

REVIEW

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## Introduction, classification and applications of 3D bioprinted hydrogels for cancer treatment: a review

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Polymeric hydrogels have become effective materials in cancer therapy because of their biocompatibility, biodegradability and tunable chattels. This review presents a thorough investigation of the synthesis and medicinal uses of different naturally occurring and synthetic hydrogels, for cancer therapy, mainly *via* 3D modeling and printing. The exceptional biocompatibility of hydrogels, coupled with their remarkable potential for replicating the intricate extracellular matrix (ECM), positions them as ideal materials for constructing scaffolds used in the synthesis of *in vitro* 3D tumor constructs. Hydrogels can also be used for 3D printing to treat cancer by aiding in accurate control over the composition of hydrogel scaffolds. 3D modeling and printing play an important role in cancer treatment by enabling drug screening. This review distinguishes itself by integrating a comparative analysis of both conventional and emerging hydrogel systems—including natural, synthetic, and hybrid types particularly designed for 3D bioprinting in cancer modeling. This study paves the path for new researchers to explore cancer treatment by combining hydrogel-based materials with advanced techniques.

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### 1. Introduction

Hydrogels are hydrophilic polymers with a three-dimensional (3D) structure. For a material to be called a hydrogel, it must contain a minimum of 10% water by weight. The presence of a significant proportion of water molecules makes the polymeric material swell and renders softness and flexibility. The structural integrity of a hydrogel is due to the physical or chemical linkages of the polymer chains.<sup>1,2</sup> The hydrophilicity can be attributed to the presence of hydrophilic functional groups on its surface including the amino group ( $-NH_2$ ), carboxylic group, ( $-COOH$ ), hydroxyl group ( $-OH$ ), amide group ( $-CONH_2$ ), imide group ( $-CONH$ ) and sulfonic acid group ( $-SO_3H$ ).<sup>3,4</sup> Their soft, tissue-like structure enables them to integrate with biological tissues, without any antagonistic reaction.<sup>5</sup> Wichterle and Lim's development of soft contact lenses in 1960 using natural hydrogels marked a significant breakthrough in the biomedical field.<sup>6</sup> A significant advancement in this area involves the development of multi-responsive hydrogels, exemplified by materials like propyl acrylamide, chitosan and polyvinyl alcohol. These materials exhibit high sensitivity to environmental changes, making them suitable for applications in smart sensors, actuators, and targeted drug delivery

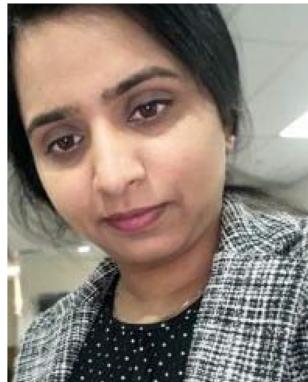
systems for cancer treatment.<sup>7,8</sup> In this context, several researchers have explored the use of hydrogels in cancer treatment.<sup>9–13</sup> This review paper aims to bridge the gap between bench studies and clinical practice for the application of both natural and synthetic polymer hydrogels in cancer treatment. The study indicates some key challenges in utilizing laboratory-based studies in practical applications. It further suggests an optimized application of these materials, discussing the latest technological advancements.

Keeping in view the above discussion, the study aims to (i) explore the utilization of 3D-printed hydrogels in cancer treatment, (ii) investigate the synthesis, characterization, and classification of both natural and synthetic hydrogels, (iii) analyse the integration of these hydrogels in 3D modeling and bioprinting techniques to accurately replicate the complex tumor microenvironment, (iv) study the advantages of combining natural and synthetic hydrogels to optimize drug delivery and improve their mechanical properties, (v) discuss the advancements in hydrogel-based therapies as photothermal and magnetic hyperthermic agents for targeted cancer treatment and (vi) provide valuable insights into the challenges and opportunities associated with scaling up hydrogel-based cancer therapies for clinical application. This review offers a unique contribution by providing an integrated comparative analysis of both established and cutting-edge hydrogel systems encompassing natural, synthetic, and hybrid forms specifically engineered for 3D bioprinting applications in cancer modeling. The paper consolidates recent advancements in stimul-

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responsive hydrogels, such as those that react to reactive oxygen species (ROS) and hypoxia, and investigates their incorporation into constructs designed to precisely replicate the tumor microenvironment (TME). Furthermore, it details



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various hydrogel types, crosslinking strategies, and their specific uses in different cancer models, offering a structured overview that is often absent in previous academic works, thereby providing invaluable direction for future translational research in hydrogel-based cancer therapies.

In the field of polysaccharide science, particularly concerning their application in biomedical engineering, a pertinent hypothesis derived from the provided review is that combining natural polymeric hydrogels, such as those made from polysaccharides like alginate, chitosan, and hyaluronic acid, with synthetic polymers can effectively address the inherent limitations of natural hydrogels. While natural hydrogels are highly valued for their biocompatibility, biodegradability, and non-toxic nature, they often exhibit weaker mechanical properties and limited versatility. The hypothesis posits that integrating synthetic components can enhance crucial characteristics like mechanical strength and enable more precise control over drug release, thereby improving their overall suitability and efficacy for various biomedical applications, including cancer treatment. This approach aims to leverage the benefits of both natural and synthetic materials to create optimized hydrogel systems.

## 2. Classification of polymeric hydrogels

Polymeric hydrogels can be grouped into two categories *i.e.*, natural and synthetic.<sup>14–17</sup> Natural polymeric hydrogels are obtained from biological sources like plants, animals, or microorganisms.<sup>18</sup> They are biocompatible, biodegradable, and inherently bioactive, which promotes their usage in biomedical applications.<sup>19</sup> These hydrogels can encapsulate cells and can be used as therapeutic agents. Their bioactive nature may be enhanced by modifying their structures through physical and chemical crosslinking methods.<sup>20</sup> Physical methods like ionic crosslinking and hydrogen bonding depend on weak polymeric chain interactions. The formation of alginate hydrogels by crosslinking alginate chains with calcium ions is a common example. Chemical methods rely on the formation of covalent bonds within the polymeric chains and free radical polymerization. The choice of method depends upon the desired properties of the hydrogel.<sup>21</sup> The bioactive nature of natural hydrogels has led various researchers to work on their extraction. Sohrabi<sup>22</sup> focused on biometric hydrogels like collagen, fibrin, and hyaluronic acid having an extracellular matrix. Gong *et al.*<sup>23</sup> further revealed that these materials effectively improve cell adhesion, proliferation, and differentiation. Elsabahy and Wooley<sup>24</sup> worked on functional nanomaterials and hydrogels for controlled drug delivery using the polymerization-induced self-assembly (PISA) technique. Peppas<sup>25</sup> used alginate and chitosan for the release of drugs to specific body tissues. The study by Kang *et al.*<sup>26</sup> focused on obtaining stimuli-responsive hydrogels, which are sensitive to changes in system pH, temperature or light. Liao *et al.*<sup>27</sup> worked on regenerative medicines.<sup>28</sup> Wu *et al.* used silk fibroin and gelatin to



produce biodegradable hydrogels having adjustable mechanical properties. These polymers can be used in tissue engineering and organ implants.<sup>29</sup>

Synthetic polymeric hydrogels are synthesized in laboratories from monomers to obtain better control of their structural and chemical properties.<sup>30,31</sup> These hydrogels can further be modified to obtain precise drug release and are sensitive to pH and temperature.<sup>32,33</sup> These polymers mostly used in hydrogels include polyethylene glycol (PEG), poly acrylic acid (PAA), polyvinyl alcohol (PVA), poly *N*-isopropyl acrylamide (PNIPAA) and poly hydroxyethyl methacrylate (pHEMA).<sup>34-37</sup> Miyata *et al.* (1999) developed polyacrylamide hydrogels by chemical cross-linking and modified it using rabbit IgG antibodies.<sup>38</sup> It is worth noting that the characterization of polymers post-synthesis and post-modification is important to explore the properties and nature of the synthesized product.<sup>39-42</sup> Hydrogels based on polysaccharides like chitosan, dextrin, fibrin, and gelatin can be synthesized using covalent crosslinking and polymerization techniques.<sup>43</sup>

Natural polymeric hydrogels, although biocompatible and biodegradable, possess weak mechanical and non-versatile properties, limiting their applications. In this aspect, synthetic hydrogels promote added mechanical strength and other properties. However, unlike natural polymers, synthesized hydrogels are not environmentally friendly.<sup>44</sup> This problem can be addressed by combining natural polymeric hydrogels with the synthetic ones.<sup>36</sup> As an example, chitosan-based natural hydrogels can be combined with those based on synthetic polymers like polymethacrylic acid or polyacrylic acid to improve their properties. Such combinations with synthetic hydrogels may improve the mechanical strength and drug release control of natural hydrogels. A practical method for combining natural and synthetic hydrogels is to use interpenetrating network (IPN) hydrogels obtained by crosslinking different polymeric materials. IPNs show enhanced mechanical properties and more precise drug release control compared to single-network hydrogel polymers. Natural polymers like alginate, carrageenan, and gum arabic may also be combined with synthetic polymers to improve the control over the delivery of drugs to specific organs and offer improved tissue engineering.<sup>34</sup> The work by Nair *et al.* can be regarded as one of the foundational research on multi-functional materials for controlled drug

release and tissue engineering. They successfully developed strong and biocompatible hybrid hydrogels using thiol-Michael addition click chemistry.<sup>45</sup> The use of non-covalent, supramolecular interactions like  $\pi$ - $\pi$  interactions and hydrogen bonding to synthesize self-assembling hybrid hydrogels was explored by Hu *et al.* The self-healing abilities and stimuli responsiveness of these hybrid hydrogels render them a good candidate for biomedical and environmental applications.<sup>46,47</sup> The development of hydrogels using 3-D printing, as investigated by Zhang *et al.* helps achieve precise control of the structure and multifunctional properties of hydrogels (Table 1).<sup>48</sup>

### 3. Industrial and medical applications of polymeric hydrogels

Polymeric hydrogels, due to their salient features, may be widely used in different industrial, medical and biomedical applications. Some of these possible uses are discussed below:

Polymeric hydrogels are excellent adsorbents.<sup>57,58</sup> They can be used in wastewater treatment to adsorb the organic and inorganic pollutants like dyes,<sup>59-61</sup> heavy metals and pharmaceuticals from polluted water.<sup>40,62</sup> In agriculture, acrylic acid-acrylamide-guar gum hydrogels have shown efficacy in improving the growth of guava plants affected by drought.<sup>43</sup> In the medical and bio-medical fields, as already discussed, the ability of polymeric hydrogels for the controlled release of drugs to specific targets can effectively be utilized in the encapsulation of drugs.<sup>63</sup> They are sensitive to changes in pH, temperature and concentration of the substance. This property, especially pH sensitivity, is very helpful for drug delivery, as different tissues and cellular components in the body are at different pH levels.<sup>64</sup>

In engineering applications, like bone tissue engineering, hybrid polymers based on calcium carbonate and alginate have shown effective performance being biocompatible.<sup>65</sup> Similarly, nanomaterials obtained from xanthan gum or chitosan impregnated with ferric oxide nanoparticles have shown improved mechanical strength. This combination has been used in developing magnetically responsive tissue engineering systems.<sup>66</sup> Mokhtari *et al.* developed an injectable hydrogel of Kappa-carrageenan functionalized with *C*-phycocyanin which

**Table 1** Comparative overview of natural and synthetic polymeric hydrogels outlining their key merits and demerits specifically for 3D bioprinting applications in cancer modeling

	Types	Examples	Advantages	Disadvantages	Ref.
Natural polymeric hydrogels	Polysaccharides	Alginate, chitosan, hyaluronic acid	Biocompatible, biodegradable, non-toxic	Lower mechanical strength, batch-to-batch variability	49-52
	Proteins	Collagen, gelatin, fibrin	Biocompatible, cell adhesive, biodegradable	Less stable, can be immunogenic	53-55
Synthetic polymeric hydrogels	Acrylic acid-based	Poly(acrylic acid) (PAA)	Tunable swelling properties, pH sensitivity	Can be toxic, less biodegradable	30
	Vinyl alcohol-based	Poly(vinyl alcohol) (PVA)	Good mechanical properties, high water content	Less biocompatible, difficult to crosslink	56
	Polyethylene glycol-based	Poly(ethylene glycol) (PEG)	Biocompatible, non-toxic, highly water-soluble	Low mechanical strength, difficult to crosslink	50



is mechanically strong and promotes cell growth. The synthesized hydrogel possesses anti-oxidant, anti-microbial and anti-inflammatory properties, thus helping in quick wound recovery.<sup>67</sup> Similarly, the research by Dev *et al.* was focused on  $\kappa$ -carrageenan-*C*-phycocyanin-based injectable hydrogels for quick recovery of wounds, especially for patients with intensive tissue damage due to burning or diabetes. *C*-Phycocyanin accelerates hemostasis in severe injuries, which helps in quick wound healing.<sup>68</sup> Another wide application of polymeric hydrogels is cancer treatment, through 3-D modeling. The controlled release of drugs to the target cancer cells and improving the immune response, these hydrogels promote therapeutic ability and also minimize side effects of the treatment drugs.<sup>69</sup>

Hydrogels and their biological performance is profoundly influenced by their chemical composition and the specific functional groups they possess. For example, the inclusion of moieties like amino ( $-\text{NH}_2$ ), carboxyl ( $-\text{COOH}$ ), and hydroxyl ( $-\text{OH}$ ) groups significantly affects their degradation, bioactivity, and cellular interactions.<sup>70,71</sup> Amino groups, with their positive charge, enhance electrostatic interactions with negatively charged cell membranes, thereby facilitating cell adhesion and proliferation, while also playing roles in buffering intracellular pH and aiding endosomal escape in drug delivery. Carboxyl groups, conversely, contribute to pH responsiveness and degradability, especially in acidic tumor microenvironments, enabling hydrogen bonding and ionic crosslinking vital for stimuli-responsive drug release. Meanwhile, hydroxyl ( $-\text{OH}$ ) and sulfonic acid ( $-\text{SO}_3\text{H}$ ) groups improve hydrophilicity, swelling behavior, and facilitate protein adsorption, modulating cellular uptake and immune responses.<sup>33</sup> These functional groups also influence enzymatic degradation; for instance, gelatin and collagen-based hydrogels, rich in natural  $-\text{COOH}$  and  $-\text{NH}_2$  groups, are more susceptible to matrix metalloproteinase (MMP)-mediated degradation, a process highly relevant in tumor microenvironments.<sup>3,18</sup>

The very design of these hydrogels is intricately tied to their crosslinking chemistry, a fundamental aspect that controls their architecture, mechanical integrity, degradation rate, and cellular compatibility. Three major crosslinking strategies are commonly employed: photocrosslinking, which uses UV or visible light and photoinitiators to activate polymerization in materials like GelMA (gelatin methacryloyl), offering precise spatial and temporal control for fabricating complex cancer models with tunable stiffness and porosity.<sup>72</sup> Another approach is click chemistry, particularly thiol-Michael addition and azide-alkyne cycloaddition, enabling mild, efficient, and cytocompatible gelation ideal for bio-orthogonal and stimuli-responsive hydrogels, such as PEG-thiol and vinyl sulfone-based systems that incorporate bioactive cues with minimal cytotoxicity.<sup>45,73</sup> Finally, enzymatic crosslinking utilizes biologically compatible enzymes like horseradish peroxidase (HRP) and transglutaminase to form hydrogels under physiological conditions, often mimicking the natural extracellular matrix (ECM) and proving suitable for cell-laden constructs.<sup>74</sup>

Beyond their chemical makeup and crosslinking, the structural properties of hydrogels, including their porosity, cross-

linking density, and degradation rate, also profoundly influence their biological interactions. Porosity, for example, directly impacts nutrient diffusion, oxygen transfer, and cell migration; highly porous scaffolds might promote angiogenesis and faster tissue ingrowth but could compromise mechanical robustness. Conversely, crosslinking density is inversely related to degradation rate and permeability; hydrogels with a high density exhibit prolonged drug retention and sustained release profiles, which are beneficial for chemotherapeutic delivery in cancer. The degradation rate itself must be precisely synchronized with tissue regeneration or treatment timeframes. While natural hydrogels like gelatin or alginate degrade enzymatically or hydrolytically, synthetic ones like PEG or PVA offer better control over breakdown through targeted structural modifications, allowing for tailored performance in diverse biomedical applications.<sup>75,76</sup>

This intricate interplay of the composition and structure often culminates in advanced designs such as interpenetrating polymer networks (IPNs), which integrate two or more polymer systems to provide a synergistic blend of mechanical robustness and biological functionality, effectively simulating the mechanical heterogeneity and signaling complexity of native ECMs. For instance, gelatin-alginate IPNs have been extensively used in breast and colorectal cancer models, where gelatin supports cell adhesion while alginate provides structural stability and ionic crosslinking, mimicking *in vivo* tumor stiffness and heterogeneity.<sup>77-79</sup> Similarly, PEG-chitosan IPNs merge the pH responsiveness and biodegradability of chitosan with the structural flexibility and non-immunogenicity of PEG, enabling responsive drug delivery in acidic tumor environments.<sup>34</sup> These sophisticated ECM-mimetic systems offer advanced capabilities for spatial patterning and matrix stiffness tuning, and thus are essential tools for studying cancer cell invasion, metastasis, and drug resistance within complex 3D platforms.<sup>80</sup>

#### 4. Cancer: a disease of uncontrolled cell growth

Cancer is a disease caused by abnormal cell growth and division. Genetic mutations can lead to uncontrolled cell proliferation, forming tumors that may invade surrounding tissues or spread to distant parts of the body through a process called metastasis.<sup>81</sup> Various factors, including lifestyle, environmental exposure, and genetic predisposition, can increase cancer risk. Cancer can develop in various tissues and organs.<sup>82,83</sup> Some of the most common types of cancer are discussed below:

- **Carcinoma:** This type of cancer originates in epithelial tissues, which line organs and cavities. Examples include lung cancer, breast cancer, and colon cancer.<sup>84</sup>
- **Sarcoma:** Sarcomas develop in connective tissues, such as bone, muscle, and cartilage. Examples include osteosarcoma and liposarcoma.<sup>85</sup>

- Leukemia: This cancer affects blood-forming tissues, primarily bone marrow. It leads to the overproduction of abnormal white blood cells.

- Lymphoma: This cancer develops in the lymphatic system, a network of vessels and glands that help fight infection. Hodgkin lymphoma and non-Hodgkin lymphoma are its two main types.<sup>86</sup>

Treatment options, such as surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapy, depend on the specific type and stage of cancer. The stage of cancer refers to the extent of disease, which helps determine the best course of treatment and predict the patient's prognosis.<sup>87</sup> Cancer is typically staged from 0 to IV:<sup>88</sup>

- Stage 0 (carcinoma *in situ*): abnormal cells are present but are not spread.
- Stage I: cancer is small and localized.
- Stage II and III: cancer has spread to nearby lymph nodes or tissues.
- Stage IV (metastatic): cancer has spread to distant parts of body.

Cancer is a dangerous disease because it disrupts normal growth and division of cells. When abnormal cells grow uncontrollably, they can form tumors that invade nearby tissues or spread to distant parts of the body through a process called metastasis. This can lead to severe health problems, organ failure, and ultimately, death.<sup>89</sup>

#### 4.1. Conventional cancer treatment methods

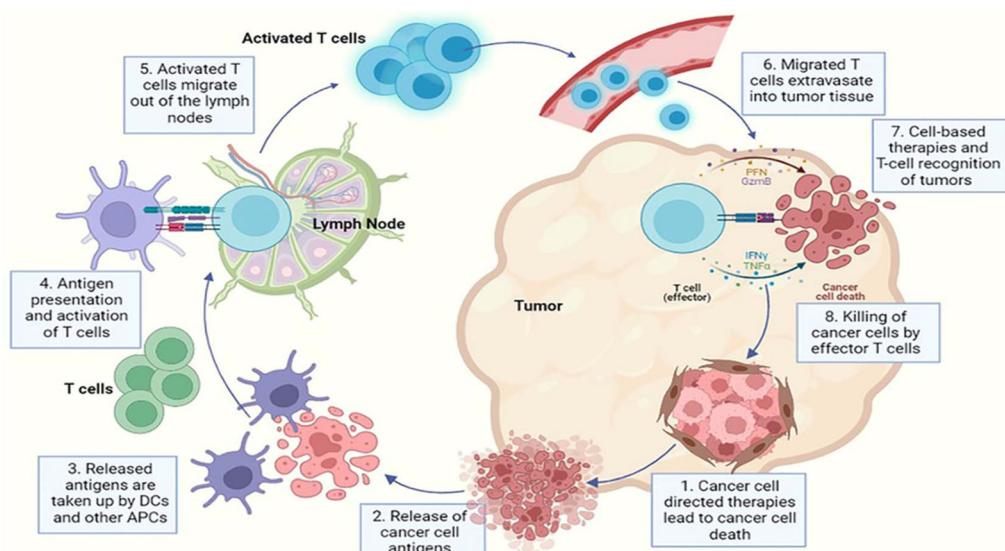
Cancer treatment involves a combination of therapies, tailored to a specific type in addition to the stage of disease. Traditional methods (Fig. 1) include surgery to physically remove tumors, chemotherapy to target rapidly dividing cancer cells, and radiation therapy to damage cancer cells with

high-energy rays. In recent years, more advanced therapies have emerged, such as immunotherapy, which harnesses the body's immune system to fight against cancer cells, and targeted therapy, which specifically targets cancer cells and minimizes damage to healthy tissues.<sup>90</sup> Additionally, emerging fields like gene therapy and nanotechnology offer promising avenues for future cancer treatments. The patient's health, type, stage of cancer, and possible side effects of various treatments are some of the key variables that affect treatment type selection.<sup>91</sup>

#### 4.2. Polymeric hydrogels for cancer treatment

Polymeric hydrogels, with their exceptional properties such as biocompatibility and biodegradability, are promising materials for the treatment of cancer owing to improved drug delivery, reduced side effects, and improved therapeutic efficiency.<sup>12</sup> There are several benefits to treating cancer with 3D printing and 3D modelling that uses hydrogels as hydrogels offer a 3D environment that closely resembles the extracellular matrix (ECM) found naturally in the human body owing to its high-water content and biocompatibility. Furthermore, when compared with traditional 2D cell culture systems, the 3D environment makes it possible to create more accurate tumour models.<sup>93–95</sup>

**4.2.1. Hydrogel based 3D modeling.** To address the limitations of traditional methods of cancer treatments, researchers are nowadays working on developing novel methods for the analysis of anti-tumor medications.<sup>69</sup> In recent days, a non-cellular scaffolding *i.e.*, the extracellular matrix (ECM, that is majorly composed of elastin, laminin, fibronectin, and proteoglycans) aids in the integrity as well as growth of tissue in the neighbourhood of solid tumours.<sup>96</sup> This can be done by developing some interactions of the ECM with cells and external



**Fig. 1** Diagrammatic overview of cancer treatment showing the multifaceted approaches for combating cancer. Reproduced from ref. 92 with permission from Frontiers, copyright 2025.



cues resulting in the formation of a tumour microenvironment (TME). The precise illustration of the TME can be achieved using 3D cell culture modelling by favouring cells to show their interactions with their environment and with one another (so that they can be comparable to how they act *in vivo* by offering a 3D matrix). This would then lead researchers to examine the cells' response to different inputs such as growth hormones and mechanical pressures.<sup>97</sup> The advancements in biomedical and tissue engineering fields make it possible to develop more complicated 3D models that elaborate interactions between tumour cells and their surroundings. A clear understanding of tumor progression and metastasis in addition to drug resistance can be achieved by using 3D scaffolds together with tumor spheroids and scaffold-based models.<sup>98</sup> A study looked into the application of hybrid self-assembling peptide (EFK8)-carbon nanotube (SWNT) hydrogels for *in vitro* 3D cancer spheroid production and tissue engineering (TE) (Fig. 2). The results of the study revealed that by the addition of SWNTs, there was an improvement in cell behaviour in comparison with hydrogels that contained only EFK8.<sup>99</sup> NIH-3T3 cells showed enhanced attachment, proliferation, and movement on hybrid hydrogels under 2D and 3D culture conditions, with increased EFK8 peptide concentration corresponding to increased hydrogel compressive modulus.<sup>100</sup> The study reveals that the use of SWNTs in self-assembling peptide hydrogels improves their biocompatibility and

mechanical characteristics thereby broadening their potential applications in tissue engineering.<sup>101</sup>

Hypoxia-inducible factor-1 (HIF-1) is a key factor in hypoxia, that is characterized by low oxygen levels and is a characteristic of advanced malignancies. Because oxygen gradients within tumours are vital, scientists have now worked on the synthesis of O<sub>2</sub>-controllable hydrogels to investigate how hypoxia affects sarcoma cell invasion and migration. A useful tool for assessing the early phases of the metastatic process are these synthetic hydrogels.<sup>103</sup> Additionally, it has been confirmed that 3D collagen scaffolds are more resistant to cisplatin having larger amounts of reactive oxygen species (ROS) when compared with 2D models. A more physiologically appropriate microenvironment that resembles the circumstances faced by tumour cells *in vivo* can lead to enhanced ROS generation in 3D cultivated cells.<sup>104,105</sup> The efficient distribution of therapeutic medicines is hampered by elements such as cancer cell adherence and cellular packing density. Nowadays, researchers use 3D hydrogel-based models for simulating the physiological conditions inside the TME for addressing this limitation.<sup>106</sup> 3D hydrogels, made from alginate, are widely employed to study anticancer agents and drug resistance pathways. These models offer a more physiologically relevant platform when compared with conventional 2D culture systems. They also better study the unique characteristics of tumor necrosis factor (TNF), as cancer stem cells (CSCs), in their specialized niches.<sup>107</sup>

A major area of preclinical study has been breast cancer, a cancer that is common in women. Creating 3D models of breast cancer can speed up research resulting in more accurate results by offering valuable information.<sup>108</sup> A study reported the use of an engineered hydrogel-based human adipose/collagen model for investigating breast cancer cell migration and exploring the effect of adipocytes on this migration. The model highlights the improved cell migration when its efficiency was compared with the empty scaffold controls revealing its cancer therapeutic efficacy in personalized medicine approaches.<sup>109</sup> Furthermore, 3D hydrogel-based platforms were used to model different kinds of cancers beyond pancreatic ductal adenocarcinoma. For example, 3D hydrogel models have been developed to replicate the microenvironment of ovarian cancer.<sup>110</sup> For lung cancer, 3D hydrogel models have been shown to more accurately mimic cell invasion and metastasis when compared with traditional 2D models.<sup>111,112</sup>

Researchers have employed hydrogels to mimic the pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment (TME) by incorporating components such as hyaluronic acid (HA), collagen, and Matrigel, which are derived from or inspired by the TME. Osteosarcoma (OS) is the most common primary malignant bone tumor. Studies have shown that both scaffold-free and scaffold-based 3D OS culture models are valuable tools for replicate complex TME, with spheroids being particularly well-suited for this purpose.<sup>113</sup> A 3D endothelialized vesicle equivalent, developed using a bladder collagen-based cancer model<sup>95</sup> can potentially reduce animal experimentation and aid in toxicological evaluation of

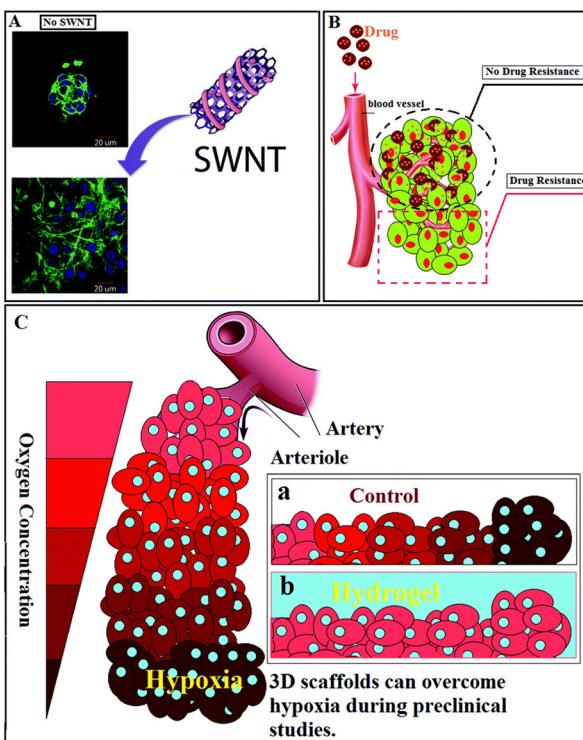


Fig. 2 (a) Hypoxia within tumor sites affects cancer cell response to therapy. (b) Hydrogels and cell culture media aid in facilitating oxygenation to the tumor site, possibly enhancing therapeutic efficiency. Reproduced from ref. 102 with permission from RSC copyright 2025.



anti-cancer drugs. To accurately model cancer progression and human tumors, a 3D environment that mimics the extracellular matrix (ECM) is crucial. 3D hydrogels facilitate *in vitro* study of various tumor cells and modeling of tumor angiogenesis.<sup>114</sup> By manipulating conditions and parameters, researchers can more easily conduct drug screening and obtain reliable results.<sup>115</sup>

Reactive oxygen species (ROS) exhibit a complex, dual nature in cancer progression and treatment. While tumor microenvironments often exhibit elevated ROS levels due to heightened metabolic activity and mitochondrial dysfunction, these same ROS can be strategically exploited for therapeutic gain. Specifically, ROS-sensitive hydrogels offer a promising avenue; they undergo cleavage, swelling, or degradation in response to oxidative stress, enabling precise, controlled drug release.<sup>116–118</sup> Consider, for instance, hydrogels that incorporate disulfide bonds. These materials effectively degrade when exposed to both glutathione (GSH) and ROS, facilitating on-demand drug release directly within oxidative tumor sites.<sup>104,119</sup> Similarly, thioketal-based polymers have been integrated into hydrogel matrices to release agents like doxorubicin or siRNA. Their cleavage by ROS in inflamed or cancerous tissues allows for targeted delivery.<sup>120</sup> Such systems are vital for minimizing systemic toxicity by ensuring drug delivery is localized to these ROS-rich cancerous regions. 3D hydrogels provide a robust platform for simulating *in vivo*-like tumor conditions, including critical hypoxia and oxidative stress gradients. By embedding ROS-sensitive probes or reporter systems within these hydrogels, researchers can visually assess drug efficacy based on subsequent ROS generation. For example, 3D collagen scaffolds have been effectively used to evaluate the varied ROS responses and cisplatin resistance in both ovarian and lung cancer cells.<sup>104</sup> Notably, higher ROS levels observed in 3D models correlated with greater drug resistance compared to 2D models. This underscores the significant utility of these advanced models in screening drugs under realistic stress conditions. Furthermore, ROS-inducible fluorescence reporters can be leveraged for real-time monitoring of antioxidant or pro-oxidant drug activities within 3D matrices. This methodology allows for dynamic assessment of redox-modifying therapeutics, such as paclitaxel, curcumin, or selenium nanoparticles.<sup>121</sup>

**4.2.2. Cancer-model 3D printing with hydrogels.** 3D bioprinting has emerged as a revolutionary technology, building upon conventional 3D printing principles. It involves the layer-by-layer deposition of bioinks, which are materials containing living cells, to create three-dimensional biological structures.<sup>122</sup> Common bioink materials include alginate, collagen, fibrin, gelatin, gellan gum, silk, polycaprolactone (PCL), and poly(ethylene glycol) (PEG). To create *in vitro* cancer models, various techniques are employed, including cell seeding, microsphere encapsulation, microfluidic systems, 3D printing (3DP), and 3D bioprinting (3DBP). Understanding complex cancer development and designing effective therapies is challenging due to the intricate interplay of biological factors. 3D bioprinting has emerged as a powerful tool to address this

challenge.<sup>123</sup> 3D bioprinting (3DP) provides precise spatial control over cell placement and biomaterial deposition, enabling the recreation of the tumor microenvironment (TME) with high fidelity. This technology overcomes drawbacks of traditional tissue engineering methods, as constructing complex biological architectures. It offers a unique approach to fabricate intricate 3D microarchitectures in tissues such as cartilage, skin, bone, and blood vessels. It allows for simultaneous incorporation of multiple cell types and polymers, while also facilitating nutrient and oxygen diffusion through integrated micro-channels.<sup>93</sup>

Hydrogels are versatile for 3D bioprinting in cancer research. Their adaptable characteristics, including mechanics, degradation, and optics, along with cell-supporting capabilities, make them well-suited for constructing 3D tumor models. Table 2 compares properties of natural and synthetic hydrogels polymers for cancer treatment.<sup>124</sup> Natural polymers while offering good biocompatibility (e.g., alginate, collagen) often suffer from poor mechanical properties that can hinder bioprinting. Semi-synthetic polymers like GelMA address these limitations by enhancing stability and tunability while maintaining biocompatibility. Synthetic polymers, such as PEG, demonstrate excellent bio-printability and shape fidelity but may exhibit limited biocompatibility. The choice of cross-linking method significantly impacts hydrogel properties. Chemical crosslinking, involving irreversible covalent bonds, often results in strong hydrogels but may introduce cytotoxicity due to the use of chemical crosslinking agents. On the other hand, physical crosslinking, relying on non-covalent interactions (e.g., thermal or ionic), is generally more cell-friendly but may produce weaker hydrogels. By carefully selecting the polymer and the crosslinking method, researchers can tailor hydrogel properties to specific 3D cancer bioprinting applications.<sup>125</sup>

Breast cancer remains a significant global health concern, with metastasis and recurrence being major contributors to mortality. While advancements in screening and awareness have improved survival rates, the complex interplay between tumor cells and the surrounding microenvironment poses significant challenges in modeling this disease. 3D bioprinting has emerged as a valuable tool for developing sophisticated breast cancer models.<sup>135</sup> Utilizing patient-derived materials, such as human mammary-derived extracellular matrix (ECM), has further enhanced the physiological relevance of these models. Photothermal therapy, which utilizes infrared radiation, has emerged as a valuable modality for cancer treatment. To effectively implement photothermal therapy, the development of scaffolds/hydrogels with robust photothermal properties is crucial. One study demonstrated the potential of 3D printing (3DP) to fabricate porous scaffolds (dopamine-modified alginate and PDA) that mimic the mechanical properties of breast tissue while exhibiting impressive photothermal effects. Elba E. Serrano *et al.*<sup>136,137</sup> developed methodologies for the ultrastructural analysis of hepatocellular carcinoma 70 (HCC70) cells cultured in both monolayer and 3D hydrogel environments. The triple-negative breast cancer



**Table 2** Comparative analysis of hydrogels derived from diverse origins for 3D bioprinting applications

Hydrogel	Origin	Properties	Crosslinker	Application in cancerous models
Alginate	Natural (brown algae)	Biocompatible, forms stable gels, easy to handle <sup>126</sup>	Ionic crosslinking (calcium ions), enzymatic crosslinking	Breast cancer, ovarian cancer, prostate cancer, pancreatic cancer <sup>78,127</sup>
Gelatin	Animal derived protein (from collagen)	Biodegradable, support cell, attachment and growth	Chemical crosslinking	Used for 3D culture model & breast cancer, liver cancer, glioblastoma, melanoma <sup>128</sup>
Hyaluronic acid (HA)	Natural (animal tissues)	Biocompatible, anti-inflammatory properties, found in extracellular matrix	Chemical crosslinking (hydrazide chemistry), photopolymerization	Breast cancer, ovarian cancer, skin cancer, brain cancer, colorectal cancer model <sup>79</sup>
Collagen	Natural (animal sources)	Excellent cell–matrix interactions, high biocompatibility	Chemical crosslinking (EDC/NHS), enzymatic crosslinking	Breast cancer, liver cancer, lung cancer, head and neck cancer, pancreatic cancer model <sup>129</sup>
Fibrin	Natural (blood plasma protein)	Promotes cell adhesion and tissue growth	Covalently cross linked	Tissue engineering for cancer therapy <sup>130</sup>
Polyethylene glycol (PEG)	Synthetic	Biocompatible, tunable properties, low immunogenicity	Photopolymerization	Cancer of breast, liver, prostate, pancreas, lung adenocarcinoma model <sup>131</sup>
Poly(vinyl alcohol)	Synthetic	Biocompatible, good mechanical strength, stable	Freeze–thaw cycles, chemical crosslinking	Glioblastoma, brain cancer, pancreatic cancer <sup>132</sup>
Poly(lactic-co-glycolic acid)	Synthetic	Biodegradable, adjustable degradation rate & mechanical properties	Chemical crosslinking	Scaffolds for cancer therapy <sup>133</sup>
PLGA	Semisynthetic (modified form of gelatin)	Maintained biocompatibility, biodegradable, increased stability and tunability in comparison with unmodified form	Free radical polymerization	Bladder cancer model <sup>134</sup>
GelMA				

(TNBC) cell line HCC70 was maintained in a commercially available membrane matrix hydrogel for a 2D monolayer culture. In contrast to a disorderly spread of flattened cells in the 2D culture (Fig. 3A–C), cells cultured in 3D hydrogels formed multi-layered spheroids (Fig. 3D–F). This approach provides a valuable tool for studying and analyzing transmission electron microscopy (TEM) images of both cancer and healthy cell line hydrogel cultures.

Lung cancer remains a leading cause of cancer-related deaths worldwide, with late diagnosis significantly impacting mortality rates, particularly in aggressive small-cell lung cancer. 3D bioprinted models enable the study of various aspects of lung cancer, such as cellular metabolism, metastatic progression, muscle cachexia, and drug response. Common cell lines used in these models include A549 and NL20, while patient-derived tumor cells can be utilized to create personalized preclinical models. Compared to traditional tumor organoid cultures, 3D bioprinting offers potential advantages such as faster development, reduced technical complexity, and increased success rates. This study investigated the impact of scaffold architecture on liver cancer model development. Gelatin-based 3D liver models were bioprinted with varying angles between adjacent layers: 90 degrees (A) and 60 degrees (B). The study assessed cell viability of HUH7 liver cancer cells after three days of culture within these scaffolds and evaluated the formation of bile canaliculi, which are crucial structures for liver function.<sup>138</sup> Colorectal cancer, a leading cause of cancer-related deaths worldwide, predominantly arises as an adenocarcinoma originating from the inner lining of the colon

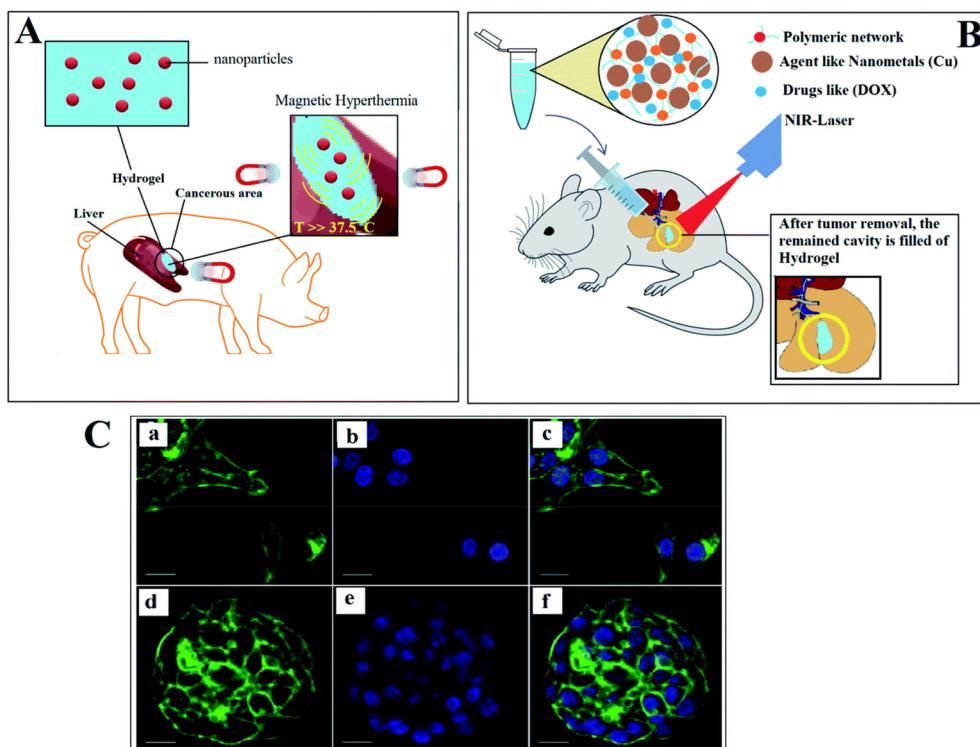
or rectum. Accurate modeling of this disease presents significant challenges due to the complex interplay between cancer cells, the gut microbiota, and the intricate layered structure of the gastrointestinal tract.<sup>139</sup> A recent study has shed light on cell of colon cancer migration through leveraging 3D printing (3DP) technology. By combining hydrogels with 3DP, researchers can conduct more accurate and cost-effective studies. For instance, 3D-printed mandible templates can accelerate product development and reduce manufacturing and clinical costs after cancer treatment. Despite significant advancements in this field, there remains a pressing need to further develop and integrate these methods to establish a comprehensive strategy for cancer treatment.<sup>140</sup>

## 5. Challenges and clinical translation of 3D-bioprinted hydrogels

### 5.1. Technical limitations

A paramount concern in 3D bioprinting is ensuring cells remain viable throughout the entire printing process. Various factors, such as high extrusion pressure, exposure to UV light during photocrosslinking, or elevated temperatures, can lead to cellular damage or even apoptosis. Research indicates that printing pressures exceeding 100 kPa and high concentrations of photoinitiators substantially reduce cell survival rates.<sup>141</sup> To combat this, optimizing nozzle diameter and extrusion speed, alongside incorporating protective additives like gelatin or trehalose, has proved effective in boosting post-printing cell viability.





**Fig. 3** (A) Hydrogels loaded with nanoparticles for enhanced magnetic hyperthermia. (B) Scaffold/hydrogel with photothermal properties. (C) Confocal microscopy of HCC70 cells: (a–c) in monolayer culture and (d–f) in a hydrogel environment. Reproduced from ref. 102 with permission from RSC copyright 2025.

lity. Another limitation in 3D bioprinting is bioink rheological behaviour. The successful printability of bioinks is intrinsically linked to their rheological characteristics, including shear-thinning behavior, viscosity, and gelation kinetics. Hydrogels like alginate and gelatin-methacryloyl (GelMA) are frequently chosen for their customizable rheological profiles. Rapid crosslinking immediately after deposition is crucial for preserving the printed structure's fidelity. If the rheological properties aren't properly tuned, issues such as nozzle clogging or the collapse of the printed construct can occur.<sup>142,143</sup> Batch-to-batch inconsistency is another limitation of 3D bioprinting. Biological components commonly used in hydrogels, such as gelatin, Matrikel, and collagen, often exhibit variability in their composition, which depends on their source and processing methods. Employing synthetic or semi-synthetic bioinks, such as PEG or GelMA, synthesized under stringent, controlled conditions, helps in reduction of batch-to-batch inconsistency.<sup>126,143,144</sup>

## 5.2. Clinical translation and regulatory limitations

Scaling up hydrogel production for either clinical application or industrial use demands highly reproducible processes that maintain consistent viscosity, crosslinking, and biological performance. Sterilization methods must also be carefully chosen to preserve the hydrogel's integrity. While advancements such as automated extrusion and robotic bioprinting platforms have improved the process, the associated costs and need for

greater standardization continue to present significant challenges.<sup>145</sup> In the U.S., the Food and Drug Administration (FDA) classifies hydrogel systems that combine a biological matrix with embedded drugs, growth factors, or cells as combination products.<sup>146–148</sup> The regulatory review process thoroughly evaluates manufacturing processes (CMC), sterility, biodegradation, and potential device–drug interactions. Early engagement with regulatory bodies, often through mechanisms like the FDA's INTERACT meetings, is highly recommended for developers.<sup>149,150</sup>

## 6. Conclusion

In conclusion, this comprehensive review has underscored the transformative potential of polymeric hydrogels, particularly when integrated with 3D bioprinting technologies, in revolutionizing cancer modeling and therapy. 3D bioprinting enables precise manipulation of hydrogel scaffolds, controlling their architecture and composition. This allows for the creation of intricate structures that mimic the tumor microenvironment, advancing cancer research and treatment. We have explored the synthesis and diverse medicinal applications of both naturally occurring and man-made hydrogels, demonstrating their critical role in advancing cancer research through precise compositional control afforded by 3D bioprinting, which is crucial for high-throughput drug screening.



By engineering stimuli-responsive hydrogels with enhanced mechanical and biochemical properties, researchers can develop more effective and personalized therapies. To ensure widespread adoption of hydrogel-based treatments, scalability and affordability are critical considerations. Addressing these aspects will position hydrogels as foundational technologies for next-generation cancer therapies. While holding significant promise in cancer treatment, the clinical translation of polymeric hydrogels faces several hurdles. These include the need to develop hydrogels with improved mechanical properties, controlled degradation, and efficient drug delivery. Additionally, successful clinical translation requires careful consideration of biocompatibility, toxicity, and regulatory approval. Despite these challenges, the future of polymeric hydrogels in cancer treatment remains promising.

## Data availability

The data are already available within the article.

## Conflicts of interest

There are no conflicts to declare.

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