodology, they chose to focus on two fundamental biological functions: autonomous cell growth and selective cell adhesion. As a result of the autonomous development of cells that are used as active cross-linking sites, CxGels have the potential to both self-grow and self-degrade, and this effectively demonstrates that these are distinctive qualities of CxGels. Furthermore, the capacity of cells incorporated into CxGels to preferentially stick to surfaces is the source of the selective adhesion shown by CxGels.

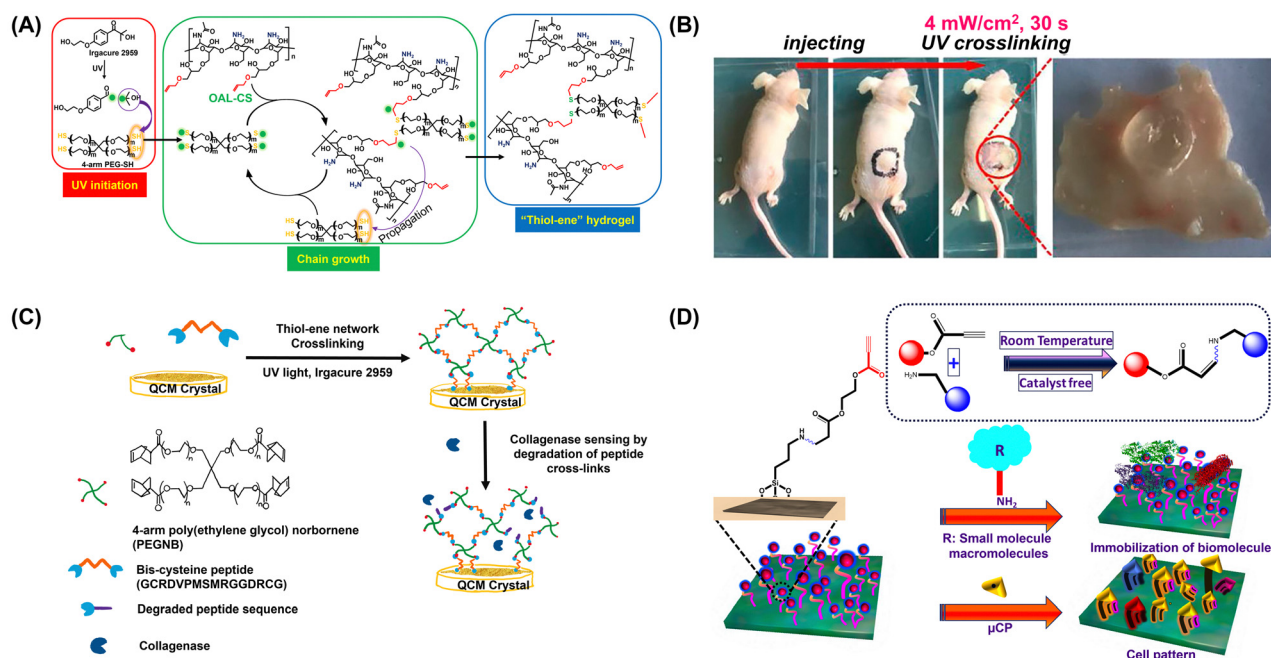


Fig. 3 (A) Click reaction mechanism of organo-hydrogel preparation and (B) *in situ* cross-linking of the hydrogel in the mouse model within 30 s. Reproduced with permission.<sup>102</sup> Copyright 2020, Elsevier. (C) Click chemistry preparation of the collagenase sensing organo-hydrogel. Reproduced from ref. 103. Copyright 2019, MDPI. (D) Schematic depiction of quick surface immobilization of native bioconjugates *via* spontaneous click chemistry. Adapted from ref. 104.

Therefore, they hypothesise that the selective adhesive ability of CxGels is unparalleled in comparison to that of hydrogels currently on the market. Together, they have successfully demonstrated that the functions of cells that have been covalently incorporated into CxGels as active cross-linking points are helpful for endowing CxGels with unique functionalities.

The 'click' reaction of CuAAC was utilised by Li and her colleagues in order to successfully build novel thermo-responsive polyurethane–poly(ethylene glycol) (PU–PEG) hydrogels. These hydrogels were based on azido-pendent PU–PEG and dialkynyl PEG (DAKPEG). The PU–PEG hydrogels were equipped with a number of exceptional qualities, such as thermo-responsivity, remarkable mechanical properties, and excellent bio-orthogonality, and because of this they were considered to be a promising choice for the fabrication of injectable hydrogels.<sup>106</sup> The production of bio-orthogonal hydrogels based on PEG will likely play a significant role in the field of future biomedicine. Bio-orthogonal PEG hydrogels were produced by Hodgson and colleagues using the SPAAC 'click' reaction. The synthesis was based on aza-dibenzocyclooctyne (DIBAC) and azide ended polymers. The gelation duration could be adjusted from 10 to 60 seconds at room temperature, and the Young modulus of each hydrogel could range from 1 to 18 kPa by adjusting the ratio and concentration of the two precursors. Both parameters were governed by the gelation time. After that, the PEG hydrogel exhibited remarkable biocompatibility.<sup>107</sup> A new thermosensitive and dual-crosslinking furyl-modified hydroxypropyl chitin polymer was produced by Bi *et al.* and it was used to fabricate injectable

hydrogels. The component that was responsible for temperature sensitivity was hydroxypropyl chitin, and the component that was responsible for chemical crosslinking was a Diels–Alder 'click' reaction that took place between maleimide-terminated PEG and furyl-modified hydroxypropyl chitin polymer. The injectable hydrogels possessed high bio-orthogonality and outstanding biocompatibility. The encapsulated cells exhibited sustained proliferation and a remarkable capacity for producing multicellular spheroids within the injectable hydrogels. The authors believed there was a significant amount of scope for the application of hydrogels in tissue engineering.<sup>108</sup>

On the basis of bio-orthogonal chemistry, the Yang group conceived and created innovative injectable bio-orthogonal dendrimer hydrogels.<sup>109</sup> The platform provides a great degree of capability and is also quite modular, as depicted in Fig. 4(C). To fine-tune the chemical and physical properties of dendrimer hydrogels, it is possible to readily manipulate a wide variety of structural parameters, such as the dendrimer generation, degree of PEGylation, loading density of clickable DBCO groups, PEG–BA chain length, and the ratio of the dendrimer to PEG–BA. In particular, the injectable bio-orthogonal DH that was synthesised under the conditions that were disclosed in this study displayed great cytocompatibility as well as prolonged drug release. It was utilised in the production of an injectable formulation for the anticancer medication 5-FU that included both sustained release and localised administration. The DH/5-FU formulation dramatically reduced the formation of tumours in HN12 tumor-bearing mice and increased the overall survival rate of the animals. This was accomplished by

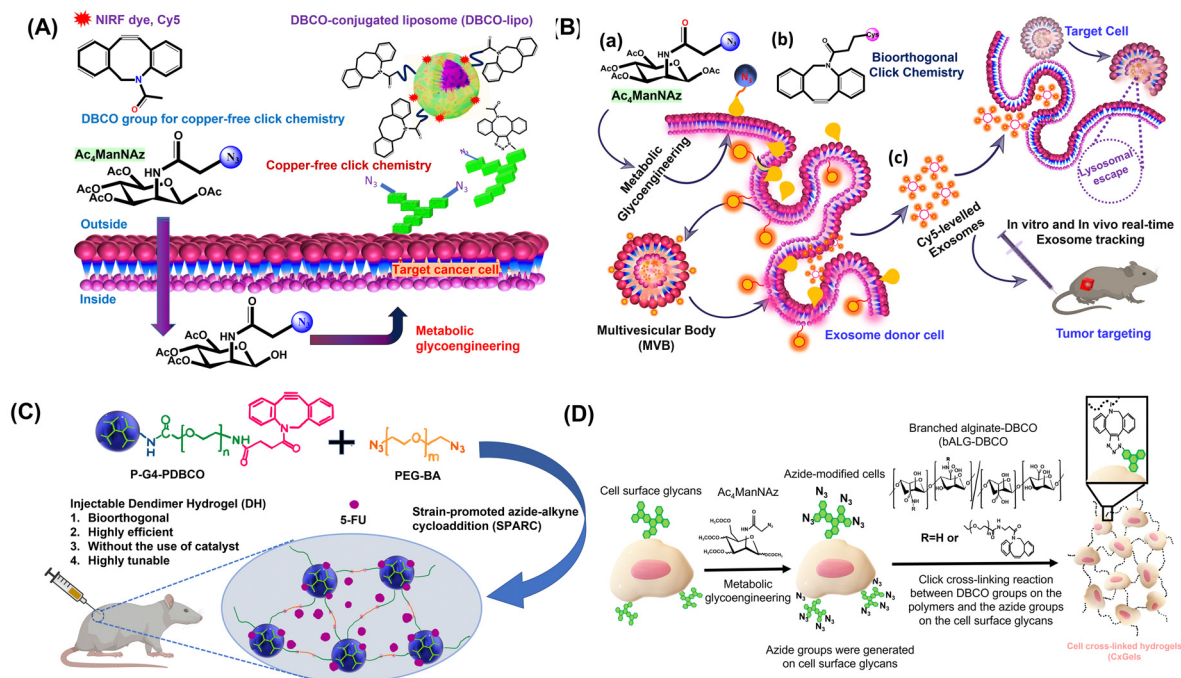


Fig. 4 (A) *In vivo* tumour-targeting *via* bio-orthogonal copper-free click chemistry. Adapted from ref. 110. (B) One-step bio-orthogonal click chemistry of metabolite-treated cancer cell exosomes. Adapted from ref. 111. (C) Synthesis and localized drug delivery using the injectable bio-orthogonal dendrimer organo-hydrogel. Reproduced with permission.<sup>109</sup> Copyright 2017, American Chemical Society. (D) Illustration of construction of cell cross-linked hydrogels (CxGels) *via* a bio-orthogonal click cross-linking reaction between azide-modified cells and alkyne-modified polymers. Reproduced from ref. 105. Copyright 2018, Springer Nature.

increasing the rate of cancer cell death as well as decreasing the rate of cancer cell proliferation and tumour angiogenesis.

### 4.3 An avant-garde design stratagem for the development of hyaluronic acid (HA)-based injectable organo-hydrogels *via* click chemistry

Click chemistry based injectable hydrogels obtained using hyaluronic acid (HA) and its derivatives are utilized for synthesis of novel injectable hydrogels. Hyaluronic acid-based hydrogels exhibit structural and mechanical properties mimicking the extracellular matrix (ECM).<sup>112</sup> Owing to its properties such as biocompatibility, biodegradability, and low immunogenicity, HA is fabricated into a desirable biomaterial for regenerative medicine, tissue engineering and cosmetology.<sup>113,114</sup> However, due to the enzymatic activity of hyaluronidase which degrades hyaluronic acid inside the body, chemical crosslinking of HA is essential.<sup>115</sup> Viscosity, shear thinning and self-healing are important parameters which impart injectable properties to a hydrogel.<sup>116</sup>

#### 4.3.1 Click-chemistry-based injectable organo-hydrogels.

We propose the development of injectable hydrogels *via* click chemistry using hyaluronic acid (HA) and its derivatives. The synthesis of a click tetrafunctional poly(ethylene glycol) hyaluronic acid hydrogel is illustrated in Fig. 5(A), where 4-arm PEG and hyaluronic acid serve as precursors. Extensive studies have shown that 4-arm PEG imparts high strength and yields an ideal homogeneous network structure to the hydrogels.<sup>117</sup> Furthermore, the synthesis of a hyaluronic acid-thiolated gelatin azide injectable hydrogel (Fig. 5(B)) involves the crosslinking of gelatin with cystamine,

followed by click reaction with cyclooctyne-hyaluronic acid. The exceptional reactivity of cyclooctyne rings with azide functional groups allows for a rapid click reaction to occur under mild conditions, obviating the need for copper catalysts.<sup>118</sup> Additionally, the inherent stability of the cyclooctyne ring facilitates the formation of a stable triazole ring upon reaction with the azide group. The resulting triazole linkages provide long-term stability to the modified molecules.<sup>119</sup>

#### 4.3.2 Click-chemistry-based injectable organo-hydrogels with inherent antimicrobial properties.

Further, we propose the development of a click chemistry derived injectable hydrogel using functionalized HA and its derivatives possessing antimicrobial properties. Combination of two or more forms of heterocycles in a molecule impart an enhanced antimicrobial spectrum due to rich electron density clouds.<sup>120</sup> The hydrogel consists of 1,2,3-triazole rings along with benzimidazole, thereby increasing its antimicrobial potency. Hence, in addition to the technological advancements, these hydrogels will possess bioactive properties from the click monomeric unit exhibiting remarkable efficacy as an antibacterial agent, in conjunction with injectable properties. The new click monomer (CM) (1-(2-(1*H*-benzo[*d*]imidazole-1-yl)ethyl)-*H*-1,2,3-triazol-4-yl)methanol is synthesized *via* an azide-alkyne cycloaddition 'click reaction'. The CM is further co-polymerized with HA or its derivative, wherein, the benzimidazole functionalized monomer containing 1,2,3-triazole groups is synthesized *via* a copper catalysed 1,3-dipolar cycloaddition reaction. Post that HA was amalgamated with free radical aqueous polymerization, under physical crosslinking of co-networks, leads to the formation

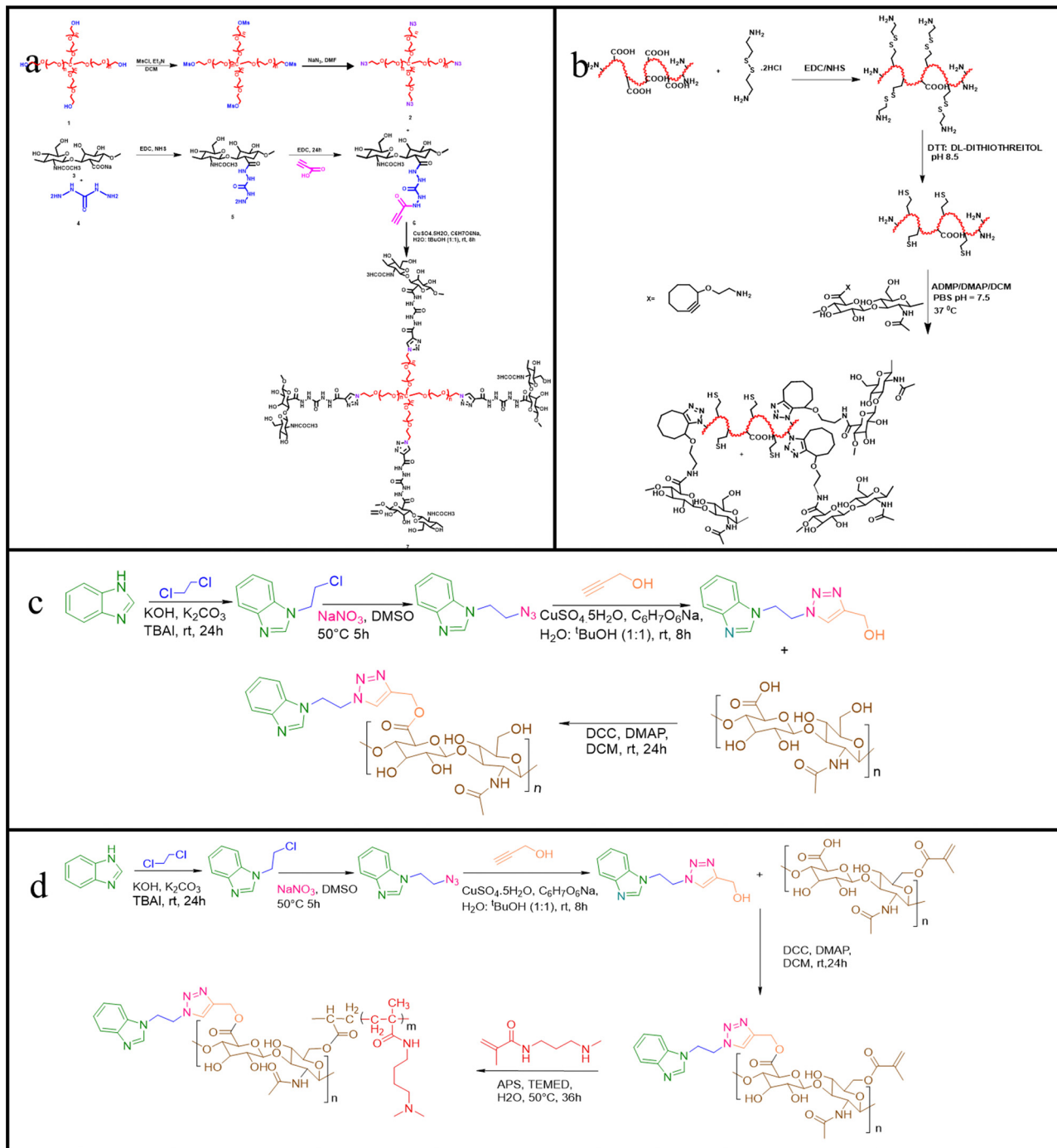


Fig. 5 Intelligent design strategies for the development of HA-based injectable organo-hydrogels.

of (1-(2-(1*H*-benzo[*d*]imidazole-1-yl)ethyl)-*H*-1,2,3-triazol-4-yl)-methanol is depicted in Fig. 5(C). In Fig. 5(D), methacrylated HA (HA derivative) is further combined with dimethylamino-propyl methacrylamide (DMAPMA) *via* free radical aqueous copolymerization. Methacrylated HA (HA derivative) is further combined with dimethyl aminopropyl methacrylamide (DMAPMA) *via* free radical aqueous copolymerization. Injectable hydrogels being life-saving carrier agents require special emphasis on their design and development and click chemistry can offer a wide

designability to such systems, an area that has huge scope to be explored.

## 5. Bench-to-bedside implementation of injectable organo-hydrogels

The translation of materiobiological innovations to clinical therapy is frequently referred to as bench-to-bedside, which is

mediated by governing institutions such as the FDA and EMA.<sup>121</sup> The regulation of the marketability of pharmaceutical products provides medical professionals agency over the pharmacokinetics of the products they prescribe to patients. Bio-inspired injectable organo-hydrogels were primarily synthesised on a pilot-plant scale on the grounds of conducting pre-clinical studies. This stipulates the notion of them being manufactured in small-scale batches, thus implying the need to scale up their fabrication techniques, while also adhering to the official guidelines and practices that have been proposed and established for their successful synthesis and commercialisation. As is evident, the subtle sophistication of crosslinking various biomimetic/synthetic/natural starting materials in an effort to devise clinically sound hydrogels makes it even more important for the manufacturing giants to comply with the cGMP (Current Good Manufacturing Practices) which have been put forward for aspects encompassing materiobiological procedures in an effort to strike out any chances of batch variations, therapeutic efficacy, mechanical robustness and adulteration during scale-up of manufacturing techniques. Moreover, the fabrication techniques of hydrogels of natural/biomimetic grade are more demanding as such natural polymers are of heterogeneous nature due to which their molecular characteristics, sterilization and storage have to be taken care of effectively.<sup>8,122</sup>

### 5.1 Pilot-scale organo-hydrogel fabrication

The pilot-scale fabrication of organo-hydrogels as an injectable aid requires addressing the critical challenges associated with the final batch size, dispersion process, homogeneous heating of biopolymer solution, and purification of organo-hydrogels.<sup>23</sup> These parameters can be summarised as follows:

- The use of industrial reactors of a volume relating to the final batch size (~10 kg) is necessitated.
- Dispersing elements along with the heating system enable the homogeneous heating of the organo-hydrogel solution.
- The dispersion process is regulated *via* time, speed, as well as size parameters of the dispersing element.
- The reproducibility of the dispersing element must be consistent for maximum performance.
- Organo-hydrogel purification is conducted with a cartridge device to facilitate concomitant purification of numerous membranes.
- Scale-up of organo-hydrogel production is to be done with minimal manipulation by preparing specifically tailored solution for respective manufacturers.
- Commercially marketed bench-top filling devices enable automatic filling of syringes.

### 5.2 Regulatory implications

Presently, under Section 201(g) of the FD&C Ac, the FDA has classified hydrogels under 'devices' as opposed to drugs which are available commercially. The aforementioned act covers "any product which does not achieve its primary intended purposes through chemical action within or on the body" which clearly implies that 'a product that has chemical action could be a device



Fig. 6 Procedure of clinical investigation of injectable medical products. Adapted from ref. 124.

if it does not achieve its primary intended purposes through that chemical action', as stated by the FDA in its regulatory guidelines.<sup>123</sup> Furthermore, with a few exceptions, the majority of hydrogel-based products must go through additional FDA review of a 510(k) PreMarket Notification submission in order to obtain legal marketing rights in the United States. Hydrogel scaffolds containing a drug or drug-secreting cells are considered combination products, and thus regulatory approval can take up to 7–10 years, further limiting their commercial viability.<sup>122</sup> Fig. 6 schematically summarizes the regulatory implications and procedure of clinical investigation of injectable medical products.

The procedure for injectable organo-hydrogels to receive the CE mark to demonstrate their compliance with the European regulation is summarised as follows:

- Requires determination of the regulatory guideline to which the medicine applies; hydrogels are active implantable medical devices;
- Classification of medical devices into one of the following categories: class I, IIa, IIb, or III; hydrogels are class III;
- Enforcing a quality management system (QMS);
- Elucidation of all available technical information regarding chemical and physical characteristics as well as properties, along with clinical data about the organo-hydrogel to validate its safe use;
- Audit of the technical information and QMS by a notified body, which includes clinical evaluation and post-marketing supervision to be conducted;
- Receiving the CE marking certificate, which has a validity for three years for hydrogels, and the ISO 13485 certificate, which has a validity of one year for the manufacturer facilities (some countries require manufacture license);
- Declaration of conformity and compliance of the organo-hydrogel to regulatory guidelines;

• State-wise registration of hydrogels where their national regulatory guidelines mandate it, and the process can be repeated on expiration of the CE mark/ISO13485 validity.

## 6. Conclusion

In conclusion, this perspective proposes biomimetic injectable organo-hydrogels to be translated in the emerging regime of pharmaceutical revolution with the development of bioresponsive and self-healing injectable organo-hydrogels by providing a bird's eye view on their tissue engineering and regenerative medical applicability. Although these classes of organo-hydrogels have not yet made to the market, they exhibit optimistic results in wound healing and drug delivery, and can effectively unleash their versatility in other tissue engineering and regenerative medical applications. Furthermore, with the focus of pharmaceutical revolution, it is imperative to address concerns regarding the demand for novel drugs and efficient techniques of mass production. Along these lines, click chemistry complimented by bio-orthogonal chemistry comes as a paramount stratagem in the conveyor belt of pharmaceuticals. Ultimately, it is necessary for them to conform to the regulatory guidelines put forward by governing institutions such as the FDA and EMA for their safe distribution, which have been extensively investigated and elucidated in this perspective. In this approach, we believe that the clinical advancement of injectable organo-hydrogels *via* click chemistry is an elixir in the arsenal of pharmaceuticals.

Nevertheless, looking at the future directions of establishing click-synthesis-derived injectable organo-hydrogels for various biomedical applications, there are certain bottlenecks associated with organo-hydrogels that need to be overcome. These challenges include achieving desired mechanical properties, immunogenicity and inflammation as some organo-hydrogels may trigger immune response and/or inflammation when implanted. When cells are injected into ischemic tissue for therapeutic repair, they are exposed to a hypoxic microenvironment, which results in immunogenic response at the injection site. Furthermore, the difficulty in biofunctionalization and inhomogeneity is a critical aspect to look at. Some organo-hydrogels are sensitive to common sterilization practices, and finally having an appropriate shelf-life would strongly benefit the medical implementation of these injectable organo-hydrogels.

## Author contributions

Abhyavartin Selvam: conceptualization, investigation, methodology, data curation, writing – original draft preparation, writing – review & editing. Misba Majood: investigation, methodology, data curation, writing – original draft preparation, writing – review & editing. Radhika Chaurasia: conceptualization, investigation, writing – original draft preparation, conceptualization of the HA-based injectable organo-hydrogel. Rupesh: conceptualization of the HA-based injectable organo-hydrogel. Akanksha Singh: investigation, writing – original

draft preparation. Tapan Dey: visualization. Omnarayan Agrawal: writing – review & editing. Yogesh Kumar Verma: writing – review & editing. Monalisa Mukherjee: conceptualization, methodology, resources, supervision, formal analysis, project administration, funding acquisition, writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

MM thanks the Department of Biotechnology (DBT; BT/PR21866/NNT/28/1145/2016) and MM and RC thank the Department of Science and Technology (DST; DST/WOS-A/CS-106/2021). The authors would like to acknowledge the Biorender software for figures. We are also thankful to Amity Institute of Click Chemistry Research and Studies, for providing the research infrastructure.

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