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Topical Review

Robotic *in situ* bioprinting for cartilage tissue engineering

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Abstract

Articular cartilage damage caused by trauma or degenerative pathologies such as osteoarthritis can result in significant pain, mobility issues, and disability. Current surgical treatments have a limited capacity for efficacious cartilage repair, and long-term patient outcomes are not satisfying. Three-dimensional bioprinting has been used to fabricate biochemical and biophysical environments that aim to recapitulate the native microenvironment and promote tissue regeneration. However, conventional *in vitro* bioprinting has limitations due to the challenges associated with the fabrication and implantation of bioprinted constructs and their integration with the native cartilage tissue. *In situ* bioprinting is a novel strategy to directly deliver bioinks to the desired anatomical site and has the potential to overcome major shortcomings associated with conventional bioprinting. In this review, we focus on the new frontier of robotic-assisted *in situ* bioprinting surgical systems for cartilage regeneration. We outline existing clinical approaches and the utilization of robotic-assisted surgical systems. Handheld and robotic-assisted *in situ* bioprinting techniques including minimally invasive and non-invasive approaches are defined and presented. Finally, we discuss the challenges and potential future perspectives of *in situ* bioprinting for cartilage applications.

Keywords: *in situ* bioprinting, cartilage tissue engineering, robotic *in situ* bioprinting, minimally invasive surgery, bioinks

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1. Introduction

Three-dimensional (3D) bioprinting comprises a group of additive manufacturing techniques that create 3D constructs using bioinks, formulations containing cells, and biomaterial inks, based on 3D models developed via computer-aided design (CAD) software [1, 2]. The bioprinted structures can encapsulate cells (cell-laden constructs) or be seeded with cells (scaffolds) and are fabricated in the machine working environment, optionally matured in a bioreactor, and implanted into the surgical site [3] (figure 1). This strategy, referred to as *in vitro* bioprinting, generates complex multi-material 3D structures outside of a defect site that allows cell attachment, proliferation, differentiation, production of tissue specific extracellular matrix (ECM), with tissue formation ideally matching the degradation rate of the fabricated structure [4–6]. These tissue constructs can be matured in a bioreactor and stimulated through mechanical and biochemical cues in an attempt to obtain functionally anatomic scale tissues. Alternatively, *in situ* bioprinting, also referred to as intraoperative or *in vivo* bioprinting, is a technique that utilizes robotic-assisted systems or handheld devices and is attracting significant attention as a novel approach in tissue engineering and surgery. This strategy enables real-time lesion treatment by accurately depositing tissue-specific bioinks and biomaterial inks that match the defect shape and can modulate a complex microenvironment to prevent further deterioration and promote tissue repair [7–9]. By depositing cells or biomolecules precisely in the defect site the biological microenvironment can be improved and can allow the recruitment of endogenous cells to the lesion site and thus foster tissue regeneration in an efficient manner [10–12]. Moreover, the anastomosis between the cell-laden construct/scaffold and the defect site can be better controlled as the design of the structure and biomaterials will require a strategy to immediately integrate with the surrounding tissue, which is typically lacking during development of *in vitro* approaches. This integration can be achieved through *in situ* crosslinking or adhesives and be part of the *in situ* bioprinting surgical plan [13].

In situ bioprinting technologies have been investigated for several tissues such as skin [7, 14, 15], bone [16, 17], muscle [18, 19], and cartilage [20–25]. For example, a handheld extrusion-based skin bioprinter has been successfully developed by Hakimi *et al* [7], which allows the covering of full-thickness skin wounds via *in situ* deposition of a homogenous sheet that was also feasible on inclined and compliant wound surfaces that are sensitive to respiratory motion. More recently, fibrin-based sheets containing mesenchymal stem/stromal cells (MSCs) were directly deposited onto wound surfaces to enhance full-thickness skin regeneration [15]. Keriquel *et al* [16], on the other hand, used laser-assisted bioprinting (LAB) to print a bone construct with ring and disc pattern *in situ*, allowing the regulation of cell density and distribution. Additionally, a six-degree-of-freedom (DoF) robotic-controlled printer was developed to directly print bone scaffolds in a large defect living animal model [17]. Moncal *et al* [26], presented an *in situ* bioprinting approach to fabricate bone, skin and hard/soft composite tissues using extrusion-based and droplet bioprinting in complex craniomaxillofacial defects of rats. An osteogenic biomaterial ink was able to induce bone regeneration covering a high (∼80%) defect area after 6 weeks. A stratified arrangement was also successfully achieved using a hybrid bioprinting approach with a controlled spatial bioink deposition.

Unlike bone and skin, articular cartilage (AC) has limited ability to self-heal, and is difficult to repair or regenerate due to its unique microenvironment that is avascular with low cell metabolic activity. A major barrier to cartilage regeneration is the failure of integration of the implant with the host tissue, causing the degradation of the surrounding cartilage [27–30]. Hydrogels, the primary biomaterials used for bioprinting, are relatively weak in mechanical properties and require strictly sterile clinical conditions, making *in vitro* bioprinted constructs difficult to handle, transfer, and implant. Additionally, prefabricated *in vitro* scaffolds must conform to complex defect morphologies (e.g. curved surfaces), which may only be discovered during arthroscopic investigation. Therefore, multiple surgeries and high-resolution imaging and image reconstruction may be required to generate a patient specific scaffold. Furthermore, scaffolds are difficult to suture with the host tissue and non-adhesive tissue-scaffold interactions would result in further deterioration of the defect [31]. *In vitro* bioprinting requires cells to be expanded prior to construct fabrication and implantation, unless using an acellular strategy and recruiting cells *in vivo*, with the implant needing maturation in a bioreactor. Indeed, these are time- and cost-consuming procedures that mismatch the urgent clinical need and increase the economic burden on financial stretched clinical settings.

In contrast, *in situ* bioprinting can provide real-time treatment during arthroscopic exploration and correlates geometry and high integration with the defect site providing unique advantages for cartilage surgery. The amount of time and resources spent in cell expansion can be also reduced without the need for bioreactors, as the human body is a perfect 'native' bioreactor providing biochemical and biophysical cues [3, 32, 33].

This review provides an insight into current clinical therapies and tissue engineering strategies for cartilage regeneration followed by an overview of minimally invasive and robotic-assisted surgical systems to provide a perspective on developments within *in situ* bioprinting. The development of cartilage specific bioinks and *in situ* bioprinting techniques are described with alternative micro-robotic solutions highlighted. Finally, the challenges and opportunities associated with *in situ* bioprinting and subsequent clinical translation are thoroughly discussed providing a future perspective.

2. Cartilage tissue repair

AC is a specific form of hyaline cartilage found on synovial joints (e.g. knee and shoulder) that endows the joint with a low-friction and load-bearing surface, allowing smooth movement and bearing up to 3.5 times of the body's weight [34]. The lack of vascularization, innervation, and a lymphatic

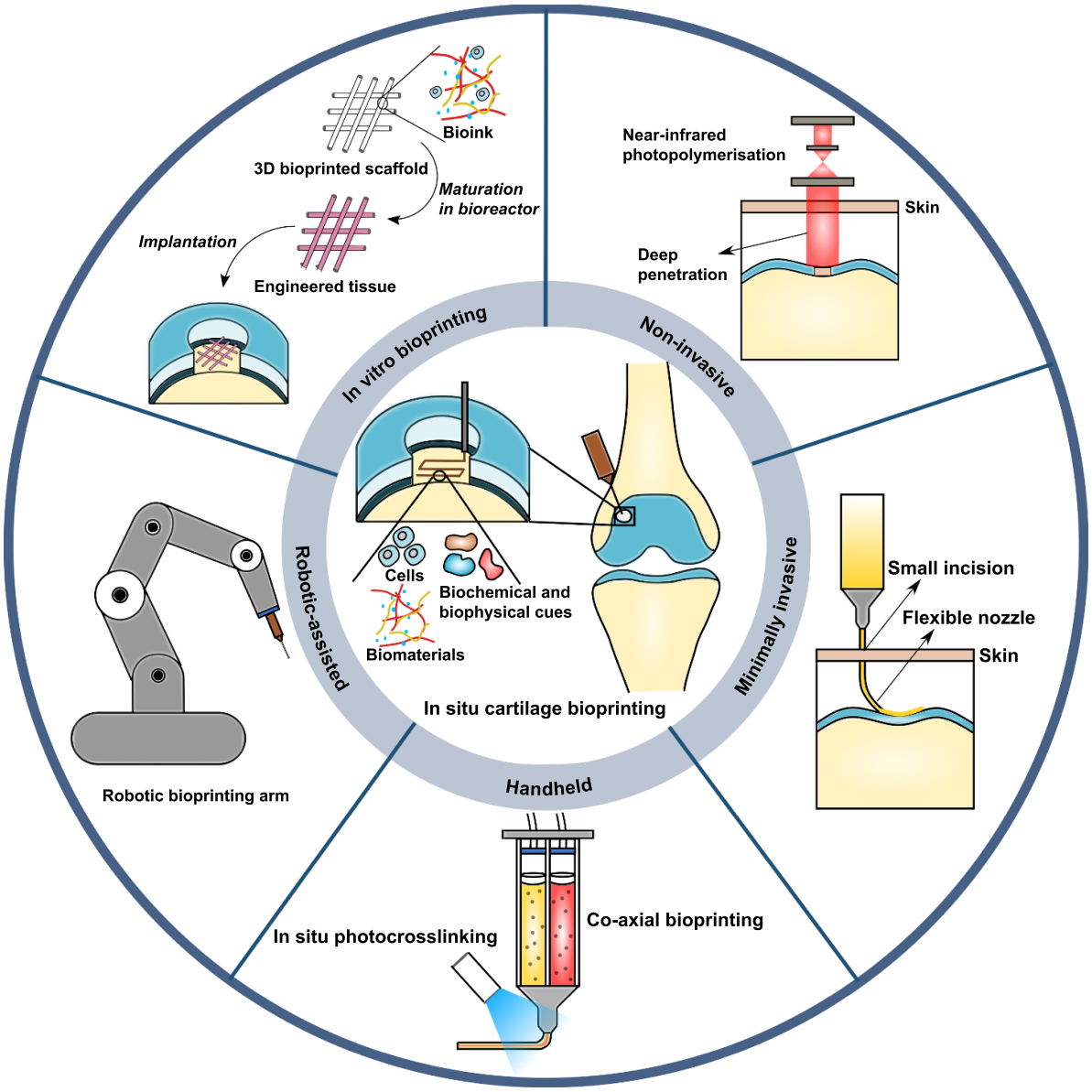

Figure 1. Schematic illustration of key strategies for *in situ* cartilage bioprinting. *In vitro* bioprinting allows the fabrication of constructs outside of a defect location, which can be matured in a bioreactor, followed by implantation in the surgical site. Robotic-assisted, handheld, minimally and non-invasive *in situ* bioprinting allows the direct deposition of bioinks in the defect site.

system, coupled with low metabolic activity of resident chondrocyte cells, the inability to obtain circulating progenitor cells, and a limited nutrient supply results in poor innate tissue self-regeneration [35]. Consequently, traumatic injuries or degenerative pathologies and the subsequent inadequate repair leads to the progressive and irreversible degeneration of cartilage tissue, as well as changes in the adjacent synovium and bone tissue, eventually resulting in osteochondral problems, pain and mobility issues, and even disability [36]. The global health and economic burden of AC and osteochondral related diseases such as osteoarthritis is increasing due to an ageing population thus requiring improved interventions when clinically appropriate [37].

Current non-surgical cartilage therapies are typically palliative and aim to provide pain relief and slow down the progression of tissue degeneration but fail to cure the joint disease. The increase in cartilage defect size and depth as the grade of injury deteriorates eventually requires surgical intervention and in the end-stage of the disease a total joint replacement [30, 38]. Surgical procedures that aim to repair cartilage defects are available, but the appropriateness of the intervention is dependent on the underlying pathology of the defect, for example, arthroscopic debridement is typically not recommended for osteoarthritis [37, 39]. Marrow stimulation such as microfracture is widely used but can lead to the development of fibrocartilage instead of hyaline cartilage, which exhibits inferior mechanical properties and longevity comparing to the native AC tissue [40]. Autografts deliver mature hyaline cartilage to the defect but requires a source from a minimal load bearing region such as the distal femur, but this can result in donor site morbidity and is only able to repair small defects less than 2 cm^2 [41]. Alternatively,

allografts, although granted with a degree of immune privilege due to its avascularity [42], can treat larger defects but are difficult to match up with the native cartilage in geometry, thus leading to the imbalance of biomechanical cues and the degradation of joint [43, 44]. Additionally, grafts have a high failure rate of up to 55% after 10 years due to poor integration [45]. Cell-based therapies such as autologous chondrocyte implantation (ACI), matrix-induced ACI, autologous matrix-induced chondrogenesis, and bone marrow aspirate concentrate are promising approaches to repair large defects [30, 31, 46]. However, the long-term efficacy, integration, and phenotypic stability is unclear. Furthermore, the need for multiple procedures, including the extraction and expansion of chondrocytes from healthy AC and subsequent implantation, increases the complexity of the surgery, patient hospitalization time, and economic burden [30, 31].

Tissue engineering is becoming a rapidly emerging and appealing approach to repair and regenerate damaged cartilage tissue [30, 31, 40, 47–49]. Natural biomaterials such as collagen [50], hyaluronic acid (HA) [51, 52], chitosan [53], gelatin [54, 55], and silk [54–56], as well as synthetic biomaterials such as polycaprolactone (PCL) [57], polylactide [58], and poly (lactic-co-glycolic acid) (PLGA) [59, 60] have been used and processed via 3D printing technologies to produce scaffolds for cartilage and osteochondral engineering.

However, how engineered tissue constructs/scaffolds integrate with the native cartilage tissue remains unsolved. The efficient integration of implant to the native AC tissue is achieved by continuous deposition of ECM at the defect site, whilst the interfacial strength is derived from the deposition of collagen and the formation of collagen fibrils through the enzyme lysyl oxidase which allows covalent bond formation and collagen crosslinking [34]. However, due to the intrinsic properties of cartilage, this integration is a significant challenge especially in trauma or disease induced inflammatory environments common in AC degradation. Osteoarthritis is a highly inflammatory disease so the regulation of this response by biomaterials is key [37, 61]. The engineering of immune-responsive scaffolds has been demonstrated by Zhang *et al* [62] who developed a 3D printed bone scaffold incorporating manganese carbonyl that reduced inflammation by upregulating the M2 phenotype of macrophages. Furthermore, cartilage defect sites are surrounded by dead cells that AC is not able to resorb, thus generating a physical barrier for hyaline cartilage formation and implanted graft adhesion [27]. The resident chondrocytes with limited ability to migrate might be further blocked by the layer of damaged tissue while the viable chondrocytes are critical for integration and seamless ECM formation [28, 63–65]. Additionally, proteoglycans and glycosaminoglycan (GAG) present anti-adhesive properties. Moreover, it has been shown that the metabolism of chondrocytes is changed at the defect site as demonstrated by Karim and Hall [66], as inflammatory factors in the permeated synovial fluid resulted in abnormal chondrocyte morphology, doubled cell volume, and clustering of chondrocytes which are all associated with osteoarthritis. Mechanotransduction pathways are essential to regulate the matrix synthesis of chondrocytes, whilst injurious compression changes the morphology of chondrocyte organelles which is responsible of biosynthetic functions [67, 68]. These unique characteristics of damaged chondral and osteochondral defects places a high standard on clinical applications that current *in vitro* approaches struggle to satisfy. In the following sections, *in situ* bioprinting, an emerging approach for directly bioprinting into a defect and promoting tissue-scaffold integration, is discussed in detail, providing an insight view of this approach for future cartilage engineering.

3. Minimally invasive and robotic-assisted surgery

The development of *in situ* bioprinting technologies requires an understanding of invasive and robotic-assisted surgical techniques. As the development of *in situ* bioprinting should not develop in isolation and unaware of developments in surgical technologies, but rather in a complimentary direction in partnership with clinicians and commercial companies active in the field. Furthermore, many technological advancements such as in kinematics (movement), control and feedback systems, surgical tools, and visualization processes can be adopted and adapted for *in situ* bioprinting. Since many of the existing systems have had significant development there may be a direction of travel that the *in situ* bioprinting technologies become part of the suite of end-effector tools, thus allowing quicker adoption of the technology.

Minimally invasive surgery (MIS) or arthroscopic (keyhole) surgery is rapidly becoming the gold standard for cartilage assessment and intervention. Arthroscopic surgery typically involves the use of endoscopic devices (e.g. vision systems and surgical tools) that are inserted via small incisions into a joint [69, 70]. Compared to the open surgery, the trauma is significantly reduced which decreases pain, improves recovery rate, lowers the incidence of post-surgical complications, shortens the hospital visit, and reduces the cosmetic scarring. However, MIS is not suitable for all situations and presents limitations for surgeons such as a reduction in surgical operating space and vision, reduction in haptic feedback, decreased hand-eye coordination, lack of stereo vision, longer operations, and higher costs. The development of flexible endoscopes with 3D cameras and high-resolution images can improve visualization of the surgical site and tool handling. But the miniaturization of surgical tools to fit within an endoscope or trocar whilst maintaining functionality is challenging. These tools are difficult to handle and operate precisely as they lack the articulation and manipulation of conventional tools with human hands. Subsequently, MIS requires extended trainings with a steeper learning curve.

Hence, there is a requirement for robotic systems that can support the surgeon with the precise operation of instruments and reduction in the cognitive burden of complex and time-consuming surgeries. Over the past decades, robotic-assisted surgical systems have been developed to overcome the limitations of pre-existing surgical procedures such as surgical precision [71]. In robotic-assisted surgery, robotic arms are controlled remotely through computer-aided devices

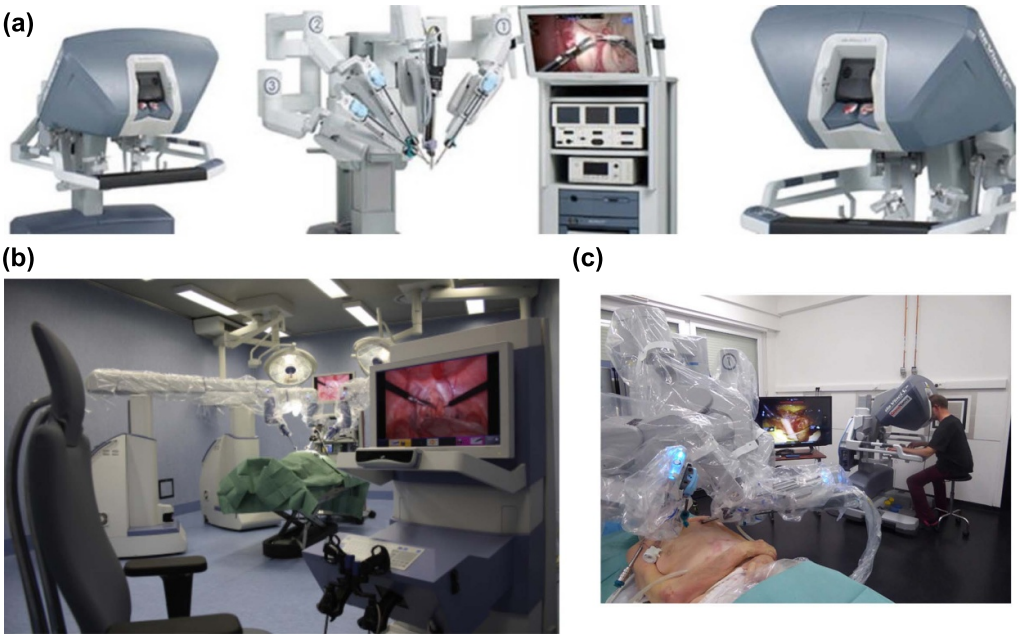

Figure 2. Examples of current robotic assisted surgical systems. (a) The da Vinci® Surgical System (Intuitive Surgical, USA). Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, Surgical Endoscopy [86], Copyright (2012). (b) The operating room with the Senhance Surgical System (Asensus Surgical, USA). The console is in the front, with the patient table, manipulator arms, laparoscope, and instruments. The secondary monitor displays the same image of the operating field as the surgeon. Reprinted from [87], Copyright (2020), with permission from Elsevier. (c) Endoscopic robot-assisted da Vinci SI® robotic system in minimally invasive surgery. Reproduced from [88]. CC BY 4.0.

to execute the actual operation for surgeons. These robotic arms can be equipped with cameras (e.g. flexible endoscope) and articulated end-effectors mimicking the freedom of movement of the human fingers and wrist. Robotic arm assisted total knee arthroplasty (TKA) and unicompartmental knee arthroplasty (UKA) have been utilized to treat knee osteoarthritis, which improves patients' functions and quality of life [72]. Since ACROBOT (Active Constraint Robot, Imperial College, UK), the first developed robotic system for TKA [73], several commercial robotic assisted systems were developed, such as the da Vinci Surgical System (Intuitive Surgical, USA) (figures 2(a) and (c)), Senhance Surgical System (Asensus Surgical, USA) (figure 2(b)), Robotic Arm Interactive Orthopaedic System (RIO MAKO, Stryker, USA), ROBODOC system (Zimmer Biomet, USA), and the Rosa Knee System (Zimmer Biomet, USA) is the latest robotic system approved by the FDA in 2019 [4].

Kayani *et al* [74, 75], performed TKA/UKA using a robotic assisted system and compared this with conventional jig-based TKA/UKA regarding early postoperative functional results and time from hospital enrolment to discharge. The findings showed that the robotic arm aided TKA improves the implant placement accuracy and reduces outliers. The early functional recovery is promoted, and the time of hospital discharge is shortened. Robotic arm aided UKA results in reduced postoperative pain and increases the maximal knee flexion after discharge compared with conventional UKA. Theoretically, robotic-assisted TKA is more precise in terms of the component location than mechanical-assisted TKA. Deckey *et al* [76], found that robotic assisted TKA is statistically more accurate in planning the component placement as well as the final polyethylene insert thickness. However, previous studies demonstrated that the precision of bone resection and the implant is dependent upon the surgeon's expertise and experiences [76–78]. It is also unclear whether enhanced precision and accuracy improve patient satisfaction and clinical outcomes. Additionally, Kim *et al* [79], conducted a randomized long-term study (with a minimum follow-up of 10 years) to evaluate the clinical results of patients who had traditional TKA against robotic-assisted TKA; however, no difference was observed between the two groups in terms of their outcome ratings, mean implant or limb alignment, survivorship, or complications.

There are three types of robotic surgical systems: shared controlled, supervisory controlled, and tele-surgical systems, depending on the guidance provided by the surgeons. A robotic-assisted surgery procedure can be divided into several phases. Figure 2(b) shows a typical operating room set up with the Senhance Surgical System (Asensus Surgical, USA) [80, 81]. The surgical tools and robotic instruments are firstly prepared followed by sterilization and the positioning of the patient in the operating room. After the skin incision, the ports are inserted followed by the trocar installation by the surgical team to access the target anatomy. In the docking phase, the patient cart moves to the operating table and the robotic arm is positioned to avoid collision. After the visualization system is installed, the operating procedure is performed by the surgeon. When the surgeon announces the completion of the surgery, the instruments are moved away, and the robotic system can be undocked from the ports.

The robotic assisted surgical systems are continuously being optimized to improve the human-machine interface. For example, consoles and joysticks enable surgeons to sit in the operation [82]. The integration of high-resolution visual systems (e.g. improved optics) allows surgical procedures with better precision and stability. The torque and force sensors at the tip of instruments receive and transfer the feedback to surgeons, allowing them to control the movements of surgical instruments carefully based on the applied forces [83, 84]. In addition, novel approaches are being explored such as the incorporation of haptic interactions and remote HD-3D-technology with eye-tracking control system endowing surgeons with capabilities to zoom in/out the image via moving their head forward or backward and center the screen by pressing buttons on the handles [85].

4. *In situ* bioprinting technologies for cartilage tissue engineering

Bioprinting technologies currently utilized for *in situ* bioprinting include extrusion-based, inkjet, light-based, and LAB systems [8, 9, 16, 26, 89–91]. Inkjet bioprinting dispenses droplets onto a substrate, but can be limited due to the potential clogging of printing heads with the use of high viscosity bioinks and cell density [5, 92]. As a result, bioinks with low viscosity and low cell density are desirable, but this can lead to additional constraints such loss of printing precision [92–94]. LAB based on light-induced forward transfer technology normally employs an energy-absorbing layer, a highly intense laser pulse, and a substrate [95, 96]. LAB allows high printing resolution and high cell density of bioinks, but the complexity may be a hindrance for utilization in a surgical environment. As one of the most popular bioprinting techniques, extrusion-based systems assisted by either a pneumatic or mechanically driven mechanism allows the continuous deposition of bioinks with a broad range of viscosity and high cell density [97]. The flexibility of extrusion-based bioprinting enables the integration of various physical and chemical crosslinking methods and allows the integration into MIS, both of which are significantly attractive for *in situ* bioprinting [13, 98, 99]. This section will provide a discussion on bioink considerations with a focus on their use in extrusion-based bioprinting. Current developments regarding *in situ* bioprinting for cartilage applications are highlighted and potential future directions demonstrated through minimally and non-invasive approaches.

4.1. *Bioinks for* in situ *cartilage bioprinting*

Bioinks can be defined as a formulation of cells eventually containing biomaterials and bioactive factors that can be processed through automated biofabrication technologies [2]. To assist the fabrication of biologically functional products, bioinks must fulfill essential criteria including printability, cytocompatibility and ability to instruct desired cell responses. Therefore, bioink design is a challenging task as it needs to consider multiple properties influencing not only the bioprinting process, but also the biological functionality of bioprinted constructs. In addition, requirements such as the gelation kinetics, presence of synovial fluid, cell sedimentation, printing resolution as well as host tissue adhesion and integration should be considered when engineering bioinks for *in situ* cartilage bioprinting.

The development of bioinks is a multifactorial process that should start by considering the characteristics of the construct to be bioprinted at structural (e.g. architecture, complexity, resolution), compositional (e.g. cells, biomaterials, bioactive factors) and biofunctional (e.g. mechanical performance, cell-instructive properties) levels (figure 3). Indeed, such construct characteristics determine the selection of biomaterials, crosslinking reactions and the most suited bioprinting technology, among others. Natural polymers such as HA and gelatin have been widely used to create bioinks for *in situ* cartilage bioprinting due to their role in native cartilage tissue and cell adhesive properties, respectively [21–23]. Synthetic polymers like poly(ethylene glycol) (PEG) have been less explored as they require further biofunctionalization to support cell functions, though they can also be used to improve mechanical properties of bioprinted multi-material constructs [24, 100]. Regarding the bioprinting technology, inkjet and LAB bioprinting provide higher resolution than extrusion bioprinting [94], being suited for applications where superior resolution is a key requirement. However, bioink rheological properties and cell density are dependent on each technology, which further increases the complexity in bioink design.

At rheological level, *in situ* bioprinting can alleviate some challenges related to construct shape fidelity as bioinks are usually deposited into the defect site as macroscopically solid constructs, which are contained within the lesion site. Despite this can potentially reduce the complexity in bioink design, the bioprinting of porous constructs, along with the gravity and injury site location, impose additional constrains to the bioink rheological properties. For extrusion bioprinting, bioinks typically exhibit a shear thinning behavior and high viscosity, which has been mostly achieved by pre-crosslinking or by using high molecular weight polymers (e.g. HA) or rheology modifiers (e.g. nanomaterials) [101–103]. Such bioinks can also be bioprinted using LAB technology, reducing cell settling and sedimentation issues throughout the bioprinting process, while improving shape fidelity without the need for immediate shape consolidation post-printing. On the other hand, low viscosity bioinks are required for inkjet bioprinting restricting the fabrication of complex constructs. To overcome this issue, bioinks exhibiting fast thermal gelation at body temperature or undergoing rapid sol-gel transition through *in situ* photocrosslinking have been explored as valuable alternatives [104–106]. In this regard, some natural polymers undergo gelation under mild conditions without the need for chemical modification such as the case of collagen and methylcellulose, with thermal gelation occurring at physiological temperature (37 °C), as well as alginate that can be physically crosslinked in the presence of divalent cations. Additionally, thermosensitive synthetic injectable hydrogels have been developed for cartilage applications that undergo sol-gel transition at physiological temperature [106]. Despite the attractive features of

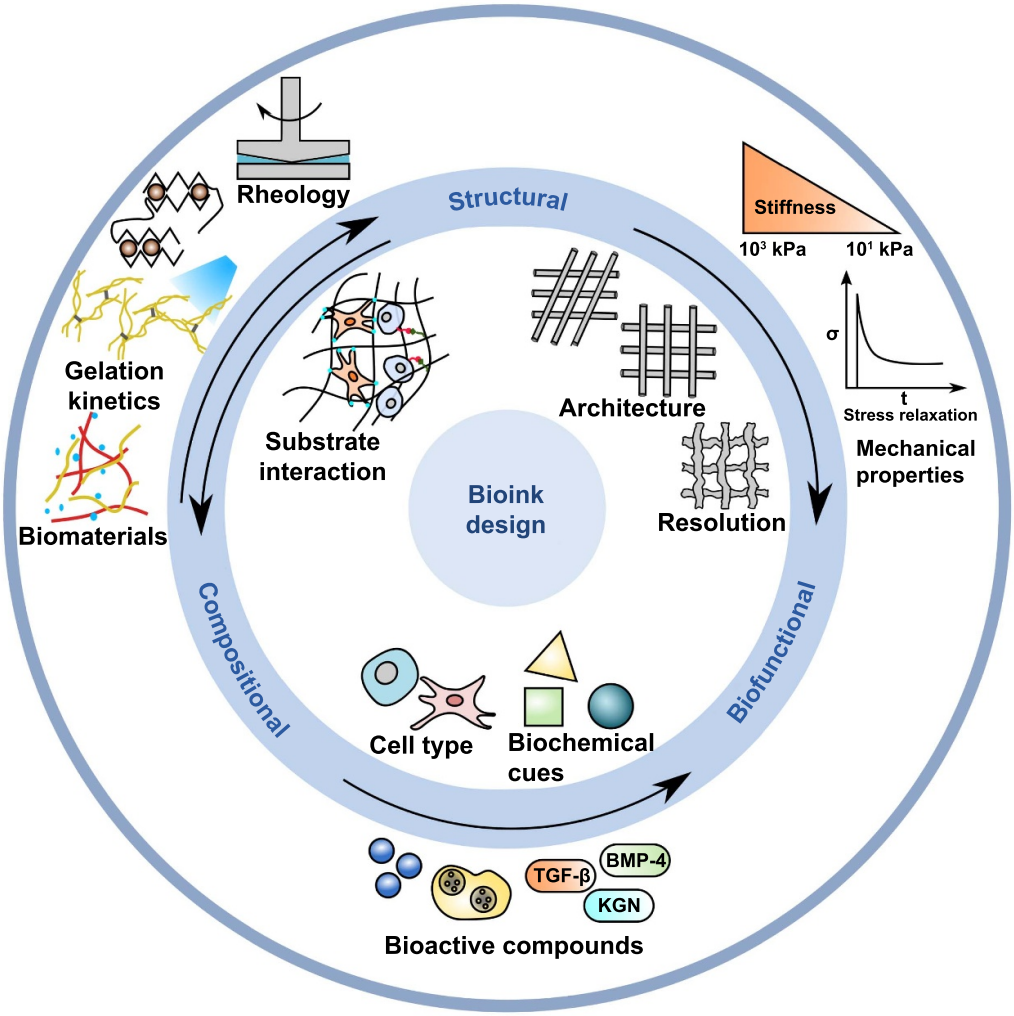

Figure 3. Schematic illustrating key factors of bioink design for cartilage tissue repair.

physical crosslinking and the relatively simplicity to perform, its relatively slow gelation kinetics and, in some cases, sensitivity to the composition of biological fluids (e.g. ionic gelation), along with the limited mechanical properties and stability of bioprinted constructs arise as major drawbacks. To address these issues, significant developments have been made on the design of photocrosslinkable bioinks, which allow for very fast *in situ* sol-gel transition in the presence of cells and surrounding tissues [94]. Examples to impart photocrosslinking ability include the methacrylate modification of HA and gelatin polymers, two major components of bioinks for cartilage regeneration [23, 107]. Although chemically crosslinked constructs exhibit superior mechanical properties due to the stable nature of covalent bonds, the formation of gel network heterogeneities can affect local mechanical properties and cellular responses, while the degradation behavior can also be difficult to control [108]. Another important aspect concerns to the dual role of photopolymerization, which can be useful to improve construct adhesion to biological tissues, but can also induce harmful effects regarding the cell viability. Furthermore, photocrosslinking may not be easy to implement due to its limited accessibility to cartilage defect site during the keyhole surgery and the poor tissue penetration of ultraviolet (UV) and blue light.

A major challenge in bioinks for cartilage regeneration relies on the mismatch between the implant and the native cartilage tissue, which can affect the functional properties of bioprinted cartilage tissue. Cell source is key in the development of cellular-based therapies including for bioinks and cell seeded scaffolds. Typically, for cartilage repair, chondrocytes are the primary cell type to be considered since they are the resident cell population of cartilage and able to produce native ECM. However, the difficulty of maintaining the chondrogenic phenotype *in vitro* and *in vivo,* along with the limited sources of supply present a barrier for their application. MSCs due to their chondrogenic differentiation capacity, high proliferation rate, immunomodulation, anti-inflammatory effects, and low immune response, are widely used in cartilage applications [109–112]. They can be isolated from a variety of tissues such as bone marrow, adipose tissue, umbilical cord, and placenta, and dental pulp. However, the efficacy of first-generation MSC-based clinical trials in a variety of diseases is unclear [113, 114]. Alternatively, resident skeletal stem cells, distinct from MSCs, have been shown

to be recruited during microfracture surgery and differentiation steered towards hyaline cartilage lineage through localized delivery of bone morphogenic protein-2 (BMP-2) and VEGFR1 [115]. Induced pluripotent stem cells and embryonic stem cells can also be differentiated towards a chondrogenic lineage and can potentially provide a supply of unlimited differentiated chondrocytes and chondroprogenitor cells [112, 116–118]. However, these pluripotent cell types need to be pre-differentiated towards chondrogenic linage before transplantation and carefully used in clinical applications due to the issue of tumorigenicity. If undifferentiated/immature cells and reprogramming factors remain in the final product, or genetic mutations occur during *in vitro* culture there is a risk of tumor or teratoma formation. Finally, as biological outcomes can significantly vary between different cell sources, understanding the underlying biological processes and cell-material interactions is key to differentiating and maintaining an AC chondrogenic phenotype during the development of a bioink [119].

Cell fate is widely recognized to be modulated by manipulating biophysical and biochemical cues of cell microenvironment [120]. The functionalization of biomaterials to allow the sequestration and presentation of biomolecules (e.g. growth factors and nucleic acids) to the cells is another attractive strategy to guide chondrogenesis and improve the biological functionality of bioprinted constructs [121]. As the presence of biomolecules is essential in modulating cellular activities such as cell proliferation, migration, secretion, differentiation, and maturation in the process of cartilage repair [117, 122]. For example, transforming growth factor β1 (TGF-β1), promotes deoxyribonucleic acid (DNA) synthesis and GAGs and collagen type II expression, and BMP-4 which induces MSC chondrogenic differentiation, were incorporated into two distinct and separate alginate layers and injected into a osteochondral defect *in vivo* for up to 6 months [123–125]. TGF-β1 and BMP-4 loaded hydrogels resulted in efficient restoration of hyaline cartilage whilst disorganized hypertrophic tissue and fibrocartilage was observed in unloaded defects. Kartogenin (KGN) is a promising small non-protein molecule that can upregulate chondrogenic gene expression and stimulate MSC differentiation into chondrocytes was loaded with a PCL matrix and co-printed with a bioink containing MSCs and methacrylated HA [126, 127]. Cell proliferation was accelerated and chondrogenic gene expression increased including TGF-β1, SOX-9, and aggrecan. However, the burst release (10 d) is a non-negligible challenge considering the long-term *in vitro* and *in vivo* studies required to assess stable phenotypic AC formation.

Moreover, AC has a dense matrix and biomolecule penetration and clearance within the joint via either vasculature/lymphatic system or via phagocytosis poses an extra contradiction and challenge [128]. For example, small molecules ($<$1 kDa) such as non-steroidal anti-inflammatory drugs can penetrate the dense matrix, but it is also rapidly cleared from lymphatic vessels and diffuses into surrounding biological fluids. Microscale biomolecules can remain in a joint regardless of the joint inflammatory status, but diffusion is limited through the nanopores of the AC matrix [129] and particles below 5 μm will be cleared by macrophages [128, 130]. Therefore, various aspects such as particle size, loading efficiency, delivery, and binding mechanism should be considered in bioink design. Commonly used delivery strategies are physical entrapment or immobilization. Physical entrapment simply mixes biomolecules with bioink before or after solidification, whilst immobilization binds biomolecules via physical adsorption, chemical bonding, or secondary associations. The release kinetics strongly depend on the matrix pore size, degradation, dissociation rate, and diffusion properties [128]. Physical entrapment endows bioinks with higher loading efficiency and retains functionality but with an uncontrolled release manner, whilst immobilization can provide an engineered controlled release rate via chemical modification but accompanied with the risk of conformational change [131, 132]. Micro- and nanoparticles can either be independent delivery systems or combined with a macroscale systems allowing sustained and controlled release of drugs [131]. Nanoscale drug loaded particles are particularly attractive for cartilage repair due to their penetration capacity. Cai *et al* [133] used biomimetic copper sulfide nanoparticles loaded with plasmid DNA encoding TGF-β1 and surface-coated with phosphatidylcholine to engineer MSCs, allowing their promoted chondrogenic gene expression, GAG deposition, and collagen type II expression. Feng *et al* [134] loaded KGN into cyclodextrin nanoparticles and incorporated them into microgels containing MSCs, contributing to high cell viability, storage and processing, and chondrogenic differentiation. Moreover, micro- and nanoparticles can be incorporated with 'smart' biomaterials which delivers drugs responded to endogenous/exogenous stimulus [135]. This strategy will allow the release of drugs at the target sites and at the desired rate.

Apart from biochemical cues, the biomechanical properties of bioprinted constructs act as a master regulator of cell fate and tissue morphogenesis [136–139]. Therefore, bioinks should be designed to match the mechanical properties and anisotropy of native cartilage tissue towards providing an optimal niche for the cells. A common strategy to improve construct mechanical properties include the development of multi-material bioinks [140], though it increases the complexity in independently controlling bioink printability, construct properties and cell response [103, 141]. Despite alternative strategies for hydrogel reinforcement such as the use of thermoplastic polymers are useful for bioprinting outside the body [142], their translation for *in situ* bioprinting is challenging due to the processing conditions. In addition to mechanical properties, the functionalization of biomaterials to allow the sequestration and presentation of growth factors to the cells is another attractive strategy to guide chondrogenesis and improve the biological functionality of bioprinted constructs [103, 121].

4.2. In situ *bioprinting approaches*

Two main *in situ* bioprinting approaches, handheld and robotic-assisted systems, are discussed in this section. Furthermore, the utilization of minimally and non-invasive techniques is highlighted although they have not yet been demonstrated in cartilage repair. Table 1 summarizes recent

Table 1. Summary of handheld and robotic-assisted *in situ* bioprinting utilized in cartilage applications. Novel minimally and non-invasive *in situ* bioprinting and micro- and nanorobotic therapies for *in situ* repair and regeneration that could potentially be used in cartilage applications are highlighted.

	Printing devices and techniques	Bioinks (with cells)/biomaterial inks (without cells)	Defects/biological study	Results		References
				3D printing	Tissue formation	
Handheld device	Pneumatic extrusion-based Biopen equipped with UV light source	GelMA/hyaluronic acid methacrylate (HAMA), allogeneic adipose-derived MSCs	*In situ* 8 mm circular full-thickness chondral defect	A handheld biopen allowing the simultaneous coaxial extrusion was designed and fabricated.	The new hyaline cartilage tissue formation was found in Biopen printed area.	[23]
	Co-axial core/shell Biopen with UV light	GelMA/HAMA, infrapatellar adipose-derived mesenchymal stem/stromal cells (ADSCs)	*In vitro* study in the core/shell structure	The coaxial printing delivered a hydrogel of uniform chemistry.	The printed hydrogel showed sufficient stiffness at the chondral lesion and high cell viability, potentially for cartilage engineering.	[21, 22]
Robotic-assisted device	Remote center of motion (RCM) based robotic system with minimal invasive extrusion 3D printing	Alginate, Poly(ethylene glycol) diacrylate (PEGDA)	Osteochondral defect	The RCM-based mechanism has been used for the extrusion 3D printing for the first time, which allows the minimization of the risk of scaffold contamination.	/	[24]
	Robotic extrusion system	Alginate, demineralized bone matrix, powered gelatin	*In situ* chondral circular defect with 16 mm diameter and 4 mm depth, and osteochondral defect with 4 mm extended underlying deep	Both defects had less than 0.1 mm mean surface errors due to novel geometric feedback and path planning methods.	/	[25]
	6 DoF robotic, extrusion-based bioprinting unit	HAMA and 4-Armed (acrylate-terminated polyethylene glycol) PEG-ACLT, ADSCs	*In situ* osteochondral defect with 4 mm height and 5 mm diameter	A 6-DOF robot was developed with a fast tool center point calibration method to enhance printing accuracy.	*In vivo* results showed the defects were fully filled by new tissue after 12 weeks.	[20]
	Extrusion-based printer and 3D handheld scanner	Alginate, HAMA, PEGDA	Large segmental defects of long bones, free-form fracture of femoral condyle, and chondral lesion	Precise 3D constructs were obtained rapidly (10–15 min, including scanning and printing), and printed *in situ* perfectly using photocurable hydrogels.	/	[100]

(Continued.)

Table 1. (Continued.)

	Printing devices and techniques	Bioinks (with cells)/biomaterial inks (without cells)	Defects/biological study	Results		References
				3D printing	Tissue formation	
Minimally invasive	Ferromagnetic soft catheter robot (FSCR)	Hyaluronic acid, Pluronic F127, Ecoflex, polydimethylsiloxane, silver flakes, PEDOT: PSS, Polycarbophil, human bladder epithelial cells	*In vitro* study on the surface of porcine tissue; *in vivo* minimally invasive bioprinting on the rat liver surface	A digitally controlled *in vivo* minimally invasive bioprinting with high accuracy was achieved by the FSCR system.	/	[143]
	Flexible miniaturized high DoF robotic arm extruder fixed on snake-like body	GelDAT, L929 cells	*Ex vivo* bioprinting on a porcine kidney; *in situ* bioprinting onto the inner surface of the colon phantom	This system enables the printing onto multiple tissues and allows bioprinting through MIS or using natural orifices to reach confined anatomical locations.	/	[144]
Non-invasive	Digital near-infrared (NIR) photopolymerization (DNP)-based 3D printing system	GelMA, articular chondrocytes, ADSCs	*In vivo* bioprinting of acellular and cell-laden ear-shape constructs	Ear-like tissue was successfully bioprinted using noninvasive *in vivo* 3D bioprinting via a digital NIR DNP-based technology.	Printed chondrocyte-encapsulated construct was maintained after 1 month culture *in vivo*. The cartilage-like tissue was formed with the growth of chondrocytes.	[90]
	The NIR associated with high-spatial resolution intravital multiphoton microscopy	7-Hydroxycoumarin-3-carboxylate, polyethylene glycol, gelatin, 7-carboxymethoxy-4-methylcoumarin, human umbilical vein endothelial cells, human embryonic stem cell, muscle-derived stem cells, human embryonic stem cell-derived neural stem cells	Intravital bioprinting for skin, skeletal muscle and brain tissues of live mice	The injectable bioinks were used to successfully fabricate spatially controlled complex 3D constructs into the anatomical site, followed by photocrosslinking via NIR laser light.	The printed hydrogels showed high biocompatibility, spatially controlled donor-cell grafting, and incorporation of a functional vascular network.	[91]

progress and relevant studies for cartilage tissue repair and regeneration.

4.2.1. Handheld device approach. Handheld *in situ* bioprinting is an approach that uses the surgeon's own hands for freeform control over the movement of the deposition device rather than a computer-controlled system. Subsequently, this could be considered a quasi-3D bioprinting approach due to the lack of computer control. However, simple scaffold-like structures, multi-material printing, and *in situ* crosslinking are all possible with currently developed handheld *in situ* bioprinting devices. Although when only utilized for defect or wound filling they could be considered as an advanced *in situ* injection device rather than a printing tool. The handheld approach is advantageous as bioinks and biomaterial inks can be directly deposited into the defect or wound site without requiring medical imaging data, toolpath generation, or in the case of robotic-assisted devices the ensuing complex multi-system surgical theatre set-up. The simplicity,

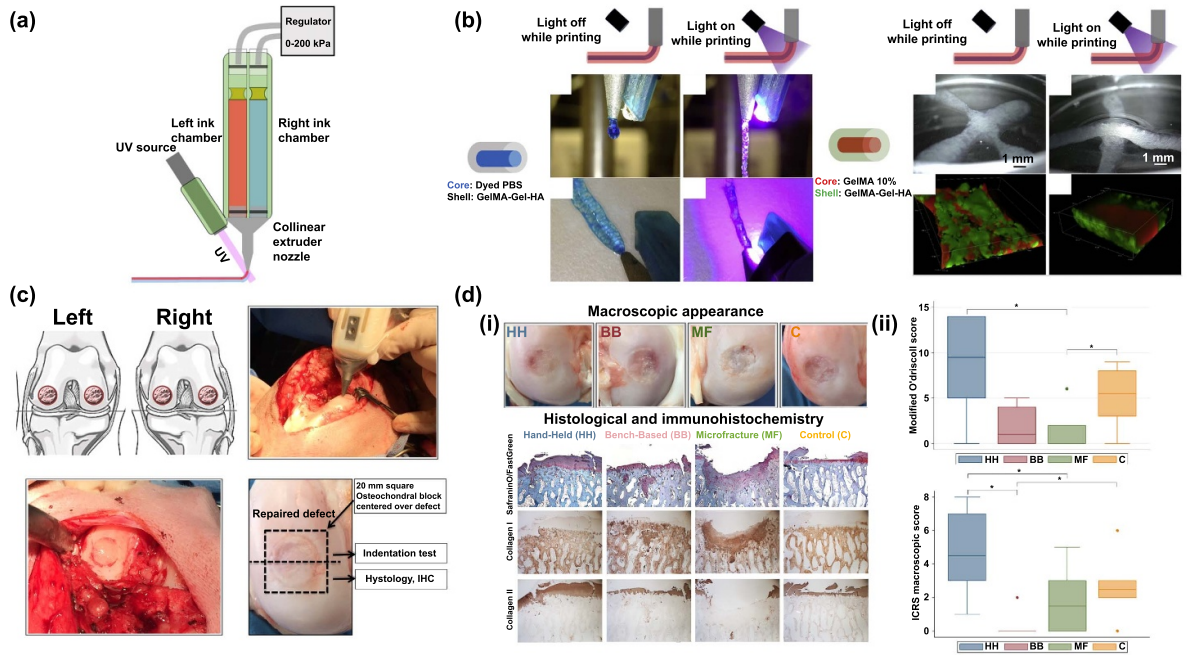

Figure 4. Handheld device for *in situ* bioprinting. (a) Design of the 'Biopen', which includes two chambers connecting to the orienting head, allowing printing core–shell structure with two different bioinks. Reproduced from [21]. © IOP Publishing Ltd. All rights reserved. (b) *In situ* crosslinking with and without light on for printed coaxial filaments (core: dyed PBS, shell: GelMA/gelatin/hyaluronic acid) of the 'Biopen', and confocal microscopy images. Reprinted from [104], Copyright (2020), with permission from Elsevier. (c) Full-thickness chondral defects filled *in situ* with bioinks using the handheld device. (d–i) Macroscopic appearance and histological and immunohistochemistry analysis of *in situ* printed and comparison groups with Safranin O staining, collagen I and collagen II analysis; and (d–ii) Modified O'Driscoll score and international cartilage repair society macroscopic score. [23] John Wiley & Sons. Copyright © 2017 John Wiley & Sons, Ltd.

portability, and speed that a handheld device can be deployed is potentially beneficial in emergency trauma situations and during exploratory surgery (e.g. arthroscopy). As a significantly simpler system compared to robotic-assisted devices, the devices will be cheaper thus can be more widely deployed and potentially much simpler to train to use. However, handheld bioprinting requires accessible wound sites such skin and muscle or the use of invasive open surgery.

The application of handheld *in situ* bioprinting in cartilage tissue engineering is limited. However, a novel and portable handheld device called the 'Biopen' has been developed by O'Connell *et al* [21] in 2016 (figure 4(a)). The system enables the deposition of bioinks with high cell viability by a direct-write method. The Biopen comprises two ink chambers, a 3D printed extruder head, and a UV source for photocrosslinking of bioinks. A pneumatic extrusion system allows surgeons to regulate each chamber individually. The bioink can be extruded from either the left or right chamber, or both at the same time creating material gradients using foot pedals. This allows the deposition of bioinks to create small and simple structures in a small area and through visual inspection the material flow can be modified in a freeform manner, providing a surgeon flexibility during operation. The small size allows portability and facile sterilization. However, the printing resolution is limited due to the larger outer diameter of the extruder nozzle (∼1 mm). As the device does not provide a bioink temperature control system, temperature alterations can occur during the handling impacting the rheological properties of temperature-sensitive bioinks.

The 'Biopen' was further developed to include a co-axial nozzle and a motorized extrusion system to generate scaffolds with appropriate structural stability and good cell viability [22] (figure 4(b)). The nozzle provides suitable core/shell distribution, allowing photocrosslinkable shell biomaterials to provide sufficient stiffness for bioinks in the inner core. During the bioprinting, biomaterial inks containing gelatin methacryloyl (GelMA), HA methacrylate (HAMA), and the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) were bioprinted and photocrosslinked after the deposition to provide structural stability. It allows the inner core containing adipose-derived mesenchymal stem (ADSCs) to be successfully separated from the potential harmful effects of LAP photoactivation during crosslinking, resulting in a high cell survival (>95%) of printed constructs 7 d post-printing. Furthermore, the co-axial 'Biopen' has been further optimized to allow rapid photocrosslinking (less than 1 s) using *in situ* photocrosslinking rather than typical post-crosslinking [104]. The core material could be made of soft or even liquid materials owing to the rapid crosslinking of the shell structure. This optimisation allows the use of a wider range of bioinks. Furthermore, the fast *in situ* crosslinking reduces the overall bioprinting time and reduces the exposure of the surrounding

tissues to light irradiation, which is useful from a clinical perspective.

After the development and preliminary research of the handheld 'Biopen', Di Bella *et al* [23] studied the co-axial handheld bioprinting approach for cartilage repair in an animal model, a step towards the clinical translation of *in situ* bioprinting (figure 4(c)). The construct containing GelMA, HAMA, and ADSCs was successfully bioprinted to repair a full-thickness chondral defect created in a sheep model, without perioperative complications after 8 weeks assessment. *In situ* bioprinted constructs had a greater Young's modulus than other groups, ∼0.5 MPa, although slightly lower than native cartilage (0.5–8 MPa). Notably, early tissue formation of hyaline cartilage and columnar chondrocyte alignment was observed for the *in situ* printed group by histological analysis (figure 4(d)). Despite the maintenance of the integrity of the subchondral bone, the lateral integration of the construct was restricted.

4.2.2. Robotic-assisted bioprinting. Robotic-assisted *in situ* bioprinting, driven by a movable robotic system, can fabricate highly complex structures with greater accuracy compared to the handled approach. Predefined by a CAD model based on image reconstruction of the defect or wound, complex 3D organs or tissues can be fabricated, with the toolpath adjusted and monitored by the surgeon. Multiple printing heads and material chambers can be in the robotic-assisted device [4, 145, 146]. Key elements typically include a high-resolution 3D defect scanner, a bioprinting unit (e.g. extrusion and inkjet printhead), a robotic manipulator (e.g. Cartesian, parallel, or articulated) with three or more DoF, a control and monitoring cabinet, and a workbench. The components of the system must be able to operate in a complex surgical environment and for example should be easily sterilized, moveable, and simple to operate.

Currently, there are no commercial *in situ* bioprinting systems available and all are still in the laboratory research phase. There is a significant challenge in developing solutions for *in situ* bioprinting in articular joints and there are only limited examples compared to other tissues such as skin and muscle. A key factor is the anatomic positioning required for different AC defect pathologies (e.g. medial and lateral regions of the tibia plateau or femoral condyles) and the limited access into the defect site because of the surrounding ligaments, tendons, meniscus, and bones. However, there have been significant developments within the field, for example, Li *et al* [100] successfully printed photocrosslinkable hydrogels in three types of *in situ* defects including large segmental bone defect, osteochondral defect on the femoral condyle, and chondral defect on the tibia plateau, using an extrusion-based 3D bioprinter (figure 5(a)). The 3D digital models were created using a high-resolution (up to 5 μm) 3D scanner, and a 365 nm UV lamp was used for photocrosslinking the hydrogels during the printing. The entire scanning and bioprinting process was completed in 15 min. This work demonstrated the feasibility of robotic-assisted extrusion *in situ* bioprinting for cartilage defects.

Non-planar deposition with a high degree of calibration is a complex process in conventional 3D printing and is an especially challenging in irregular non-planar defects in AC, posing a serious difficulty for precise bioprinting. The development of an advanced path planning algorithm was used in a robotic platform which allows the fabrication of 3D structures onto irregular surfaces (figure 5(b)) [147]. This algorithm can determine the joint angles for each location in order to keep the end effector perpendicular to the surface at all times. Furthermore, a 6-DoF robotic-assisted system was developed for cartilage *in situ* bioprinting coupled with an extrusion-based bioprinting unit and an off-line programming software to determine printing parameters (figure 5(c)) [20]. To decrease the calibration error, a fast tool center point calibration method utilizing a laser tracker was developed. The surface error was reduced to 30 μm which is significantly lower than the manual operation. Biomaterial inks including HA and PEG were deposited in a rabbit osteochondral lesion and a fully filled lesion was observed after 12 weeks. The presence of GAGs and collagen II in the ECM, and chondrocytes organized horizontally on the surface and vertically in the deep zone indicated a predisposition to develop into hyaline cartilage (figure 5(d)).

4.2.3. Minimally invasive and non-invasive bioprinting. Open surgery in cartilage repair can have significant complications such as risk of infection, larger tissue trauma, and longer recovery time. Therefore, a minimally invasive or non-invasive approach for *in situ* bioprinting is a promising research trend although still in the early stages of development. Robotic-assisted bioprinting through MIS has been used to repair bone and cartilage defects in the joint, providing a viable option for *in situ* cartilage restoration and the reduction of invasiveness in the arthroplasty [24]. However, the rigid or semi-rigid tools (e.g. printing nozzles) with limited flexibility and dexterity increase the complexity of the MIS procedure. Consequently, flexible robotic systems provide an attractive alternative strategy, however, these have not yet been explored in cartilage applications [148–150]. For example, a ferromagnetic soft catheter robot (FSCR) system enabling minimally invasive *in vivo* bioprinting and remote control has been developed (figure 6(a)) [143]. The FSCR is a slender rod-like structure composed of hard-magnetic particles and a polymer matrix, allowing *in situ* bioprinting of various biomaterial inks through a small incision with a magnetoactive soft printing nozzle and reaching the defect region in the body by remote magnetic actuation. However, further optimisation of the system is needed regarding a more versatile magnetic field and the miniaturization of the system body [143, 151]. Additionally, Thai *et al* [144] have demonstrated a miniaturized high DoF extrusion-based soft robotic arm printhead fixed onto a flexible snake-like structure (figure 6(b)). This system enables bioprinting through MIS or using natural orifices to reach confined anatomical locations. A colon model was used to validate the flexibility and bioprinting capability. The bioink loaded with L929 cells showed high cell viability and proliferation when bioprinted *ex vivo*. Furthermore,

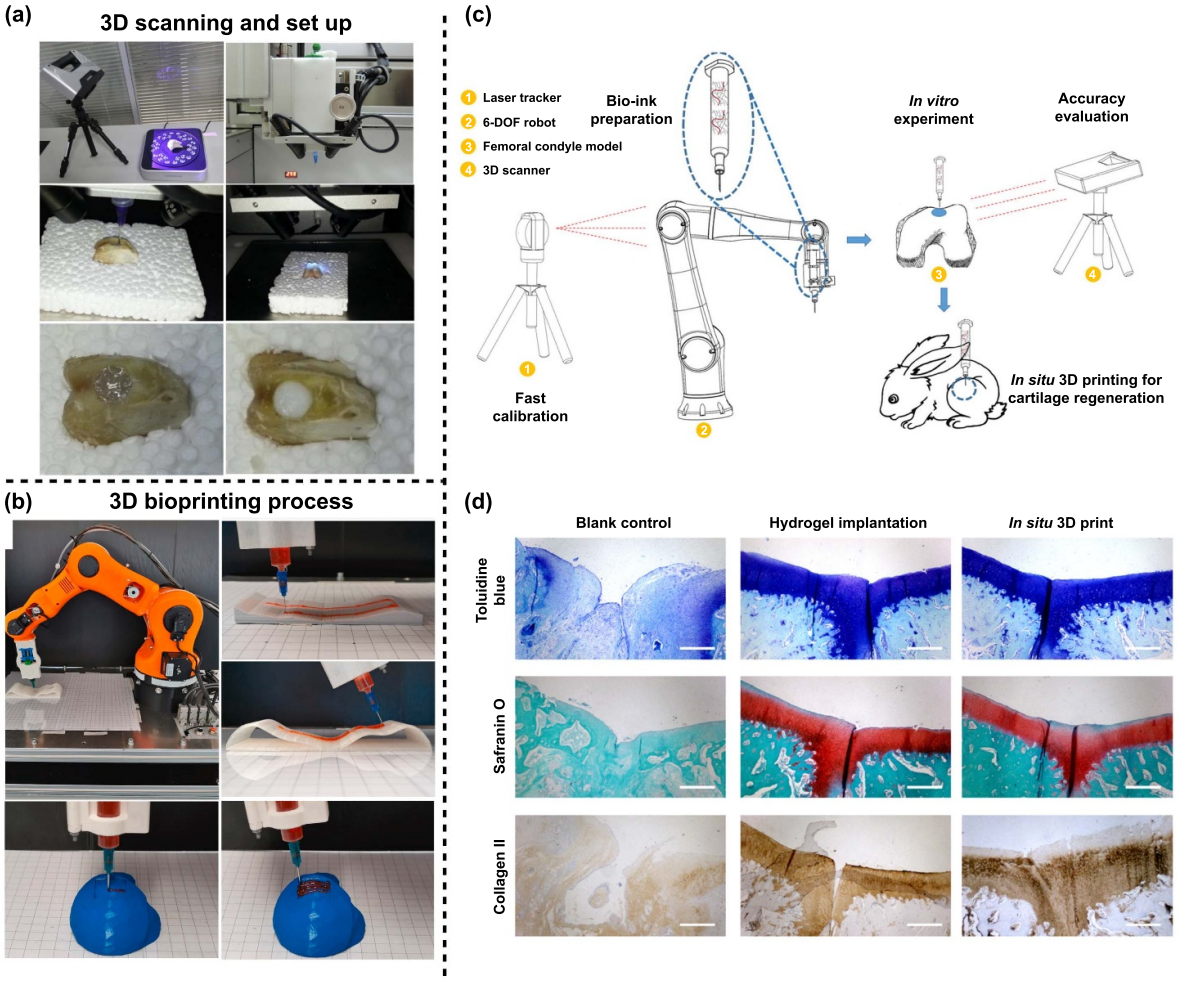

Figure 5. Robotic-assisted extrusion-based *in situ* printing for cartilage regeneration. (a) Three-dimensional scanning, setup and 3D printing for an osteochondral defect. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, Scientific Reports [100], Copyright (2017). (b) *In situ* bioprinting with different slopes of surfaces, the developed algorithm allows the printing nozzle to keep perpendicular to the surface. Reprinted from [147], Copyright (2021), with permission from Elsevier. (c) Schematic of extrusion-based *in situ* bioprinting for cartilage defects in a rabbit model using a robotic arm. The osteochondral defect (diameter of 5 mm and a height of 4 mm) is created before *in situ* printing. (d) Histological characterization of *in situ* bioprinted, hydrogel implantation and control groups, with toluidine blue, Safranin O, and collagen II staining respectively (Scale bar: 500 μm). Reprinted from [20], Copyright (2020), with permission from Elsevier.

tissue dissection was shown by utilizing the printing nozzle as an electrosurgical knife. Thus, the device can act as both a bioprinting and dissection surgical tool.

Alternatively, non-invasive *in situ* bioprinting has been explored as a novel approach. For instance, digital near-infrared (NIR) based photopolymerization (DNP) *in situ* bioprinting was used to fabricate ear-like constructs non-invasively (figure 6(c)) [90]. Although photopolymerization is an efficient and attractive strategy to assist the bioprinting process, it is difficult to penetrate deep into tissues using UV and blue light, limiting their use for non-invasive bioprinting. However, in this study, NIR light was used to initiate the photocrosslinking process in the deep tissue. The bioinks were injected into the tissue cavity followed by photopolymerization through spatial patterning with NIR light. A similar technique has been proposed by Urciuolo *et al* [91], through which cell-laden photocrosslinkable hydrogels were bioprinted within tissues (brain and muscle) of live mice. At a wavelength longer than 850 nm, the femtosecond pulsed NIR can penetrate through soft tissues and allow crosslinking via bio-orthogonal two-photon cycloaddition using a multiphoton laser-scanning microscope. However, this technique might be restricted by the multiphoton microscope, for example, the size and depth of hydrogel crosslinking at millimeter scales. These non-invasive approaches are highly promising and a significant advancement for *in situ* bioprinting. However, significant development is required to enable clinical translation and their suitability for cartilage repair and regeneration applications remains to be determined.

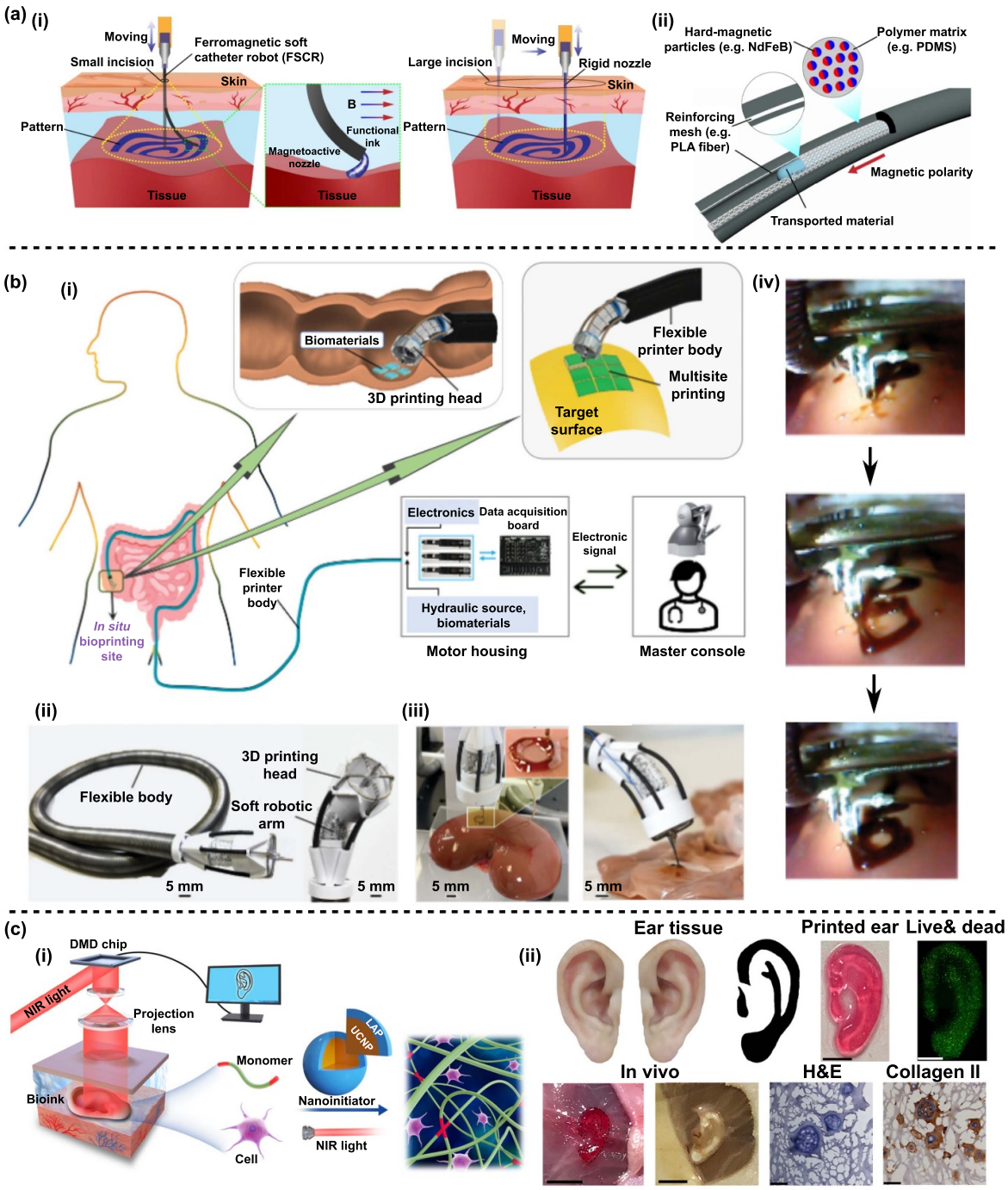

Figure 6. Minimally invasive and non-invasive *in situ* bioprinting. (a–i) Schematic of MIS bioprinting with functional inks using FSCR system control through remote magnetic actuation versus an open surgery approach; and (a–ii) schematic of FSCR system that contains hard-magnetic particles dispersed within polymer matrix and reinforcing mesh. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Nature, Nature Communications [143], Copyright (2021). (b–i) Schematic of flexible *in situ* bioprinting system; (b–ii) key elements of the system; (b–iii) *ex vivo* bioprinting on a porcine kidney and porcine colon; and iv) *in situ* bioprinting a shape onto the inner surface of a colon model. [144] John Wiley & Sons. © 2023 The Authors. Advanced Science published by Wiley-VCH GmbH. (c–i) Schematic of DNP-based non-invasive *in vivo* bioprinting; and (c–ii) bioprinted structure and biological evaluation (top images scale bar: 2 mm; bottom left two images scale bar: 5 mm; bottom right two images scale bar: 50 μm). From [90]. Reprinted with permission from AAAS.

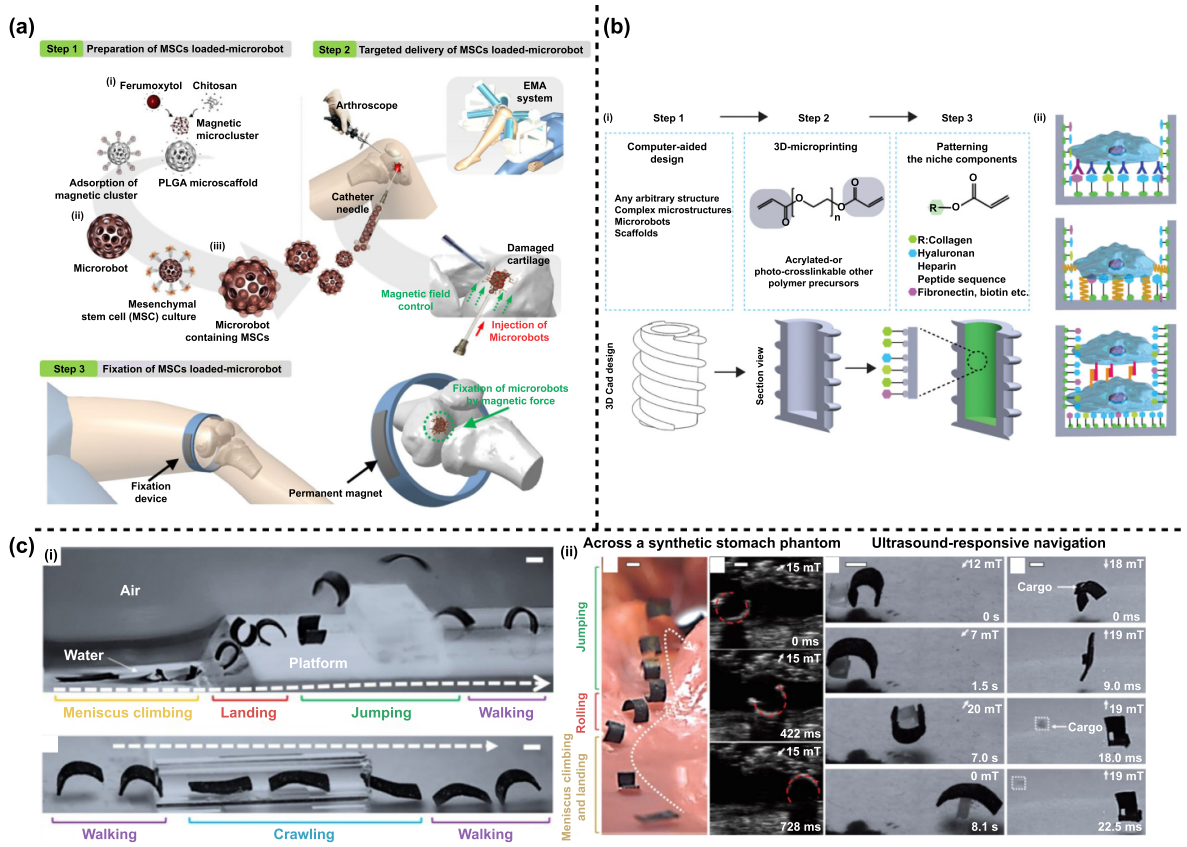

Figure 7. Micro- and nanorobotic cell delivery and locomotion. (a) Schematic illustration of cartilage regeneration using targeted magnetic microrobot-mediated stem cell-based delivery system. From [156]. Reprinted with permission from AAAS. (b–i) Fabrication steps of the microrobotic transporter; and (b–ii) illustration of the interaction between encapsulated cells and the niche. [157] John Wiley & Sons. © 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (c–i) Multimodal locomotion of soft robot; and (c–ii) the potential medical applications of soft microrobot. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Nature [159], Copyright (2018).

5. Micro- and nanorobotic cartilage therapies

Micro or nano robots are machines fabricated on the scale of micro or nanometres [152]. They present unique capabilities to access into the body non-invasively and navigate in narrow and complex areas to perform biomedical operations [153, 154]. Microrobots can be precisely actuated and accomplish tasks, such as the delivery of cells or therapeutic agents, offering considerable potential in the area of non-invasive surgical treatments and as a complimentary strategy for *in situ* bioprinting. This section briefly highlights the significant progress in capabilities of these systems in cartilage applications to provide understanding of a novel alternative robotic approach that may have future applications for *in situ* bioprinting.

The targeted delivery of stem cell laden magnetically actuated microrobots has been assessed *in vitro* [155] and in a rabbit knee cartilage defect *in vivo* [156] (figure 7(a)). The microrobot was fabricated using a PLGA micro-scaffold and equipped with an electromagnetic actuation (EMA) system for the targeted cell transport. The magnetically actuated microrobots were injected into the joint cavity via minimally invasive procedures and moved to the defect site using the EMA system. Subsequently, the microrobot system was immobilized to the defect by a strong permanent magnet. The results showed that the magnetic guidance of the microrobot by the EMA led to a stronger targeting capability than the injection-only group. With the magnetic field generated by the EMA, the microrobots are free from gravity and able to move to the defect site in a controlled manner. The microrobot delivered stem cells to the cartilage defect providing a suitable environment for cell proliferation and chondrogenic differentiation both *in vitro* and *in vivo* and eventually degrading within 3 weeks *in vivo*. An alternative approach developed by Yasa *et al* [157] was the use of a 3D printed magnetically actuated microrobotic cell transporter (MCT) for stem cell delivery that recapitulated a stem cell niche through patterning of biomolecule components (figure 7(b)). The MCT was wirelessly steered using the magnetic field and the encapsulated cells were transported to the target position without invasive intervention. Moreover, this system also increased the adhesive stability of the cells minimizing off-site delivery and regulated the cell fate towards the pre-designed osteogenic lineage.

During robotic-assisted surgical operations, there is a risk of tissue damage due to deformation or perforation because of the size and stiff nature of the robotic systems. The clinical transition of miniature soft robots is becoming feasible. Robots fabricated with soft materials can allow locomotion, elastic deformations, and physical adaption to the environment [154].

However, the challenges of designing soft microrobots remain, such as the incorporation of functional materials, miniaturization, and navigation. Moreover, as a multiphasic tissue, cartilage has a dense structure formed by the solid components of the matrix, the interstitial fluid, underlying subchondral bone, and the surrounding synovial fluid [158]. This nature of cartilage brings difficulties for the locomotion of microrobots. Nevertheless, a small-scale soft robot with multi-modal locomotion might offer the solution (figure 7(c)) [159]. The magneto-elastic robot can switch between different modes of locomotion and transition between fluid and solid environments through remote magnetic actuation. By deforming the shape of the robot body, it is capable of multiple navigation modalities, such as swimming in biological fluids, walking and rolling, climbing the air–liquid meniscus onto a platform, and transiting a synthetic stomach model.

The integration of micro- and nanorobotics with *in situ* bioprinting is an intriguing prospect as the micro- and nanorobots could be included in the bioink formulations to enhance cell positioning and guide differentiation, enable access to highly confined anatomical sites for bioprinting technologies, and enable feedback on tissue formation. However, these are hypothetical and requiring long-term significant developments in both robotic systems and *in situ* bioprinting.

6. Challenges and future perspectives

In situ bioprinting, through the direct deposition of bioinks within the human body to target defects in a tissue-specific manner, has emerged as a promising strategy for cartilage tissue regeneration. Although being a rapidly developing technique, several improvements in current *in situ* bioprinting approaches are still required to enable successful clinical translation. To be clinically effective, *in situ* bioprinting of cartilage will require multiple advancements including: the rational design of cartilage-like functional biomaterials, improvements in robotic bioprinting strategies, enhanced imaging and toolpath planning systems, incorporation of surgical feedback mechanisms, utilization of artificial intelligence (AI), simplifying access and set-up of the bioprinters, and the consideration of the intra-articular environment. Furthermore, a clear understanding of the regulatory environment and clinical requirements (surgeon and patients) is needed to enable commercialization.

6.1. Tissue complexity, functionality, and integration

Cartilage tissue has a complex hierarchical and zonal structure with a functional gradient in composition and properties. To repair cartilage defects, the composition, structure, and biological function of *in situ* bioprinted constructs must be considered as key parameters. The gradient structure of cartilage from the superficial to deep zone requires bioinks exhibiting various biomechanical and biochemical functions. Multi-material bioinks with gradient functions can be used to mimic native cartilage features, such as zonal organization and anisotropic properties [160–163]. However, bioprinting approaches developed firstly for *in vitro* use may not be able to translate into a viable *in situ* bioprinting strategy. Furthermore, currently both *in vitro* and *in situ* bioprinting studies have difficulty in recapitulating the complexity of native tissue or promoting the long-term phenotypically stable hyaline cartilage and integration with surrounding tissue.

A main aspect of improving *in situ* cartilage bioprinting is the development of bioinks. Current *in situ* bioprinting studies use bioinks based on several materials such as HA, gelatin, and alginate derivatives, which have not been specifically optimized to recapitulate key properties of cartilage ECM. Major advancements in bioink design and *in vitro* bioprinting have been demonstrated and these now should be translated into the *in situ* bioprinting application. For example, in the development of multi-cellular constructs, Levato *et al* [164] demonstrated that GelMA-based bioinks containing AC-resident chondroprogenitor cells displayed superior tissue formation than chondrocytes and expressed lower levels of collagen type X, a marker of hypertrophy, and proteoglycan 4, a marker of lubrication, compared to MSCs. Multi-layered and multi-cellular constructs were bioprinted with distinct ECM and cellular compositions to mimic the superficial and deep regions of AC resulting in a zonal distribution of AC-specific markers. To mimic the hierarchical anisotropic structure of AC and modulate cell behavior, Sun *et al* [165] have 3D bioprinted a gradient PCL scaffold and engineered dual-factor releasing bioink (TGF-β3 BMP-4) in a single structure. The results showed a structurally stable construct that mimicked the organization of AC and the release of growth factor was able to modulate ECM formation to resemble native AC.

Alternatively, a novel approach is the spatial patterning of spheroids or microtissues to fuse and self-assembly into a macrotissue. Melt electrowriting has been used to fabricate microwell scaffolds that can orient cellular aggregate growth and provide mechanical reinforcement [166]. This was followed by the inkjet bioprinting of cells into the microwells and spontaneous cellular aggregation. This study was followed by the fabrication of a biphasic osteochondral construct through the spatial organization of phenotypically distinct cartilage microtissues guided through a 3D printed polymer framework [167]. The results showed zonal collagen organization mimicking the native AC structure. Furthermore, the stabilization and the appearance of a normal AC surface in an *in vivo* osteochondral defect was demonstrated. Additionally, by incorporating a joint fixation device into the 3D printed framework the attachment to the subchondral bone potentially can be improved [168].

An often neglected aspect of AC is the surface lubrication that engineered constructs require to satisfy the biotribology properties of native AC. In this regard, Zhao *et al* [169] have reported a design concept of an efficient cartilage lubrication system mainly composed of a hydrophilic polyelectrolyte lubrication phase on the superficial layer of a 3D printed elastomer-hydrogel composite scaffold with excellent load-bearing properties. The strategy combining interface

lubrication and a non-dissipative mechanism resulted in superior friction reduction functionality and wear resistance under a dynamic shear process.

Moreover, the integration between the bioprinted construct and surrounding tissues must be addressed, especially for cartilage tissue which has an anti-adhesive ECM and low metabolism [27, 30, 35, 170]. *In situ* bioprinted constructs must allow lateral and vertical biological fixation and restore function. For example, fibrin glue might be used to guarantee immediate construct adhesion [23]. Other strategies can be the development of adhesive bioinks which can directly bind to the adjacent tissue. A single-step crosslinked HA-transglutaminase hydrogel was developed, showing the potential to adhere to the surrounding cartilage tissue *in situ* [171]. Moreover, an ultrafast, tough and adhesive bioink containing HA, methacrylated HA, o-nitrobenzyl-grafted HA and gelatin has been reported to strongly bind to the native cartilage tissue via photocrosslinking [172]. Despite the promising material formulation, the feasibility of the bioink for *in situ* bioprinting is unknown. The poor adhesion between solid polymeric scaffolds and hydrogels or tissues is another integration issue. This has been addressed by the development of a mechanically robust solid-hydrogel material using plasma immersion ion implantation generation of surface-embedded radicals to polymerize and covalently attach hydrogels onto solid polymer surfaces [173]. This study provides a potential solution for weak phasic binding between distinct bioprinted cartilage zones composed of different materials and adhesion between the engineered construct and the native surrounding environment.

The phenotypic instability of engineered cartilage is another well documented challenge for current tissue engineering strategies [30, 31, 35, 40, 47]. For example, chondrocytes can maintain the chondrogenic phenotype within a soft matrix (2–25 kPa) while the engineered construct needs to satisfy the stiffness of native cartilage (0.5–4 MPa) [174–176]. de Melo *et al* [177] developed a multi-material bath with the high stiffness component reflecting the macromechanical properties of cartilage and a soft matrix providing a chondrogenic environment. Moreover, control of the mechanobiology, the conversion of mechanical forces into biochemical signals via mechanotransduction pathways, is a key strategy to regulate cartilage regeneration [68, 138, 139, 178–180]. The hydrogel material composition and architecture that aim to recapitulate the ECM can serve as an important biomechanical cue in regulating cell chondrogenesis and maintaining chondrogenic phenotype. Additionally, functionalized bioinks embedded with a variety of signaling molecules (e.g. TGF-β, bone morphogenetic proteins, and insulin-like growth factors) can be developed to modulate cell–cell functions and cellular activities for developing and maturing cartilage tissue [47, 103, 181–184]. Hence, further studies involving mechanotransduction pathways, the role of mechanosensors, and different signaling pathways for *in situ* cartilage tissue engineering are necessary.

However, the requirement for cell-instructive, biomimetic, and cell-based constructs may not be necessary to alleviate the pain and mobility issues of patients [185]. For example, Zhao *et al* [186] have successfully designed a non-cellular synthetic hydrogel composite composed of crystallized polyvinyl alcohol and bacterial cellulose with wear resistance and mechanical properties that exceed that of cartilage tissue whilst having the same coefficient of friction.

6.2. Photocrosslinking

Rapid *in situ* crosslinking in clinically relevant time scales under physiological conditions is a significant benefit for *in situ* bioprinting. Typically, this can be achieved using photocrosslinking, but special attention should be focused on challenges related to incomplete or inhomogeneous crosslinking [187, 188]. Moreover, heat generation during photocrosslinking and potential cytotoxicity effects (e.g. cell membrane and DNA damage, cell cycle arrest and apoptosis) caused by shorter wavelengths and light dose all need to be taken into account in case of applying *in situ* photopolymerization at a defect site [189–192]. In this sense, it is critical to guarantee a balance between the photoinitiator's absorbance spectra and the photocrosslinking light source towards avoiding the use of high intensity light irradiation and photoinitiator concentration. Fortunately, the protective environment provided by the 3D biomaterial matrix can alleviate these effects to some extent, thus allowing variable range of light wavelengths and photoinitiator concentrations to be used [193]. Subsequently, the exploitation of longer wavelengths, including visible and NIR light to minimize cytotoxicity is under investigation and allows new strategies such as non-invasive *in situ* bioprinting approaches [90, 91, 194]. Apart from light irradiation and photoinitiator choice, other factors such as the selection of functional group, corresponding propagation chemistry, and environmental factors (temperature and pH) also influences *in situ* photopolymerization. More details can be found in the critical review reported by Lindberg *et al* [187]. Furthermore, issues surrounding photo-induced cytotoxicity may depend on the size of the defect and the quantity of biomaterials required. Subsequently, *in vivo* studies should also take into account long-term changes in cell viability and behavior in the surrounding tissue.

6.3. Integrating in situ bioprinting and surgery

To make the bioprinting process more reliable, improvements including printing speed, resolution, movement precision, and complexity of bioprinted constructs are required to conform to the complex cartilage defect environment and clinical setting. Most current bioprinting equipment uses a 3-axis motion system, which limits the system flexibility and the complexity of bioprinted structures. Indeed, robotic arms with 4-, 5-, and 6-axis enable more DoF to improve surgical dexterity and construct complexity. However, these need to be aligned with the aims of cartilage restoration surgery. The most attractive and innovative way is the provision of MIS or arthroscopic techniques allowing the accurate assessment of the pathology using high-definition arthroscopy cameras and minimal damage to the surrounding tissues. The clinical

scenarios that would benefit from *in situ* bioprinting of cartilage would be normally addressed with arthroscopic access to the cartilage defect. In most joints with discrete defects, there is no periarticular tissue contractures or osteophytes which often would need open surgery to restore range of motion. Therefore, the design parameters for the device must ensure compatibility with the arthroscopic surgery. The success of MIS is also associated with reducing soft tissue trauma which enhances recovery and minimizes post-operative pain. If the set-up of novel systems for *in situ* cartilage bioprinting requires increasing the size of incisions, it might compromise the benefits of *in situ* bioprinting compared to more established techniques. Additionally, if the delivery system requires implantation into bone either to secure the device during the use or to ensure accuracy of application, this might increase post-operative pain, which is undesirable for patients and clinicians alike. The aim should be to maintain the minimally invasive approach to surgery and ensure stable printing through arthroscopy portals, even though additional portals could be considered. Therefore, the development of soft snake-like systems and miniaturization or combination of bioprinting with surgical tools to enable precise minimally invasive manipulation within a complex or small area of cartilage tissue, although technically challenging and in the early stages of technological development, is highly desirable [24, 143, 144, 195–201]. Alternatively, and a more long-term prospect, the design and fabrication of small untethered soft-bodied robots for clinical use are appealing for MIS and cartilage repair [153, 154, 198, 202]. As previously highlighted, the navigation within confined environments to access anatomical sites is attractive. Moreover, the integration of robotics with multiple functions such as the delivery of drugs, cells and genes, and the visualization of the anatomy needs to be considerably enhanced.

Magnetic resonance imaging (MRI) has become common in the pre-operative assessment of patients with cartilage defects, however, the accuracy of MRI assessment for cartilage defects is variable [203]. Thus, a system should be proposed that is capable of unplanned *in situ* bioprinting at the time of surgery rather than a sophisticated pre-operative planning tool, which would benefit surgeons treating cartilage defects. If the defect is not identified until the time of arthroscopy, this prevents the need for postponing the cartilage therapy until a subsequent surgical intervention, saving time and further surgical insult for the patient.

Further to the challenges surrounding access and set-up of the bioprinting, there are additional challenges within the intra-articular environment. The surface tension of synovial fluid, which is altered in arthropathies, may interfere with the application of the bioprinted cartilage [204]. Additionally, the saline fluid used to visualize the joint in arthroscopic surgery would adversely affect the bioprinting ability and thus would have to be drained out of the joint prior to *in situ* bioprinting. This technique is commonly used for other cartilage therapy techniques and, although produces an inferior view to an arthroscope within saline fluid, is compatible with visualization of the pathology to perform surgery in a safe manner. Lastly, as exemplified in the knee, cartilage surfaces on either side of a joint are opposing, and so will be orientated in positions which will be influenced by gravity. *In situ* bioprinting onto the femoral condyles in the knee, a common location of full thickness cartilage defects, will be technically more challenging as bioprinting would be into an area above the nozzle, whereas in the tibial condyles, gravity would assist the extrusion. New positioning techniques might need to be considered [205]. Moreover, single use products, although controversial in their ecological profiles, have been popular in healthcare due to their ability to be stored until use as well as ensuring sterility. Therefore, design considerations should be made so that the part of the machine within the surgical field is sterile and single use.

6.4. Next generation robotic surgical systems

The potential following steps in the levels of miniaturization and autonomy of robotics systems include: (1) higher degrees of autonomy of robotic systems in conjunction with AI based control systems; (2) the long-term creation of fully implantable robots which enable the restoration and replacement of physiological processes; and (3) the realization of micro- and nanoscale robotic devices [206, 207]. These are all significant long-term challenges and aspirations. However, the next generation of robotic surgical systems should be integrated with enhanced imaging, sensing and feedback (i.e. force and haptic feedback; thermal, pressure, and positioning sensors), faster digital communication, and improved bioprinting systems. The use of imaging techniques and position sensor allows the understanding of the surrounding environment. A better imaging system can also provide 3D high-resolution real-time video, which enables the monitoring of the bioprinting process. The use of wearable eyeglasses such as virtual reality (VR) with recording capabilities can be potentially used to monitor the surgical process as well as to provide critical information for quality control assessment [195]. At the stage of bioprinting, another main challenge for robotic surgery is the lack of real-time position feedback, which may cause collisions between surgical tools and tissues. Recently, surgical systems equipped with advanced positioning, sensing, and feedback systems have been used to improve real-time correction and minimize error [195, 208, 209]. In addition, AI has been utilized in medical applications and 3D printing in both virtual and physical aspects [210, 211]. Machine learning or deep learning, using mathematical algorithms to improve learning autonomously from experiences or collected data, can help in real-time medical imaging for surgery to provide faster and optimal decision making to assist the surgeon efficiently and safely in complex tasks [212]. The current major limitations for *in situ* bioprinting strategies can be summarized as scanning of the defect, model creation, optimisation of the model and printing path, and final printing procedure [213]. The safety issue and damage to tissue might occur due to the limited sensing and control of the robotics. AI-empowered systems can be used as a useful tool to integrate with *in situ* bioprinting system. This requires AI to collect and analyze data during the surgery, minimize errors, and subsequently predict and plan commands. In this way, AI could offer a

more precise, interactive, and adaptive approach. In addition, by using big data and AI analysis, the Internet of Things (IoT) devices can collect real-time data and transmit the information to the cloud [214]. Future *in situ* bioprinting surgery could be potentially conducted remotely via VR and augmented reality (AR) technologies. This potential application might require a highly interdisciplinary approach, involving AI, IoT, VR, AR, and 5G technologies interacting with the clinical team.

7. Conclusion

AC has an important biomechanical role in the human body, but with limited self-regenerative properties, trauma and disease can seriously affect tissue functionality and result in pain and disability for patients. Since existing clinical treatments fail to effectively repair or regenerate cartilage tissue defects, tissue engineering approaches are being explored including *in situ* bioprinting strategies. This review has discussed recent progress in the use of handheld and robotic-assisted *in situ* bioprinting technologies, highlighted alternative approaches in micro- and nanorobotics, and provided a perspective on challenges and future directions to enable successful clinical translation.

Whether to use *in situ* bioprinting depends on the complexity of the tissue or organ, the anatomical location, and the required clinical outcome. Robotic-assisted *in situ* bioprinting systems are highly accurate and can facilitate automation, easing the workload on surgeons. Furthermore, they have the potential to integrate with existing robotic surgical systems. Subsequently, robotic-assisted printing system is a feasible approach for cartilage repair, especially if integrated with MIS. However, challenges remain in the development of biomaterials that are cell-instructive, enable phenotypic stability and tissue integration, and have appropriate biomechanical properties. Furthermore, advances in robotic and bioprinting systems are required such as miniaturization of components, movement and feedback, and surgical planning. To enable clinical translation, standardized bioprinting procedures will need to be developed alongside clinical trials to ascertain the efficacy and cost-benefit compared with current gold standards. Additionally, *in situ* bioprinting may not be appropriate in all clinical indications, the grade of defect and stage of tissue degeneration will require evaluation of the suitability of a minimally invasive or open surgery *in situ* bioprinting approach. Furthermore, an *in vitro* bioprinted approach may be better especially for larger full-thickness defects or complete resurfacing or total joint replacements. Finally, the challenges of robotic *in situ* cartilage bioprinting are broadly in ensuring the advances in MIS are not forced to take a backward step to accommodate the advances in another field.

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