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Synthetic Polymers Derived Single-Network Inks/Bioinks for Extrusion-Based 3D Printing Towards Bioapplications

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Abstract

Three dimensional (3D) printing, also known as additive manufacturing technique has revolutionarized the field of manufacturing with a great impact as compared to the other traditional methods. This technique has shown steep rise over the past decade owing to their benchmark capabilities of fabricating new and complex 3D constructs, especially towards tissue engineering, and regenerative medicine. Among currently applied 3D printing techniques, extrusion-based 3D printing has gathered particular attention for the employment of ink/bioink materials to enable on-demand personalized fabrication due to its low cost, broad utility for various materials and ease of controlled printability. However, there still exist a lack in diversity of the ink materials with their optimized degradation rate, rheology and bioactivity for precisely fabricating complex and self-supported cell-laden 3D printed constructs. Therefore, to develop an array of such new materials is a major challenge for synthetic polymer chemists, material scientists and biomedical researchers for widening the future applicability of 3D (bio)printing. This review aims to summarize the recent advances in the rational designing and development of ink/bioink materials based on synthetic polymers as single network precursor due to their great opportunity to tune their physico-chemical
and mechanical properties in order to design and mimic in-human 3D tissue scaffolds with shape retention for both hard and soft tissues.

**Keywords:**

3D printing, additive-manufacturing, 3D bioprinting, synthetic polymers based inks, bioinks, hydrogels

**1. Introduction**

3D printing based computer-aided additive manufacturing technique has emerged as a revolutionary manufacturing method and is currently being considered at the forefront research area in material sciences and biomedical engineering.\(^1,2\) 3D printing is a promising method for future era of personalized medicine via layer-by-layer fabricating patient-specific, customizable medical devices, organs, tissues and other bio-systems mimicking their native counterparts with complex and heterogeneous structures.\(^3,4\) In the endeavour of such biofabrication, the selection of printing technology, design and materials are critically important in order to maintain the structure-property-processing relationships in the 3D printed constructs.\(^5,6\) To date there has been considerable development towards the engineering of different types of 3D printing devices,\(^7,8,9,10,11\) and to increase their printing affordability and reliability such as with having tight control over the printing speed, higher resolutions, and multicomponents fabrications.\(^12,13\) Further, development and optimization of the printable ink materials is highly required to achieve desired properties including physico-mechanical properties, rheological properties and biofunctionality to obtain self-supporting functional and high-strength products, depending on the applications.\(^14,15\)

Although there exist different ink materials ranging from polymers to composites and ceramics,\(^16\)
there is still a lack to achieve such diversity in 3D printable ink materials. On the other hand, the preparation of 3D printable cell-laden ink materials (i.e. bioinks) have attracted great deal of interest to the researchers in the recent years,\textsuperscript{17} and have been considered as one of the most advanced tools to find new avenues in tissue engineering,\textsuperscript{18} regenerative medicine,\textsuperscript{19} drug delivery,\textsuperscript{20} cell therapy,\textsuperscript{21} etc. In the endeavor of mimicking the biocomplexity and heterogeneity of natural tissues in the 3D printed constructs at various scales, the development of such cellular embedded ink materials with “printability” (for e.g., with optimized printing process, speed, and resolution are critically important)\textsuperscript{22,23} are pretty much challenging due to the demand of various features depending on the application envisioned as discussed here. First, as cells are encapsulated within such ink materials, therefore all the components of the inks such as functionalized polymer based main ingredient along with other applied precursors for e.g. catalysts, cross-linker, drugs, bioactive molecules (like peptide sequences,\textsuperscript{24} growth and differentiation factors, etc.,\textsuperscript{25,26}) should be biocompatible in nature in order to maintain the cell survivability throughout the printing process as well as in the final printed article. Then, the ink materials should protect the cells and maintain their sensitivity to enable the survival of cells during the printing process for the biofabrication, and the desired cellular functions (for e.g., cell growth and proliferation, adhesion and differentiation) should be preserved in the fabricated structures.\textsuperscript{27} Further, the post-extrusion structural and functional integrity in the product should be maintained with standardizing different parameters such as pressure, temperature, pH or light to avoid concerned cell resistance.\textsuperscript{28,29} Finally, the materials should be biodegradable with controlled degradation kinetics and their waste compounds and intermediates should not have any toxic effect.

In the effort of developing ink materials for 3D printing with desired mechanical and biological properties, two major categories of material precursors have been adopted: (i) natural
polymers (biomaterials), and (ii) synthetic polymers. Natural polymers for e.g. polysaccharides and proteins, as typically extracted from plants, animals, bacteria, cells, etc., are currently widely applied materials as network precursor in bioink formation due to their greater biocompatible nature and higher cellular proliferation rates in comparison to synthetic polymers.\textsuperscript{30} Note that in many reports of bioink formulation natural polymers were artificially modified with functional groups, for instance to enable network crosslinking, however such artificial biomaterials have been considered in this review in the category of natural polymers only.\textsuperscript{31,32} Naturally derived polymers are often associated with some disadvantages which can arise challenges in their utility for biofabrication such as (1) variation in batch-to-batch which can led to the complication due to their variable printability and the issue of the reproducibility of constructs, and the cellular sensitivity to such variations, (2) tuning the structures, solubility, viscosity and other properties of biopolymers remains challenging as their functionalization are often more difficult, (3) fast biodegradability rate which often may not be suitable. On the other hand, synthetic polymers can overcome these limitations of natural polymers along with the opportunity of being tailored with specific physical, chemical and biological properties and can further lead to the synthesis of a broad library of new materials for (bio)inks.\textsuperscript{33} Synthetic polymers further offer the ability to tune functional properties and rheological behaviour like printability and mechanical integrity of the inks, through monomer selection, architecture control, and post-polymerization functionalization opportunities.\textsuperscript{34,35} Therefore, synthetic polymers hold great potential to be exploited as future ink materials of 3D printing for shaping into advanced and highly customisable architectures suitable for medical devices and tissue scaffolds including both hard and soft tissues.\textsuperscript{36,37} However, a careful selection of synthetic polymer has to make in order to maintain biocompatibility and biodegradation issues. For instance, poly(ethylene glycol) (PEG) corresponds to the synthetic polymer which has been
approved by food and drug administration (FDA) and illustrates good water solubility as well as biocompatibility.\textsuperscript{38,39} Poly(lactic-co-glycolic) acid (PLGA), polylactic acid (PLA), and polycaprolactone (PCL) are also FDA approved synthetic polymers for their application in medical devices and are biocompatible and biodegradable in nature.\textsuperscript{40,41} Therefore, the utilization of synthetic polymers as ink materials can provide the advancement in the 3D printing technology with obtaining the highly defined (bio)fabricated constructs with better control size, resolutions, integrity and features compared to the traditional manufacturing techniques. Gupta et al. exemplified the combination of 3D printing technique with functional plasmonic nanomaterials for exhibiting programmable release from the printed multiplexed array of stimuli-responsive capsules having shell comprised of PLGA polymer within hydrogel matrices.\textsuperscript{42} In 2019, Camacho and Busari et al. investigated RGDS(biotin)-PCL and RGES(azide)-PCL based peptide-polymer conjugates for the controlled fabrication of spatially functionalized 3D printed biodegradable scaffolds by using multiple printer heads, which displayed a significant enhancement in NIH 3T3 fibroblast adhesion on the 3D-printed fiber surface of (RGDS(biotin))-PCL.\textsuperscript{43} Another example of PCL-based 3D printed scaffold was documented by Rashad et al., where it was subsequently coated with cellulose nanofibrils material which significantly improved hydrophilicity and protein absorption of PCL scaffold surface, and further enhanced cellular response (attachment, proliferation and osteogenic differentiation) of mesenchymal stem cells (hBMSCs), in vitro for the potential application of such 3D printed PCL scaffolds towards bone tissue regeneration in vivo.\textsuperscript{44} In contrast to the recent burgeoning advancements in the 3D (bio)printing technique, the focus of this review is to highlight the examples and development of the (bio)ink materials based on synthetic polymers as a single-network precursor (or major component) employed for the extrusion-based 3D printing technique towards bioapplications, as to the best of our knowledge
this area of interest has not been reviewed till date. Note, that the formulation of (bio)ink materials based on only natural polymers or combined natural and synthetic polymers are out of the scope of this review article. Their associated existing challenges in order to enable 3D biofabrication have been also discussed in this article with an aim to motivate synthetic polymer chemists to address the current demand of new and advanced (bio)ink materials which would further help to widen the scope of this unique and advanced technique of extrusion-based 3D printing.

Figure 1. (A) Schematic illustration for working principle of extrusion-based 3D printing techniques, and (B) the concept of biofabrication window to improve the printability of polymeric hydrogel bioinks. Adapted with permission from ref 47. Copyright 2013 John Wiley and Sons. (C) Schematic illustration of different bioprinting approaches for photo-crosslinkable inks via crosslinking before (pre-crosslinking), after (post-crosslinking) or during (in-situ crosslinking) extrusion. (D) Representative images of the extruded material from nozzles and the lattice structure
of corresponding printed constructs. Reproduced with permission from ref 52. Copyright 2017 John Wiley and Sons.

2. Extrusion-Based 3D Printing Technique

Extrusion-based 3D printing is the commercially available and most common rapid prototyping technique due to its affordability, versatility, and compatibility with a wide selection of ink materials usable for biofabrication and tissue regeneration from small vessels to large constructs/organs.\textsuperscript{45,46} Here, the inks are extruded through a micronozzle of extrusion head and controlled by either an endless screw, pneumatic pressure or a mechanical piston (Figure 1A).\textsuperscript{47,48} The 3D objects are constructed via the blueprints from a computer-aided design (CAD) file by direct-ink-writing in a layer-by-layer fashion and controlling an xyz stage of the deposition path of the print-heads.\textsuperscript{49} Extrusion-based 3D printers can be successfully engineered under sterile conditions with nozzle diameters and multiple extrusion heads providing the unique advantage of depositing different types of layers in the final printed article via delivering multiple (bio)ink materials with varying components, cells and cellular density (Figure 1B).\textsuperscript{8,50} To successfully enable the “printability” of an ink, a suitable viscosity should be achieved typically by inducing either external shear force (i.e. shear-thinning property) or pre-crosslinking to enable its extrusion from the nozzle, however as the external mechanical stress is removed post-extrusion a rapid filament shape retention with efficient recovery of mechanical properties (i.e. self-healing property) is desired to keep an integer and self-supported 3D printed structures with no structural collapsing.\textsuperscript{51} For instance, UV irradiation (or heating) can be applied for such shape retention to the tip of the printing nozzle or directly over the printed structure on the deposition plate. Burdick
and coworkers developed a general extrusion-based bioprinting method of “in-situ crosslinking” for photo-crosslinkable non-viscous hydrogel inks (such as from 5 wt% methacrylated hyaluronic acid (MeHA)) via introducing light through a photopermeable capillary which enables their simultaneous extrusion and crosslinking, prior to deposition (Figure 1C and D). The printed constructs depicted the uniform filament formation along with the high viability of the encapsulated cells. Another fascinating approach has gained significant interest recently based on extrusion-based 3D bioprinting using suspension baths, illustrating their ability to suspend and completely encapsulate the extruded filament materials. Such secondary support provides the flow restriction of bioink and trigger the cross-linking (physically or chemically) immediately after the deposition, and enables the printed construct with improved resolution and controlled heterogeneity. Feinberg and co-workers presented such approach as Freeform Reversible Embedding of Suspended Hydrogels (FRESH) printing where a thermoreversible support bath (CaCl₂) was utilized to embed soft hydrogel (based on alginate) to exemplify the construction of a bioprinted full-size human heart model. Recently, a co-axial extrusion-based 3D bioprinting approach has been also introduced to enable simultaneous printing of bioink along with cross-linker solutions in a coaxial system to promote gelation during extrusion. Khademhosseini, Dentini and co-workers demonstrated a coaxial needle extrusion system where the internal needle carried the flow of cell-laden bioink materials (based on alginate and gelatin methacroyl) and the external needle was designed to the simultaneous flow of ionic crosslinking solution (CaCl₂) to generate the ionically crosslinked hydrogel microfibers at the tip of dispensing system, which was further secondary crosslinked covalently via exposure of UV light.

3. Hydrogel Inks/Bioinks Based on Synthetic Polymers as Single-Network Precursor
Hydrogels are highly hydrated three-dimensional polymeric networks and have been exploited for various biomedical applications, for e.g., as a scaffold for tissue engineering, and drug delivery.\textsuperscript{58,59} Hydrogel based ink materials can provide a perfect soft material for soft tissue engineering as to create a soft tissue is more challenging due to the demand of features like high elasticity, flexibility, viscosity and inter-layer adhesion.\textsuperscript{60,61} From a biological point of view, hydrogels having a high water content can be considered as an ideal candidate for cell-laden bioink materials for extrusion-based 3D printing because they can closely simulate the native extracellular matrix (ECM) microenvironments with having aqueous 3D environment, and provides cells survivability with retained rounded morphology and homogeneous encapsulation during the extrusion process and there after promote new tissue formation and functioning in 3D space.\textsuperscript{18,62} However, fabrication of hydrogel based scaffolds or cell-laden constructs with shape fidelity remains challenging, and there exist a current demand of optimization in the mechanical properties of the hydrogel materials within a biofabrication window (Figure 1B).\textsuperscript{47} On one hand, the soft hydrogels which are well suited for cells are disadvantageous for fabrication process to achieve the constructs with maintained pre designed structure fidelity, and on the other hand the stiff hydrogels which can typically provide the shape fidelity can affects the cellular functioning by limiting the migration and proliferation of the cells within the dense
Figure 2. (A) Chemical structure of acrylated PCL-PEG-PCL triblock copolymer. (B) Preparation of visible-light induced cross-linked single-network biodegradable hydrogel having high elasticity for bioprinting with various human cells. (C) Photographs illustrating the mechanical properties of PCL-PEG-PCL hydrogel upon twisting for four cycles and recovering after release. (D and E) Live/dead cell assay and cellular compatibility, respectively, of different cell types in the printed 10% PCL-PEG-PCL hydrogel carried out immediately after gelation (D0) and after 7 days in culture (D7) (scale bar: 500 μm). (F) Printed sample shapes by using different needle sizes (scale bar: 5 mm). Adapted with permission from ref 70. Copyright 2018 American Chemical Society (ACS). https://pubs.acs.org/doi/10.1021/acsami.8b01294 (For further permissions related to the material excerpted readers should be directed to the ACS).
polymeric network. In this context, the utilization of synthetic polymers as hydrogel ink materials can offer the tailoring into its mechanics by maintaining an adequate viscoelastic characteristics and integrity, including self-healing and shear-thinning properties, and also provides optimization opportunity into its functionality, and degradation kinetics of the printed products. In general, hydrogel ink formulation requires a specific viscous polymeric solution that can be immediately form high networking post-printing either by physically or chemically cross-linking of polymers. Prestwich and coworkers described the formation of extrudable hydrogels with suitable rheological properties for bioprinting of vessel-like constructs derived from tetrahedral polyethylene glycol tetracrylates via co-crosslinking thiolated hyaluronic acid and gelatin derivatives. Joas et al. investigated the hydrogels formulation for extrusion-based 3D printing comprised from diacrylate of PEG the anionic and cationic monomers 3-sulfopropyl acrylate and [2-(acryloyloxy)ethyl]trimethylammonium chloride, respectively, via UV irradiation based photopolymerization. The biodegradable cross-linked hydrogel network (using visible light stimulus) was further illustrated by Xu et al. based on single-component precursor of diacrylated polycaprolactone-poly(ethylene glycol)-polycaprolactone (PCL-PEG-PCL) triblock copolymer which was synthesized (theoretical block length 2k-20k-2k) via ring-opening polymerization followed by acryloyl groups insertion (Figure 2). As the hydrogels were composed of PEG and PCL based well known biocompatible synthetic materials, therefore the hydrogels exhibited support to cellular growth with good survival rates (in vitro) of the encapsulated 3T3 fibroblasts over 90% from day 1 and day 3 of culture. Further the hydrogels possessed their good mechanical properties with high flexibility and elasticity upon stretching, compressing and twisting. The viscosity of the obtained hydrogels precursors were optimized to prepare cell/polymer bioink (with PCL-PEG-PCL at 10% of polymer concentration) based on different types of human cells such as
human umbilical vein endothelial cells (HUVEC), human aortic smooth muscle cells (SMC), and neonatal human lung fibroblasts (NHLF). 3D bioprinting was performed to create cell-gel constructs upon visible-light exposure, exhibiting their high cellular viability over 83% across all three cell types, within the constructs after 7 days in culture printing. Basic shapes and complex patterns were printed using 20% (w/v) PCL-PEG–PCL solution, and their resolutions were maintained by changing the nozzle diameter (with 18G and 21G needle), indicated the printability of the hydrogels.

**Figure 3.** Principle of sol–gel extrusion printing of PEG-peptide based hybrid hydrogels. Hybrid silylated PEG polymers and hybrid GRGDSP peptides are dissolved in buffer to provide a multicomponent bioink which undergo for condensation during 3D printing to produce hydrogel scaffolds. Reproduced from Ref. 80 with permission from The Royal Society of Chemistry.
3.1 Supramolecular Polymers

Supramolecular polymers are derived from a unique combination of supramolecular chemistry and polymer science,\textsuperscript{71,72} have appeared as an advanced smart materials for their diverse applications towards energy harvesting, drug delivery, electronic devices, template synthesis, etc.\textsuperscript{73,74} Such polymeric chains involves various types of supramolecular interactions based on hydrogen bonding, $\pi-\pi$ interactions, metal-ligand coordination, interactions between ions, and host-guest interactions.\textsuperscript{75,76} Those supramolecular noncovalent interactions offers dynamic and reversible nature, providing an opportunity to tune the mechanical and viscoelastic behavior with improved rheological performance of supramolecular polymers,\textsuperscript{77} and therefore makes them potential candidates for 3D printing based additive manufacturing to create customizable and dynamic constructs for tissue engineering scaffolds and other biomedical applications.\textsuperscript{19,78,79} Echalier et al. documented 3D printable bioink derived from supramolecular peptide-functionalized synthetic polymer based hydrogels by using soft sol-gel polymerization without using photoactivation or additional organic reagents (Figure 3).\textsuperscript{80} Hydrolysis followed by condensation of the silylated precursors of hybrid bifunctional PEG polymer and integrin ligand (GRGDSP peptide) occurred during the extrusion based 3D printing process (at room temperature and in physiological buffer with pH 7.2) resulting chemically cross-linked network through siloxane (Si-O-Si) bonds. The obtained hybrid PEG-peptide based 3D scaffolds were then seeded with mesenchymal stem cells (mMSC) culture, where after 4 days of proliferation live/dead assay on the scaffolds illustrated excellent cell viability, indicating the compatibility of the hybrid PEG-peptide scaffolds for cell culture.

In another example, Lorson et al. documented thermogelling supramolecular materials as a bioink for 3D bioprinting based on a series of synthetic biocompatible amphiphilic block
copolymers comprised of poly(2-methyl-2-oxazoline) (PMeOx) based hydrophilic block and poly(2-n-propyl-2-oxazine) (PnPrOzi) based thermoresponsive segment, yielding PnPrOziₙ-b-PMeOxₙ (Figure 4). At a concentration of 20 wt% and above, the copolymers depicted thermogelation between the room temperature and 37 °C, and the formed hydrogels exhibited high mechanical strength with G' values more than 1 kPa, and pronounced isothermal shear thinning behavior with a rapid and complete shear recovery upon removal of stress. Such rheological properties of the copolymer materials along with their obtained cytocompatibility, enabled their suitability for extrusion-based 3D bioprinting to afford cell-loaded constructs. The homogeneous cell distribution was observed within the entire printed constructs generated from NIH 3T3 fibroblasts encapsulated PnPrOzi-b-PMeOx-based bioinks and no negative effects on the cell viability were observed by dispensing process, with cytocompatibility (91.5 ± 0.8%) measured 24 h post printing which was comparable to the viability of control cells incorporated in the material but not printed (92.8 ± 1.7%), and untreated cells in medium (98.9 ± 0.18%).
Figure 4. (A) Chemical structure of investigated diblock copolymer materials PnPrOziₙ-b-PMeOxₙ. (B) Photographs of 20 wt% concentration of PnPrOzi₁₀₀-b-PMeOx₁₀₀ (100_B) at different temperature. (C) Shear recovery at 37 °C and 10 rad/s of (20 wt %) 100_B (low strain: 0.5%, high strain: 150%). (D) Light microscopy image of hydrogel 3D printed constructs, and (E) cell-loaded printed constructs. (F) FACS analysis results to illustrate the effect of the printing process on the viability of NIH 3T3 fibroblasts cells stained with propidium iodide (PI) and fluorescein diacetate (FDA) (“untreated” corresponds to the cells in medium, “control” represents to the cells redispersed in the bioink but not printed, and “print” refers to the viability of cells after 24 h of printing). Adapted with permission from ref 81. Copyright 2017 American Chemical Society.
The one-pot fabrication of printable supramolecular polymer based self-healing hydrogels was demonstrated by Wei et al. derived by guanidinium-based oligopeptide via the dual-enzyme-triggered self-assembly and simultaneous enzyme-initiated polymerization approach (Figure 5). The ink material for such system was comprised of the mixed solution of monomer poly(ethylene glycol) methacrylate (PEGMA) and NapFFRK-acryloyl (peptidic hydrogelator), and enzymes horseradish peroxidase (HRP), glucose oxidase (GOx), along with glucose, acetyl acetone (AcAc), which further commenced the two enzyme-mediated redox reaction. The hybrid hydrogel provided an enlarged time window for the in situ viscosity-controlled (of the pre-gel solution) printing into 3D constructs. Further, NIH 3T3 cells incorporated hybrid hydrogels exhibited a very high survival rate 99.0 ± 0.8%, and a live/dead assay of the 3D bioprinted construct from such cell-laden bioink materials showed the cell viability of 98.0 ± 2.3%, suggesting the biocompatibility of the hybrid hydrogels. The preparation of similar hybrid hydrogels by utilizing NapFFK-acryloyl hydrogelators (along with other components GOx, HRP, AcAc, and PEGMA) for their 3D cell printing was further illustrated by Wei et al. in another report. Such supramolecular-polymeric hydrogel exhibited high mechanical strength with reusability and thermal stability along with biodegradability and cellular compatibility, and was successively employed for in situ 3D cellular printing to fabricate 3D scaffold of NIH-3T3 cells.

A self-healable supramolecular polymer materials for 3D printing under simplified thermomelting extrusion condition was further described by Wang et al. based on copolymer P(N-acryloyl glycinamide-co-1-vinyl-1,2,4-triazole) cross-linked through the dynamic hydrogen bond interactions. The prepared hydrogels exhibited thermoplasticity, self-repairability, and reprocessability for various 3D printed constructs over a lower temperature range. By tuning the
feed ratios and concentration of monomer in the copolymer, the effective mechanical properties of the formed supramolecular hydrogels were obtained with high tensile strength up to 1.3 MPa, large stretchability up to more than 1300% and increased compressibility to 11 MPa at swelling equilibrium state. Moreover, these supramolecular hydrogels biomaterials depicted medicinally desirable antibacterial, anti-inflammatory activities along with the biocompatible properties.

Other notable system include a 3D printed drug delivery implant developed by Roberts, Hayes and coworkers based on thermo-responsive supramolecular polyurethane (SPU), able to form self-assemble polymer network via hydrogen bonding and π-π stacking (Figure 6). The material displayed suitable mechanical properties (with self-supporting and stiff, yet flexible) for hot-melt extrusion-based approach at a relatively low processing temperature (100 °C). Further to establish the potential application of SPU for 3D printing of a biomedical device, the biocompatibility of the polymeric films of polyurethane was determined by MTT assay using mouse fibroblasts (L929) which illustrated their non-cytotoxicity with more than 94% of cell viability. A multicomponent synthetic material SPU-PEG-paracetamol was prepared based on SPU co-formulated with PEG (4 wt% and 8 wt%) and incorporating paracetamol drug (16% w/w) to afford 3D printed robust implant constructs which exhibited appropriated mechanical performance, and prolonged drug release over a time period of 5 to 8.5 months. Such release rates can be potentially altered by modulating the formulation with varying PEG percentage or its molecular weight. In 2019, Binder et al. developed 3D printable inks based on supramolecular polymers as linear and three-arm star biocompatible poly(isobutylene)s exhibiting multiple hydrogen-bonds and their nanocomposites to enable construction of stable and self-supported structures at room temperature with polymer filament diameters 200-300 µm.
3.2 Stimuli-responsive Polymers

Stimuli-responsive polymers are smart or intelligent materials which can undergo alteration in their chemical, physical, morphological or mechanical properties upon interactions with their surrounding environment. In the past decade these materials have been increasingly applied to a wide range applications, including controlled and triggered drug delivery, (bio)sensing, actuators, coatings, diagnostics, tissue engineering and biomedical devices. Various stimulus has been investigated for such polymers, including pH, temperature, chemicals, light, redox, mechanical force, biomolecules, electric and magnetic fields. The synergistic integration of stimuli-responsive polymer materials with the 3D printing technique can potentially provide a

Figure 5. (A) Schematic representation of the pre-gel solution. (B) Dual-enzyme-triggered self-assembly (SA) and simultaneous enzyme-initiated polymer reaction (EIP). (C) The hybrid hydrogel formation with incorporated dual enzyme. (D) Schematic illustration of the peptidic hydrogelator, enzymes, and PEGMA (AcAc and glucose are omitted). (E) The self-healing of hybrid hydrogel at the joint by placing two parts together at 3600 s. (F) The joint cannot withstand bending by placing them together at 6000 s. (G) 3D printed constructs. (H) A 3D stack of printed
cells in the hybrid hydrogel materials stained with FDA and PI (scale bar represent 300 μm).

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![Figure 6.](image)

**Figure 6.** (A) Chemical structure of dynamic self-assembling supramolecular polyurethane (SPU) materials. (B) Schematic illustration of SPU-PEG-paracetamol formulation based 3D printing of the prototype drug-release implant. (C and D) Side-on and end-on view photographs of the 3D printed bar construct from such formulation. Reproduced from Ref. 86 with permission from The Royal Society of Chemistry.

remarkable engineering and materials advancements in the field of additive manufacturing processes to create complex geometries and structures which are not possible via other manufacturing techniques.\(^\text{92,93}\) For instance, Pluronic F127 has been investigated as a stimuli-responsive ink materials for 3D printing, which corresponds to ABA type triblock copolymer PEG-
PPO-PEG and possess a inverse thermo-gelling properties and can be readily printed and removed under mild condition. Zhang et al. developed a similar type of hydrogel materials with dual stimuli-responsiveness for 3D printing, however such ABA triblock copolymers were comprised of one hydrophilic PEG segment and two hydrophobic poly(isopropyl glycidyl ether) (PiPrGE) segments arranged in a PiPrGE-PEG-PiPrGE configuration, and corresponding hydrogels were able to exhibit thermoreversibility as well as a rapid and reversible response to shear forces. Further, Hsieh et al. documented bioink materials based on the aqueous dispersions of thermoresponsive biodegradable polyurethane (PU) nanoparticles (comprised of diol of PCL and (L or D,L) PLA of $M_n \sim 2000$, mixed in 4:1 M ratio) which formed hydrogel near 37 °C without using any cross linker (Figure 7). The hydrogel stiffness was optimized by varying the PU content (25 or 30%) of the dispersion and their neural stem cell (NSC)-laden hydrogel constructs were printed by fused deposition manufacturing equipment. The filaments of the subsequently printed constructs displayed proper shape retention with reasonable swelling (<10% at 72 h), for e.g., from diameter of ~210 µm at 0 h to ~220 µm after 72 h for PU2 (30% or 25%) hydrogels. The cell viability of the encapsulated NSCs in above PU2 hydrogels (~680-2400 Pa) were found to be greater than 100% at 72 h, indicating excellent proliferation and differentiation. Moreover, the zebrafish embryo neural injury model having the injection with such NSCs (labeled with PKH26 (red fluorescence))-laden 25-30% PU2 hydrogels exhibited their wide dispersion in all brain areas, in particularly for those with 25% PU2 hydrogel, and further promoted the repair of the function of impaired central nervous system. Murphy et al. illustrated another example of stimuli-responsive ink materials for 3D printing (Figure 8) based on photoresponsive polypeptide (with functional photoresponsive crosslinking). The hydrogel ink materials, comprised of UV responsive copolypeptides incorporated with isolucine, glutamic acid and cysteine (protected with
photo-cleavable nitrobenzene groups), and alkyne functionalized 4-arm PEG-propiolate crosslinker, illustrated impressive shear-thinning properties to readily print a range of mechanically stable 3D structures. A catalyst free nucleophilic thiol-yne “click” reaction was performed upon UV curing to enable crosslinking between the cysteine and propiolate residues, providing crosslinked hydrogel constructs with more than 10 layers exhibiting improved stiffness, high fidelity and resolution. The biocompatibility of the copolypeptide hydrogel was further investigated with human dermal fibroblasts cell assay by culturing on the top and within the hydrogel, illustrating almost no cytotoxicity and a high viability of cells, respectively.

In 2011, Hennink and coworkers developed a biodegradable, photopolymerizable and thermosensitive (as dual stimuli-responsive) ABA triblock copolymer hydrogel for 3D

![Figure 7.](image)

(A) Schematic representation depicting chemical structure of polyurethane (PU), and the 3D bioprinting process for creating constructs based on the neural stem cell (NSC)-laden PU
hydrogel. (B and C) Photographs for NSCs (labeled with PKH26) in the 3D printed stacking fibers at 0 h and 72 h of corresponding 25 and 30% PU2 hydrogels bionks, respectively. (D, E, F and G) The distribution of NSCs (labeled with PKH26) in the zebrafish embryos at 48 h post fertilization injected with PBS, NSCs and NSC-laden PU2 hydrogels of 25 and 30%, respectively. Adapted with permission from ref 95. Copyright 2015 Elsevier.

Bioprinting based cartilage tissue engineering applications (Figure 9). Free radical copolymerization method was used to prepare this thermosensitive copolymer (with cloud point 11 °C) comprised of A-blocks based on biodegradable poly(N-(2-hydroxypropyl)methacrylamide lactate) (p(HPMAm-lac)) (MW ~23.5 kDa) with partially modified by methacrylate groups to access photo cross-linking, and B-block derived of hydrophilic PEG (MW ~10 kDa), yielding p(HPMA-lac)-PEG-p(HPMA-lac). The as developed p(HPMA-lac)-PEG-p(HPMA-lac)-based hydrogels, with thermally and chemically cross-linked networks, displayed strain-softening behaviour and illustrated semi-flexible properties similar to various natural polymers including collagen. Moreover, the copolymeric hydrogels (25 wt%) were found to be suitable for printing of porous 3D constructs with subsequent photopolymerization to exhibit an elastic modulus of 119 kPa (of 0.6 cm construct) and a degradation time of around 190 days (of 12 layered construct), and offer mechanical support to the encapsulated cells for long-term until new tissue is formed. Different fluorescent microspheres (fluorescent orange and lemon) loaded hydrogels displayed accurate and precise localization of such cell mimicking microspheres encapsulated within the copolymer to their 3D fiber deposition. The cell-laden photopolymerized hydrogel (25 wt%) revealed homogeneous distribution of chondrocytes over entire hydrogel with excellent viability of 94% after 1 day with no adverse effects found due to the exposure of UV.
3.3 Nanoengineered Polymers

Nanoengineered polymers are hybrid materials in which polymeric matrix are dispersed with one or more type of inorganic or organic nanofillers such as silica or metallic nanoparticles, nanoclay, nanorods, carbon nanotubes and nanofibers, graphene, metallic nanowires, quantum dots, etc.\textsuperscript{98,99} Due to the several advantages imparted by the incorporated nanomaterials (via choosing proper mixing strategies) within the polymer matrix, such fabricated polymer nanocomposite materials exhibit significantly improved rheological performance, functionality and properties (for e.g., mechanical, electrical, optical, thermal, biological and magnetic properties) with respect to the native polymers,\textsuperscript{100,101} and therefore, makes the polymer nanocomposite a suitable candidate to enable additive manufacturing based highly-customized complex and functional 3D constructs.\textsuperscript{102,103} Meng et al. documented poly(vinyl alcohol) (PVA)-based nanocomposite hydrogel as an artificial cartilage constructed by extrusion-based 3D printing (Figure 10).\textsuperscript{104} Incorporation of graphene oxide (GO)-hydroxyapatite (HAp) in the nanocomposite PVA/GO-HAp (25 wt\%) led to highly improve the dynamic viscosity of the
Figure 8. (A) Schematic illustration for photoresponsive copolypeptide hydrogels based 3D-extrusion printing. Photocleavage of nitrobenzyl protecting groups generated free thiol residues which further reacted with alkyne functional groups of 4-arm PEG-propiolate. (B) Design and development of human nose shape construct by 3D printing of hydrogel ink. Adapted from Ref. 96 with permission from The Royal Society of Chemistry.
Figure 9. (A) Chemical structure of thermosensitive triblock copolymer. (B) A cartoon representation for the effect of temperature and photopolymerization on the polymer illustrating hydrogel formation above the cloud point followed by chemical cross-linking within the hydrophobic domain. (C and D) Photographs of the 3D printed construct from 25 wt% copolymer based hydrogels with dimensions (cm): 1 × 1.5 × 0.6, and strand spacing (mm): 1.5. (E, F and G) Microscopic images of the 3D printed layers of fluorescent microspheres loaded hydrogels with 1.5 mm strand spacing of 2 and 3 layers angled constructs, and 0.8 mm strand spacing of 2 layers construct, respectively. Adapted with permission from ref 97. Copyright 2011 John Wiley and Sons.

materials and exhibited good shear-thinning properties in the extrusion shear rate range with reduced Barus effect in order to obtain the stable printed constructs with high printing accuracy. The 3D printed PVA/GO-HAp hydrogels displayed biomimetic gradient porous structure with
excellent bio-mechanical and bio-frictional behaviors, attributing to their promising potential for the exact repair of articular cartilage. Shah and coworkers demonstrated the extrusion-based 3D printing of polymer nanocomposite inks comprised of a biocompatible copolymer polylactide-co-glycolide (PLG) (85:15) with high content of graphene (60 vol % of solid) to construct mechanically robust and flexible scaffold (with strands ranging from 100 to 1000 µm in diameter) with shape-fidelity and enhanced electrical conductivities >800 S/m for electronic applications. The printed 3D scaffold further revealed their potential biomedical application towards nerve tissue engineering and regeneration by exhibiting their significant cellular response (attachment, proliferation, and neurogenic differentiation) to human mesenchymal stem cells (hMSCs) and viability to multiple, distinct cell types, including hMSCs.

Polymer/clay nanocomposite ink materials based on PEG precursor colloidal solutions incorporated with biocompatible and bioactive disk-shaped 2D laponite (XLG) nanoclay were investigated by Gaharwar group for 3D bioprinting (Figure 11). Such hydrogels based on diacrylated PEG-laponite demonstrated shear-thinning and self-healing properties due to rapid internal phase rearrangement, thus enabling to print a range of complex 3D structures upon UV based photo-crosslinking. The rheological characteristic of the nanocomposite hydrogels was appeared to be independent of addition of PEG and dominated by the behaviour of laponite network, whereas their self-recovery time was found to be controlled by the ratio of PEG:laponite. Further the PEG-(4%)laponite based bioinks exhibited high cell viability of the encapsulated murine preosteoblasts immediately post injection, and the viability was further maintained across different volumetric flow rates 500, 1000, and 200 µL/min as indicated by live/dead assay.
Figure 10. (A) Digital photograph of a 3D printing filament of PVA/GO-HAp nanocomposite solution with 25 wt% PVA concentration. (B) Digital photograph and structural morphology of the 3D printed sample for above nanocomposite based hydrogel having a reticular porous structure. (C) Schematic design of the PVA/GO-HAp nanocomposite based biomimetic gradient porous structure as an artificial cartilage. (D) Digital photograph and SEM images of the 3D printed constructs of PVA/GO-HAp (25 wt%) nanocomposite based artificial cartilage. Adapted from Ref. 104 with permission from The Royal Society of Chemistry.

Another example of nanoclay based polymeric nanocomposite bioink for 3D printing was demonstrated by Zhai et al. derived by supramolecular polymer poly(N-acryloyl glycinamide)
The polymer/clay, PNAGA-laponite XLG, based hybrid hydrogel ink provided the fabrication of a 3D high strength composite scaffold with swelling stability (via post-extrusion UV light irradiation) imparted by the dual amide hydrogen bonding interactions of polymer combine with physical cross-linking of nanoclay-polymer chain interactions. Such PNAGA(20%)-laponite bioscaffolds were able to promote the osteogenic differentiation of primary rat osteoblast (ROB) cells via release of encapsulated Mg$^{2+}$ and Si$^{4+}$ ions from the hydrogel, as suggested by the in vitro studies. Importantly, improved cellular adhesion, proliferation, and differentiation bioactivities were observed for ROB seeded within the PNAGA-laponite composite scaffolds due to the incorporation of clay into such hydrogel system. The in vivo experiments demonstrated the efficient formation of new bone upon the implantation of the PNAGA(20%)-Clay scaffold into tibia defects of rats for 8 weeks.

4. Conclusions and Future Perspectives

The field of 3D printing based additive manufacturing is burgeoning enormously over the past few years and a range of affordable 3D printers are accessible for laboratories to date. A lot of efforts have been devoted to this field, essentially towards (i) engineering and optimization of the printing techniques for enabling the creation of the complex and highly organised structures with controlled processing and higher resolution, and (ii) developing the ink materials with suitable rheological properties and functionality. The particular challenge is the development of (bio)ink materials for 3D printing of biologically relevant scaffolds/tissue replacement, which allows the cells to adhere, differentiate and proliferate, and preserves the bioactivity of their other embedded compounds such as growth factors, signalling peptides, drugs, etc. Currently, researchers are focusing on the designing of (bio)ink materials mainly based on natural polymers or combination of natural and
synthetic polymers, however this approach is associated with several challenges, and therefore the field of 3D (bio)printing is still in its infancy. The introduction of synthetic polymers as a single (or major) network precursor of ink, and the involvement of the functionalization and cross-linking chemistry in the ink formulation

![Figure 11.](image)

**Figure 11.** (A) Digital photograph of PEG-laponite colloidal solution based hydrogel and their printed structure. (B) Schematic of formation of internal structure for PEG, laponite and PEG-laponite nanocomposite solutions at initial time and after 18 hours. (C and D) 3D printed circular and crosshatch structure, respectively, of PEG-(4%) laponite with preosteoblasts and cell tracker images. Adapted with permission from ref 106 Copyright 2018 American Chemical Society.

...can lead to the development of a diverse range of new generation (bio)ink materials with the tuneable mechanical, chemical and biological properties, and therefore can emerge as a key player...
for advancing the field of 3D printing to the next frontier with the envision of creating synthetic and in-human 3D printed tissues. The fundamental understanding of engineering 3D printing technique with controlled processing parameters, evaluating new and advanced polymers-based ink/bioink materials, and designing the biomimetic hard and soft tissue scaffolds with controlled structural integrity and functioning, are critically important to fabricate the targeted tissue/organ, and therefore will drive further innovation in the field of tissue engineering and regenerative medicines.

Conflicts of interest

The authors declare no conflict of interest.

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