Incorporating nanoparticles in 3D printed scaffolds for bone cancer therapy

Nour M. Al Sawaftah, William G. Pitt, Ghaleb A. Husseini

Abstract

The low occurrence rate of bone cancer contributes to delayed diagnosis and treatment; in addition, the surgical resection of bone tumors can cause significant bone defects, further hindering the effective treatment of the disease. 3D printing can help overcome some of these limitations by enabling the design and fabrication of innovative scaffolds loaded with chemotherapeutics and growth factors, stimulating bone regeneration, and delivering targeted cancer treatment. Moreover, advancements in nanotechnology have opened up new possibilities for bone tissue engineering. Nanoparticles (NPs) possess size-dependent physicochemical properties. NPs can be harnessed by embedding NPs in 3D-printed scaffolds to develop multifunctional bone scaffolds with enhanced mechanical properties and drug delivery capabilities. This review evaluates the impact of incorporating NPs in 3D-printed scaffolds on bone cancer therapy and bone regeneration. First, various 3D printing techniques employed in the biomedical field are presented and explained. The article then highlights notable achievements by researchers in this area. Finally, the review discusses the current obstacles facing this technology and how they can be addressed to enable translation into clinics.

Keywords: Bone cancer, Cancer therapy, Nanoparticles, 3D printing, Stimulus

1. Introduction

Bone cancer is a group of diseases characterized by the uncontrolled growth and dissemination of abnormal cells in the body forming masses known as tumors [1,2]. According to the American Cancer Society, around 1.9 million new cancer cases are expected to be diagnosed in 2023 in the US alone [3]. The American Cancer Society estimated that for primary cancer of the bones and joints in 2023, about 3970 new cases will be diagnosed [4]. Bone cancer can be divided into primary and metastatic, where the former is caused by cancer cells originating in bone tissue that has metastasized from other primary tumor areas to bone tissue [5]. Primary bone cancer can be further classified into the following (refer to Fig. 1) [6–8]:

- Osteosarcoma: this is the most common form of bone cancer and usually affects children and young adults (in children and young adults, osteosarcoma accounted for 56% of primary bone cancer cases in 2022). It often develops in the growing ends of long bones, such as the legs or arms.

- Chondrosarcoma: this type of bone cancer arises in the cartilage cells, usually affecting adults and accounted for around 40% of primary bone cancer cases in adults in 2022. It tends to occur in the pelvis, thigh, or shoulder.

- Ewing sarcoma: primarily affects children and young adults and can develop in the bones or soft tissues (Ewing sarcoma accounted for 34% of primary cancer cases in 2022). Ewing sarcoma often occurs in the pelvis, chest wall, legs, or arms.

Surgery is usually the first course of action for bone cancer treatment; chemotherapy, radiotherapy, and/or targeted therapy can also be included in the treatment plan, especially if the cancer has metastasized or is in a high-risk or difficult-to-reach region [5]. Despite the availability of various cancer interventions, bone cancer treatment remains a challenge for medical professionals and researchers due to its low incidence rate leading to delayed diagnosis and treatment, tumor heterogeneity, inter-individual variability, systemic side effects associated with some treatment modalities, and cancer recurrence [9,10]. Moreover, bone tumors and surgical interventions can cause large bone defects, usually addressed using autografts, allografts, and artificial implants.
structure to induce bone formation and growth [11]. Nevertheless, au
cancer patients [22, 23]. With respect to bone cancer, 3DP was first
research and drug development [21]. The most significant breakthrough
allowing the creation of 3D-printed tissues and organoids for cancer
patients, improving treatment outcomes [20]. In the early 2000s, re
was initially used to create custom implants and prosthetics for cancer
become a significant field of research. A promising approach involves
using 3D printing to fabricate novel scaffolds from different functional
materials and to load these implants with chemotherapeutics and growth factors; such scaffolds would provide mechanical support, pro-
mote bone regeneration, and localized cancer treatment [14–16]. Recent studies have also focused on developing stimulisi-sensitive-drug-loaded-implants to further enhance the efficiency of bone cancer treatment and promote bone regeneration [5]. Therefore, incorporating stimuli-responsive materials into 3D-printed scaffolds presents a promising technique to control the bone/tumor micro-environment, enhance localized chemotherapeutic/growth fac-
tors release, and accelerate bone formation allowing better cell growth and tissue regeneration [17,18]. This review focuses on incorporating NPs into 3D-printed scaffolds to enhance bone cancer therapy and bone regeneration.

2. 3D printing methods

3D printing (3DP), also known as rapid prototyping or additive
manufacturing, refers to the process of making 3D solid objects by
depositing materials layer-by-layer via computer-aided design (CAD)
3DP was first introduced in the early 1980s in Japan and since its
invention, various 3DP techniques have been developed. In 1988, Charles Chuck Hull developed the first commercial 3D printer based on
his patented technique known as stereolithography [16,19]. In the early
1990s, the use of 3DP started expanding into the biomedical field and
was initially used to create custom implants and prosthetics for cancer
patients, improving treatment outcomes [20]. In the early 2000s, re-
searchers started exploring the potential of 3DP for personalized cancer therapy, including patient-specific tumor models for drug testing.
Moreover, in the late 2010s, bioprinting emerged as a promising field,
allowing the creation of 3D-printed tissues and organoids for cancer
research and drug development [21]. The most significant breakthrough
came in 2016 when Aprecia Pharmaceuticals launched Spritam®, the
first FDA-approved 3D-printed tablet designed to treat epilepsy. Nowa-
days, 3DP can also be used to devise individualized treatments for
cancer patients [22,23]. With respect to bone cancer, 3DP was first
introduced in the 2010s for bone tissue engineering, with a focus on
creating 3D-printed bone substitutes followed by the introduction of
3D-printed orthopedic implants designed to replace cancer-affected
bone segments. Currently, 3DP can be used to fabricate scaffolds at a
lower cost, with higher accuracy and repeatability, as well as facilitate
the incorporation of other functional agents within the scaffold [5,15,
16,24].

Various 3DP techniques have been employed in the biomedical field,
including extrusion-based, inkjet-based, laser-assisted, and stereo-
lithography (SLA)-based methods [15,25,26]. Table 1 summarizes the
working principle, advantages, limitations, and applications of 3DP
techniques relative to cancer therapy.

2.1. Biomaterial inks and bioinks

The terms biomaterial ink and bioink are sometimes mistakenly used
interchangeably; technically these are different terms as biomaterial inks refer to materials that are biocompatible or designed to interact with
biological systems and can serve as ink in 3DP. In contrast, bioinks are
defined to contain living cells as a fundamental element, irrespective
of the other materials into which the cells are seeded or that serve as the
basal matrix (refer to Fig. 3) [16,20,27,28]. Biomaterial inks can be used
to print structures such as scaffolds, matrices, and implants that can be
used independently or in conjunction with bioprinting to provide me-
chanical support for the cells [25,27]. Various biomaterial inks have been utilized, including biocompatible metals, ceramics, polymers, and
biomolecules, such as enzymes, growth factors, etc. On the other hand,
bi-inks include single cells, cells aggregated in spheroids, cells organ-
ized into mini-tissues or organoids, cells coated by a thin layer of
material, cells seeded onto microcarriers, or cells encapsulated in
tailed colloidal microenvironments such as physical hydrogels or
hydrogel precursors [27,29,30]. Active hydrogels are commonly used
in bioprinting to provide an environment that optimally mimics the
native microenvironment of the human body because their structure and
composition resemble that of the extracellular matrix (ECM). Hydrogel
bioinks can be classified by the matrix surrounding the cells as natural
(e.g., collagen, gelatin, alginate, chitosan, hyaluronic acid, cellulose,
and fibrin) or synthetic bioinks (e.g., polyurethane, polyethylene glycol
(PEG), and Pluronic F127) [15,27,28,31,32].

The properties of bioprintable materials are essential to ensure the
quality of the printed structure, as well as to meet the stringent condi-
tions placed by the cellular component(s) of bioinks. Inks should also be
composed of biocompatible, biodegradable, nontoxic materials that
promote nutrient diffusion and sustain tissue maturation during the
post-printing culture phase. Furthermore, biomaterial and bio-ink scaf-
folds must possess three main qualities [16,33]: printability, function-
ality, and mechanical strength. The ink needs to be able to be fed and
flow through the 3DP printer, be processed, and finally result in a scaffold with enough strength to maintain its shape and functionality [31,34,35].
Viscosity is another important rheological parameter because it affects
the printing process, i.e., high-viscosity inks are able to maintain their
shape and structure; however, they require higher pressures during the
printing process. Biomaterial inks with shear-thinning characteristics
are more suitable for 3DP, especially for extrusion-based methods,
because their viscosities decrease during extrusion, thus limiting the
exposure of cells to excessive shear forces that can induce cell membrane
damage and lead to reduced cell viability after printing [31,35]. Lower
viscosity materials are recommended for droplet-based 3DP, whereas
higher viscosity inks can be used in laser-assisted 3DP [16,36].

3. Nanoparticles in 3D printed scaffolds for bone cancer therapy

An emerging trend in 3D-printed scaffolds is the incorporation of
nanomaterials or nanoparticles (NPs) in the printed product to enhance
the printed systems’ properties, functionality, and drug release kinetics
[40–42]. NPs are ultrafine particles whose size ranges between 1 and
100 nm, with at least one property being considerably different from its
bulk counterpart [43,44]. The size dependence of some properties
of NPs arises from their large surface-area-to-volume ratio, which means
that there are more atoms exposed at the surface; hence, more
Table 1
Comparison of various 3D printing technologies (adapted from Refs. [14–16,20,37–39]).

<table>
<thead>
<tr>
<th>Method</th>
<th>Materials</th>
<th>Working Principle</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Fused deposition modeling       | Thermoplastic polymer filaments          | Extrusion-based method where a molten thermoplastic polymer filament is extruded through a high-temperature nozzle and is then deposited layer by layer (LbL)                                                | - Produces complex scaffolds with high mechanical strength  
- Inexpensive  
- Can be used with simple desktop 3D printers  
- Uses readily available filaments, reducing post-processing needs |
| (FDM)/Fused filament fabrication (FFF) |                                          |                                                                                                                                                                                                              | - Not suitable for bioprinting because high temperatures can cause cell degradation  
- Resolution of printed products limited by nozzle size and printing setup |
| Pressure-assisted microsyringes | Semi-liquid mixture of polymers and solvents (solution, paste, or dispersion) | Viscous and semi-liquid materials are deposited LbL through a pressurized air piston and a syringe extruder                                                                                               | - Operates at room temperature  
- Suitable for bioprinting  
- Continuous flow of aqueous-based materials  
- Simple, versatile  
- Wide range of starting materials |  
| (PAM)                           |                                          |                                                                                                                                                                                                              | - Quality depends on rheological properties  
- Requires solvents or crosslinking agents  
- Slower printing speed |
| Stereo-lithography (SLA)        | Photo-curable liquid resin               | Uses light sources from UV to visible light to crosslink or polymerize the ink for the development of 3D structures                                                                                           | - Operates at room temperature  
- Drugs cast in resin which prevents degradation  
- High resolution  
- Speed of fabrication  
- Creation of smooth surfaces |
| Laser-assisted method           | Ink solution, laser energy absorbing powders | Based on laser-induced forward-transfer (LIFT) effect. A NIR or UV- a pulsed laser that transfers energy into a liquid photopolymerizable material. Photopolymerization occurs, and the product is created LbL. | - Low heat required, suitable for bioprinting  
- High resolution  
- Solvent-free process |
| Drop-on-demand (DOD) inkjet-based printing | Ink—drug solution, substrate—polymer-based films | Two-step process: (1) formation of electrositically charged ink droplets and directing them toward predefined locations on the substrate and (2) droplet and substrate get to interact | - Higher droplet generating frequency than continuous inkjet  
- Involves high temperatures, which can cause the degradation of drugs and/or cells  
- Sterilization issues  
- Wastage of material, low resolution, and expensive |
| Continuous inkjet printing      |                                           |                                                                                                                                                                                                              | - Lower drop generating frequency than continuous inkjet  
- Involves high temperatures, which can cause the degradation of drugs and/or cells  
- Sterilization issues  
- Wastage of material, low resolution, and expensive |
| Binder jetting                  | Binder fluid, powder bed                | Liquid binder injected through DOD to selective areas of a spreading layer of powder material. The saturated area solidifies, and inkjet-solidification steps are repeated for each layer until the object is constructed | - Can be performed at room temperature  
- Wide range of starting materials  
- Fast disintegrating dosage forms can be produced |
|                                |                                          |                                                                                                                                                                                                              | - Post-fabrication processes necessary  
- Use of organic solvents  
- Wastage of powder material  
- Results in fragile dosage forms |

All schematics were created using BioRender.
The second reason for size dependence at the nanoscale is quantum confinement, i.e., when the NP size is smaller than the de Broglie wavelength of an electron or hole. Accordingly, NPs exhibit unique physical, chemical, and optical properties. Moreover, depending on their composition, NPs can be classified as organic, inorganic, and carbon-based. Another current trend in 3D-printed scaffolds for bone cancer therapy research is to co-load the scaffold or the embedded NPs with anticancer drugs and bone growth factors to simultaneously kill tumor cells and counteract bone loss/defects caused by surgery and chemotherapy. Osteogenesis and tumorigenesis are two distinct biological processes that involve the growth and development of different types of tissues, each regulated by intricate molecular pathways and cellular interactions. Osteogenesis refers to the formation of bone tissue, whereas tumorigenesis is the formation of abnormal tissue masses resulting from the uncontrolled proliferation of cells.

Bone growth factors, can exhibit effects of promoting osteogenesis while avoiding tumorigenesis through selective targeting of specific pathways or receptors, promotion of stem cell differentiation into osteoblasts while inhibiting cancerous cell differentiation, immunomodulation, and specific receptor activation/inhibition.

However, these properties require meticulous design to ensure safety and efficacy in therapeutic applications, considering factors such as dosage and delivery mechanisms. Growth factors for improving osteogenesis include vascular endothelial growth factors (VEGF), fibroblast growth factors (FGFs), and bone morphogenic proteins (BMPs). Interestingly, some of these growth factors are also being studied for their role in tumorigenesis. One such example is bone morphogenetic protein-2 (BMP-2). BMP-2 is a member of the transforming growth factor-beta (TGF-\(\beta\)) superfamily and is known for its crucial role in promoting bone formation and repair by activating Smad-dependent pathways to induce mesenchymal stem cell differentiation into osteoblasts, upregulating osteogenic transcription factors such as Runx2 and Osterix, and subsequently stimulating the production of essential extracellular matrix proteins like collagen and osteocalcin, vital for bone tissue formation. In the context of cancer therapy, BMP-2 was found to exert antiproliferative effects on specific cancer cell types by inducing cell cycle arrest and apoptosis, mediated through the regulation of signaling pathways like Smad and MAPK, while also modulating the tumor microenvironment by affecting angiogenesis and immune regulation, ultimately impeding cancer cell growth and metastasis.
Incorporating these NPs into 3D-printed matrices for bone cancer therapy due to their ability to encapsulate and target therapeutic agents, containing bone morphogenetic protein 2 (BMP-2) and Cisplatin (CDDP). In vitro and in vivo studies showed that incorporating the nanoMOFs into the scaffold helped improve drug loading and release; it also enabled the device to become responsive to the tumor microenvironment leading to tumor inhibition through the release of CDDP and inducing new bone formation by releasing BMP-2. Oladapo et al. [66] used FDM to synthesize a new poly-ether-ether-ketone calcium hydroxyapatite (PEEK-chAp) composite bone implant to investigate the impact of chAp on the mechanical behavior of PEEK bone implants. The 3D-printed composites showed improved tensile strength, stiffness, and Young’s elastic moduli compared with the pure epoxy matrix, especially at chAp of 10 and 20 wt%. Core-shell NPs, liposomes, and micelles have shown significant attention in drug delivery and cancer therapy due to their ability to encapsulate and target therapeutic agents effectively. These nanocarriers offer advantages such as controlled release, improved solubility of hydrophobic drugs, reduced systemic toxicity, and targeted delivery to specific cells or tissues [67]. Incorporating these NPs into 3D-printed matrices for bone cancer therapy has shown promising potential in enhancing treatment outcomes. Core-shell NPs consist of a core material surrounded by a shell that can protect the therapeutic cargo and control its release profile [68]. By incorporating anticancer drugs into the core, and modifying the surface with targeting ligands, core-shell NPs can efficiently deliver drugs to tumor cells while minimizing damage to healthy tissues. Integrating these NPs into 3D matrices allows for the fabrication of patient-specific implants or scaffolds that can release drugs locally, providing sustained therapeutic effects at the tumor site [14]. Micelles are self-assembled NPs formed from amphiphilic block copolymers, capable of encapsulating hydrophobic drugs within their hydrophobic core. These structures can improve the circulation time of drugs in the body and enhance their accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect [69]. When integrated into 3D printing matrices or tissue engineering scaffolds, micelles enable controlled drug release, facilitating precise spatiotemporal control over therapeutic delivery for effective cancer treatment. Moreover, liposomes are spherical vesicles composed of lipid bilayers, enabling the encapsulation of both hydrophilic and hydrophobic drugs within their aqueous core or lipid layers [70, 71]. These structures can improve drug solubility, stability, and bioavailability while minimizing off-target effects. In tissue engineering, liposomes can be incorporated into scaffolds or hydrogels to create drug-releasing constructs that facilitate the localized and sustained delivery of anticancer agents, thereby enhancing the effectiveness of cancer therapy. Sarkar and Bose [72] used porous 3D-calcium phosphate (CaP) scaffolds containing curcumin-loaded liposomes to study curcumin’s anticancer and osteogenic properties. These bifunctional scaffolds showed improved cytotoxicity towards osteosarcoma (MG-63) cells, while also promoting bone proliferation as tested in vitro using human fetal osteoblast (hFOB) cells. In another study by Bose et al. [73], curcumin was encapsulated in nanosized polymeric micelles and incorporated in TCP-3D-printed scaffolds. The developed scaffold demonstrated enhanced attachment of osteoblast (hFOB) cells, a 4-fold increase in hFOB cell proliferation, and a 73 % enhancement in hFOB cell differentiation. Additionally, it exhibited cytotoxic effects against osteosarcoma cells, resulting in 61 % lower viability compared to the control. Ma et al. [74] studied Fe3CaSiO4 composite scaffolds (30CS) to provide mechanical support to bone cortical defects and induce anti-cancer effects. The 30CS possessed remarkable compressive strength; in addition, the inclusion of Ca3SiO4 within the composite scaffolds resulted in enhanced degradation characteristics, stimulated the growth and differentiation of rat bone-marrow-derived mesenchymal stem cells and further facilitated the formation of bone tissue in living organisms. Fan et al. [75] developed Paclitaxel (PTX) 3D-printed titanium scaffolds (TS) for bone reconstruction and osteosarcoma recurrence prevention. The amphiphilic PEG-acetal-PTX (PEG-acetal-PTX) prodrug polymer self-assembled into micellar structures and was attached to the surface of the TS using polydopamine (PDA). The 3D NP-composite scaffold was tested under physiological (pH of 7.4) and acidic conditions (pH of 6.5) at 37 °C. An accelerated release rate of PTX was observed under acidic conditions (85.33 ± 0.66 %), attributed to the cleavage of the acetal bonds at a lower pH. The in vitro study results showed that the treatment of co-cultured five types of human osteosarcoma cells (143 B, MG63, HOS, U2OS, and SAOS2) with the composite scaffold group for 24 h.

**Fig. 3.** Incorporating NPs in 3D-printed scaffolds and using stimuli to trigger drug release (created using BioRender.com).
decreased cell activity compared to that of the TS treatment group. However, when the incubation time was extended to 48 and 72 h, there was no statistically significant difference in the cell activity between the two groups (the scaffold group was dominated by late apoptotic cells, accounting for 20.31%–58.21 % of the total cells while for the PTX treated group, the late-stage apoptotic cells accounted for 24.31%–59.64 % of the total cells). Moreover, the in vivo performance of the developed system was tested in nude mice. The longest survival time was reached in the composite scaffold group (survival time of 60 days), whereas the survival times of the mice in the control, PTX, and TS groups were 29, 37, and 30 days, respectively.

Another interesting feature of NPs is that they can be designed to respond to internal or external triggers forming what is known as stimuli-sensitive or “smart” NPs [76,77]. Smart NPs can undergo specific changes in their physical and chemical properties or have specific functionalities triggered by various stimuli, e.g., temperature, pH, light, magnetic fields, or chemical agents [78-80]. Depending on the desired properties and applications, these NPs can be engineered from a variety of materials, such as polymers, lipids, metals, or hybrid composites. By incorporating them into 3D-printed scaffolds, they allow for precise and targeted delivery of therapeutic agents, imaging agents, or other payloads in biomedical applications, thereby enhancing the efficiency, selectivity, and therapeutic efficacy of drug delivery systems (refer Fig. 3) [81-83]. Lin et al. [84] developed a dual-purpose 3DP scaffold to simultaneously kill cancer cells and repair tumor-associated bone defects. The scaffold was composed of polydopamine (PDA) incorporating Fe and Mg-NPs. Fe-NPs were added to the PDA-based scaffold because Fe$^{2+}$ ions can result in a synergistic effect of combining chemodynamic therapy (CDT) and photothermal therapy (PTT), leading to the effective elimination of tumor cells when triggered using NIR. On the other hand, Mg-NPs were included because Mg$^{2+}$ can enhance osteoblastic differentiation, thereby effectively promoting the repair of bone defects associated with tumors. In vitro, results showed that the FeMg-NPs scaffolds could effectively kill tumors even under a short laser irradiation time of 3 min. Similar antitumor effects were observed in mice treated with the FeMg-NPs scaffold group. In addition, the scaffolds containing Fe and Mg-NPs showed remarkably higher bone mineral density, new bone volume, and new bone volume fraction in vivo. Zhang et al. [85] fabricated gelatin-based DOX-loaded scaffolds incorporating SrCuSiO$_4$-TCP core/shell filaments to treat bone defects caused by osteosarcoma. The research demonstrated that DOX was released in response to NIR-II triggering, leading to effective chemo-photothermal therapy with synergistic effects for cancer treatment both in vitro and in vivo. Additionally, the scaffold’s transformation to a hollow channeled TCP/SC scaffold, along with the sustained release of bioactive ions (Sr, Cu, Si, and Ca) from bioceramic degradation, was found to promote osteogenesis and angiogenesis in vitro and facilitate vascularised bone regeneration in vivo. Dong et al. [86] designed MOF Cu-TCPP nanosheets interface-structured β-tricalcium phosphate (TCP) (Cu-TCPP-TCP) scaffold using the solvothermal method and were irradiated with NIR light. The developed Cu-TCPP-TCP scaffolds, when exposed to NIR light with a power density of 1.0 W/cm$^2$ for 10 min, were able to effectively eliminate osteosarcoma cells by releasing heat energy. The irradiated Cu-TCPP-TCP scaffolds increased the temperature on the surface, with the equilibrium temperatures of 5Cu-TCPP-TCP, 10Cu-TCPP-TCP, 20Cu-TCPP-TCP scaffolds at the time point of 600s reaching 84.58, 101.77, and 108.18 °C at dry conditions and 42.04, 51.79, and 57.21 °C at wet conditions, respectively. Furthermore, these scaffolds demonstrated the ability to ablate subcutaneous bone tumor tissues, inhibit their growth by converting NIR light into heat energy, and promote bone regeneration in vivo. Hyperthermia NPs, also known as magnetic nanoparticles (MNPs), are a type of NPs, composed of magnetic materials such as iron oxide, that can generate heat when exposed to an alternating magnetic field. When these NPs are targeted to cancer cells and subjected to an alternating magnetic field, they generate localized heat, which can be used to selectively destroy cancer cells. This process, known as magnetic hyperthermia, takes advantage of the sensitivity of cancer cells to elevated temperatures compared to healthy cells. The localized heating can induce cell death in the tumor while minimizing damage to the surrounding healthy tissues. Zhang et al. [87] developed a 3D-printed β-TCP bioceramic scaffold modified with Fe$_3$O$_4$ NPs/graphene oxide layers, resulting in a structure exhibiting super-paramagnetic behavior and hyperthermia effects. The magnetic intensity of the scaffold was found to increase proportionally with the Fe$_3$O$_4$ content. Careful modulation of the scaffold’s temperature between 50 and 80 °C was achieved by adjusting the magnetic intensity and Fe$_3$O$_4$ content. The hyperthermia effect of the scaffolds induced more than 75 % cell death for osteosarcoma cells (MG-63) in vitro. These findings highlighted the scaffold’s potential for treating bone defects caused by tumors through its combined magnetic and osteogenic properties. Table 2 summarizes some relevant studies discussing 3D-printed scaffolds incorporating NPs utilized for bone cancer therapy.

4. Challenges and future directions

Despite the potential demonstrated by 3D-printed scaffolds in bone cancer therapy, they still face several challenges regarding technology, safety, quality control, regulatory aspects, and their translation into clinical applications. The technological limitations of each 3DP technique have been detailed in Table 1 above; as can be seen from the table, the main limitation across different techniques is the risk of drug degradation and the incompatibility of some techniques with heat-sensitive active pharmaceutical ingredients (APIs) [16,37,99]. Choosing the appropriate ink is crucial for 3DP, as it has to fulfill the safety and biocompatibility requirements [100]. Many materials have been employed as 3D inks, but some can deteriorate or generate toxic substances when heated, extruded, or fused. For instance, certain biodegradable polymers, such as polylactic acid (PLA), are commonly used in 3DP for their biocompatibility and biodegradability. However, during the 3DP process, if the temperature and printing conditions are not properly controlled, these polymers can degrade, leading to the generation of harmful substances including particulate matters (PM) and volatile organic compounds (VOCs) such as, toluene, aldehydes, and ethylbenzene. Acrylonitrile butadiene styrene (ABS), a widely used thermoplastic, was also found to emit VOCs during the printing process [101]. Polylactic acid (PLA) is a frequently used biodegradable polymer. However, its hydrophobic nature may impede cell adhesion and proliferation, and also result in the release of acidic by-products during the degradation process [101,102]. Surface treatments have been applied to alter the structure and surface chemistry of PLA, enhancing the hydrophilicity and promoting the attachment of cells and biological compounds to PLA-based structures [102]. Plasma treatment is one of the most investigated methods developed to enhance the surface chemistry of PLA-based parts without affecting their overall properties [103]. Moreover, the crosslinking process involved in forming hydrogel structures can sometimes produce toxic residues or unreacted monomers that may be harmful to the cells or tissues they come into contact with. For example, chemical crosslinking using glutaraldehyde (GA) can lead to cytotoxicity and dysfunction in cells because the GA crosslinking mechanism is a highly nonspecific process that involves most of the lysine residues distributed on the surface of the protein. Ethyl-dimethylaminopropyl-carbodiimide hydrochloride (EDC) can be used as a chemical cross-linking agent to overcome this cytotoxicity since EDC can easily be washed away as a water-soluble urea derivative [104,105]. Although physical crosslinking can avoid the toxicity of the crosslinking agent and the possible interaction between the crosslinking agent and the drug, physically crosslinked hydrogels tend to have low strength and can cause protein denaturation [104,106]. To address these concerns, scientists are actively working on refining printing parameters, exploring innovative crosslinking methods, and developing more stable biomaterials. Their goal is to enhance the safety and effectiveness of 3D-printed biomaterials inks. Enzymatic crosslinking, an emerging
<table>
<thead>
<tr>
<th>Payload</th>
<th>NPs type</th>
<th>3DP technique</th>
<th>Scaffold material</th>
<th>Stimulus</th>
<th>Main Findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Fe and Mg NPs</td>
<td>Cryogenic micro-extrusion</td>
<td>PDA</td>
<td>NIR</td>
<td>- Fe$^{2+}$ and Mg$^{2+}$ ions enhanced antitumor effects and stimulated bone regeneration.</td>
<td>[84]</td>
</tr>
<tr>
<td>BMP-2 and VEGF</td>
<td>-</td>
<td>IBL deposition</td>
<td>HAP, gelatin, and chitosan</td>
<td>-</td>
<td>-ALP activity significantly improved, i.e., the differentiation to osteogenic cells was enhanced considerably.</td>
<td>[88]</td>
</tr>
<tr>
<td>BMP-2 and CT</td>
<td>pZIF-8 nanoMOFs and PDA-decorated- pHA NPs</td>
<td>FDM Gelatin incorporated with PDA-hybridized Calcium phosphate</td>
<td>PDA</td>
<td>pH</td>
<td>-Incorporation of nanoMOFs improved drug loading and release.</td>
<td>[65]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Liposomes</td>
<td>Binder jetting</td>
<td>PDA</td>
<td>-</td>
<td>-Tumor microenvironment responsive device.</td>
<td>[72]</td>
</tr>
<tr>
<td>BMP-2</td>
<td>-</td>
<td>-</td>
<td>HAP, chitosan and PVA</td>
<td>-</td>
<td>-MTT results showed 70 % lower cell viability.</td>
<td>[89]</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Liposomes</td>
<td>SLS</td>
<td>Polyglycolic acid</td>
<td>Magnetic field</td>
<td>-ALP assay exhibited higher osteoblast-differentiating ability in the presence of liposome-encapsulated curcumin.</td>
<td></td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>-</td>
<td>Semi-solid extrusion-based 3D-printer Calcium phosphate cement, SOL, and PEG</td>
<td>PCL</td>
<td>-</td>
<td>-Inhibition of cancer cell growth after 5 days.</td>
<td>[92]</td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>-</td>
<td>Drop-on-powder CaSO$_4$ hydrates, vinyl polymer, and 2-pyrrolidone</td>
<td>-</td>
<td>-</td>
<td>-In vitro release studies showed that about 90 % of the drug had been released within 2 h for all scaffolds.</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Fe$_2$O$_3$ NPs</td>
<td>Exrusion</td>
<td>TCP</td>
<td>NIR</td>
<td>-NIR irradiated scaffolds eliminated osteosarcoma cells and promoted bone regeneration in vivo.</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>Mo$_2$Z N nanosheets and HA NPs</td>
<td>Exrusion AKT Calcium phosphate</td>
<td>PEEK</td>
<td>NIR</td>
<td>-The incorporation of NPs in combination with PTT enhanced adhesion and proliferation of bone cells.</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>CaO$_2$ and Fe$_3$O$_4$ NPs</td>
<td>Exrusion AKT Calcium phosphate</td>
<td>PEEK</td>
<td>NIR</td>
<td>-CaO$_2$ NPs loaded into AKT scaffolds featured distinct bone-regeneration capabilities due to the release of Ca$^{2+}$ ions.</td>
<td>[95]</td>
</tr>
<tr>
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<td>-</td>
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<td>BCN nanosheets</td>
<td>Exrusion</td>
<td>AKT</td>
<td>NIR</td>
<td>-BCN-Fe$_3$O$_4$ NPs loaded into AKT scaffolds showed higher expression of osteogenesis genes in vitro and in vivo, indicating higher bone regeneration capability.</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Nb$_2$C MXene S-nitrosothiol-grafted mesoporous silica</td>
<td>Bioplotting Biactive glass</td>
<td>Biactive glass</td>
<td>NIR</td>
<td>-controlled NO release upon irradiation.</td>
<td>[97]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Polymeric micelles</td>
<td>Binder jetting</td>
<td>TCP</td>
<td>-</td>
<td>-The developed scaffold exhibited a4-fold increase in hFOB cell proliferation, and a 73 % increase in hFOB cell differentiation.</td>
<td>[73]</td>
</tr>
<tr>
<td>DOX</td>
<td>SrCoSi$_2$O$_7$-β-TCP core/shell filaments</td>
<td>Exrusion Gelatin</td>
<td>NIR</td>
<td>-</td>
<td>-The scaffold resulted in 61 % lower viability compared to the control.</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Fe$_3$O$_4$ NPs, graphene oxide nanocomposites</td>
<td>Exrusion TCP Magnetic field</td>
<td>TCP</td>
<td>-</td>
<td>-The scaffold resulted in 61 % lower viability compared to the control.</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Magnetic graphene oxide @Fe$_3$O$_4$ NPs</td>
<td>Exrusion PVA/SA/HA Magnetic field</td>
<td>PVA/SA/HA</td>
<td>Magnetic field</td>
<td>-Adding MGO@Fe$_3$O$_4$ NPs to the prepared composite scaffolds improved the biological functions and supported the differentiation of rat BMSCs in vitro and showed favourable anti-tumor effects in vivo.</td>
<td>[98]</td>
</tr>
</tbody>
</table>

*TCP/PLGA, β-tricalcium phosphate/poly(lactic-co-glycolic; PDA, Polydopamine; HAP, Hydroxyapatite; PVA, Poly(vinyl alcohol); PCL, Polycaprolactone; SLS, selective laser sintering; TCP, β-tricalcium phosphate, PEEK, Polyether-ether-ketone; PTT, Photothermal therapy; AKT, Akermanite (Ca$_2$MgSi$_2$O$_7$); BMP-2, bone morphogenetic protein 2; LbL, Layer-by-layer; pZIF-8 nanoMOFs, nanosized zeolitic imidazolate framework-8; pHA NPs, hydroxyapatite nanoparticles; ALP, alkaline phosphatase; BCN, Borocarbonitrides; PVA/SA/HA, Polyvinyl alcohol/sodium alginate/hydroxyapatite.*
method in hydrogel production, presents numerous benefits, including rapid gelation time, mild aqueous reaction conditions (e.g., physiological temperature), as well as controlled physicochemical properties. Various enzymes, such as horseradish peroxidase, transglutaminase, tyrosinase, phosphopantetheinyl transferase, and l-lysyl oxidase, have been employed for this purpose, demonstrating the diverse enzymatic options available. However, the careful selection of enzymes is crucial, because using enzymes for crosslinking may introduce unforeseen biological changes in vivo [107]. Therefore, it is essential to take sufficient safety measures and adhere to the standard guidelines to reduce the risk of exposure to harmful substances. Rigorous testing and validation protocols should be established to assess the safety and biocompatibility of the printed constructs before their application in various biomedical and research settings [37,108].

Furthermore, various obstacles can hinder the implementation of 3D technology in hospitals. First, establishing 3D printers in hospitals is expensive and demands proficient technical operators to manage the technical procedures on the premises. Second, maintaining the standard of the printed drug forms is another challenge, especially since there is currently no regulatory framework governing 3D-printed drugs. In 2017, the FDA released guidelines that outlined the regulatory standards for medical device manufacturing using 3DP. Currently, only one 3D-printed pharmaceutical product (Spritam®) has received FDA approval [109], and it is unclear whether regulatory approval will only be limited to the final product or will apply to all aspects of the product’s design and manufacturing stages [32,37,110]. Complex UK and EU regulations, along with safety, scalability, and production challenges at the regional level, have posed obstacles for both regulators and applicants, especially since many developments arise from academic institutions rather than businesses. The European Commission’s implementation of the Advanced Therapy Medicinal Products (ATMP) regulation (EC 1394/2007) and directive 2009/120 aimed to standardize market availability in the EU and provide marketing authorization guidance, including definitions for these novel technologies [111]. Therefore, improved communication between regulatory bodies and organizations that produce and market tissue engineered products (TEPs) for clinical purposes is vital to effectively tackle these challenges [112]. Furthermore, institutions that adopt such therapies must show their readiness to address the increasing need for these services, including logistical elements such as appropriate transportation, storage, and nearby manufacturing facilities. Additionally, these facilities should be equipped to source donor and autogenous tissue for bioprinting, possibly through collaboration with established clinical organizations, such as blood and transplant services, which manage tissue handling and recipient preparation [112,113]. The importance of incorporating well-designed clinical trials into the regulatory process cannot be overstated. Conducting rigorous clinical trials within hospital settings allows for the comprehensive evaluation of the safety and efficacy of 3D-printed products in real-world patient populations. By implementing well-structured clinical trials, hospitals can gather vital data on the effectiveness and potential risks associated with these innovative therapeutic interventions [114]. However, concerns regarding clinical trials stem primarily from the ethical concerns of testing tissue-engineered organ transplantation on healthy volunteers and the complexities associated with determining the impact of patient-specific cell populations on treatment outcomes versus the bioprinted product. Challenges arise from the inability of patients to withdraw post-consent and obtaining consent for participation becomes problematic when the severity of the problem is unknown. These factors present additional obstacles in trial organization. Consequently, it is necessary to establish a valid and all-encompassing method for assessing the impact of bioprinted interventions before initiating any valuable clinical trials in this field [112,115]. With the rise of artificial intelligence (AI), some researchers are suggesting integrating AI and predictive models into 3DP to ensure the quality, uniformity, release profile, and concentration of the loaded drug(s) [116,117].

As mentioned above, an area of research that is gaining popularity is the use of stimuli-responsive biomaterials in 3DP technology to produce multifunctional products incorporating NPs capable of responding to stimuli or providing diagnostic and therapeutic, i.e., theranostic applications. This trend is expected to expand rapidly in the upcoming years [37,40,118,119]. Despite the reported success in literature, further investigation is still needed to develop biomaterials with sufficient complexity to accurately emulate the bone microenvironment. In the context of bone cancer, the ideal biomaterials would have the ability to sense the most suitable bioactive molecules to release into the tumor microenvironment, thereby achieving successful tumor elimination and tissue regeneration following surgical removal [40,118,120]. In addition, the choice of NPs to be incorporated into the scaffolds is also highly affected by the 3DP method to be used [121]. Incorporating NPs into the printing process might reduce the printing efficiency or place additional demands on the 3D printer [44]. For instance, in SLA, the presence of NPs in the ink reservoir affects light penetration and scattering, resulting in a significant decrease in printing speed [63,122]. Other issues caused by the incorporation of NPs in the 3DP ink include uneven spreading of ink, difficulties in controlling the ink viscosity, and agglomeration [44,63,122]. Additionally, the toxicity and biodegradability of NPs are commonly raised issues when NPs remain in the body for extended periods. Freely circulating NPs can be cleared effectively through the kidney or GI tract to reduce long-term toxicity risks [123–125]. However, NPs incorporated in 3D-printed scaffolds pose the extra challenge of releasing the NPs from the scaffold before they can be cleared from the body, hence reducing their effective biodegradability [63,126,127]. A possible approach to address these limitations is to focus more on organic NPs, such as liposomes, micelles, dendrimers, and solid-lipid NPs, because they have lower toxicity and higher biocompatibility than inorganic NPs [63,128,129]. Moreover, adding NPs into 3D-printed scaffolds introduces complexities in terms of fabrication and processing, which would increase the costs of production and limit their applications in clinical settings [130,131].

The concept of incorporating stimuli-responsiveness in 3D-printed structures has led to the development of an intriguing technology known as four-dimensional printing (4DP) [81]. 4DP evolved from 3DP; therefore, the printing steps and printing methods are similar to 3DP; however, compared to 3DP, this technology adds one more dimension, i.e., time, to the product [129,132,133]. 4D-printed structures can ‘fold’ or ‘unfold’ into predetermined shapes in response to specific triggers, including temperature, pressure, humidity, light, magnetic and electric fields [133]. Smart materials that generate 4D-printed structures for biomedical applications include shape-memory alloys and polymers, electroactive polymers, magnetic shape-memory alloys, and smart hydrogels [134,135]. However, 4DP is a relatively new technology, and several aspects need to be further studied and researched. For instance, high-resolution printing techniques are needed to ensure precise shape fabrication and movement, limiting the choice of 3DP methods that can be employed [129]. Moreover, at present, the deformation of 4D-printed structures is relatively basic, often limited to simple actions like folding, which falls short of fulfilling the intricate requirements in clinical applications. The activation/de-activation of these responses needs to be precisely controlled since it introduces an added risk in case the structure does not behave or change its shape as required [81,132]. Smart materials used in 4DP introduce complexities in processing and fabrication that would significantly increase the costs of translating this technology into clinical applications [129]. Additionally, for 4D-printed scaffolds to be widely accepted, the manufacturing process must be both cost-effective and time-efficient. Unfortunately, existing 4DP methods have limited scalability rendering them impractical for large-scale manufacturing. Scalability is a challenge for both 3DP and 4DP bone scaffold because the printed scaffolds need to be customized or personalized to cater to the diverse needs of patients which would increase the time and costs associated with the process [136]. Nonetheless, 4DP is presenting new possibilities in biomedical engineering and is
expected to act as a versatile toolset to address medical challenges by acknowledging the dynamic nature of both the human body and the scaffold used.

5. Conclusion

Many challenges still limit the success and effective treatment of bone cancer using existing methods. The emergence of 3DP and its rising popularity in the biomedical and pharmaceutical fields promises safer and more effective treatment opportunities by enabling the development of novel scaffolds loaded with chemotherapeutics and growth factors. These scaffolds support, stimulate bone regeneration, and enable targeted delivery of drugs. In addition, embedding NPs in these scaffolds, with their unique physicochemical properties, results in versatile bone implants with improved mechanical attributes and drug delivery capabilities. Although this technology is still in its infancy, it appears to be a groundbreaking tool and is expected to revolutionize bone cancer therapy in the near future.

Ethical compliance

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References


D. Loss, Quantum phenomena in nanotechnology, Nanotechnology 20 (43) (2009), 430205.


Ehata, S. and Miyazono, K., Bone Morphogenetic Protein Signaling in Cancer; Some Topics in Recent 10 Years. https://www.frontiersin.org/articles/10.3389/fmars.20


combinatory osteosarcoma therapy and NO-augmented bone regeneration, Small 16 (14) (2020), 1906814.


[115] K. Singh, G. Sharma, 3D Printing Technology in the Pharmaceutical and Applications, Now and Future..


[136] Ramezani, M. and Mohd Ripin, Z., 4D Printing in Biomedical Engineering: Advancements, Challenges, and Future Directions..