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Topical Review

3D/4D printed bio-piezoelectric smart scaffolds for next-generation bone tissue engineering

Annan Chen\textsuperscript{1,2,3,6}, Jin Su\textsuperscript{1,2,6}, Yinjin Li\textsuperscript{1,2}, Haibo Zhang\textsuperscript{1}, Yusheng Shi\textsuperscript{1,2}, Chunze Yan\textsuperscript{1,2,*} and Jian Lu\textsuperscript{3,4,5,*} \footnote{These authors contributed equally to this work.} \footnote{Authors to whom any correspondence should be addressed.}

\textsuperscript{1} State Key Laboratory of Materials Processing and Die & Mould Technology, School of Materials Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, People’s Republic of China
\textsuperscript{2} Engineering Research Center of Ceramic Materials for Additive Manufacturing, Ministry of Education, Wuhan 430074, People’s Republic of China
\textsuperscript{3} Centre for Advanced Structural Materials, Department of Mechanical Engineering, City University of Hong Kong, Hong Kong, People’s Republic of China
\textsuperscript{4} Centre for Advanced Structural Materials, City University of Hong Kong Shenzhen Research Institute, Greater Bay Joint Division, Shenyang National Laboratory for Materials Science, Shenzhen 518057, People’s Republic of China
\textsuperscript{5} CityU-Shenzhen Futian Research Institute, Shenzhen 518045, People’s Republic of China

E-mail: c_yan@hust.edu.cn and jianlu@cityu.edu.hk

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Abstract

Piezoelectricity in native bones has been well recognized as the key factor in bone regeneration. Thus, bio-piezoelectric materials have gained substantial attention in repairing damaged bone by mimicking the tissue’s electrical microenvironment (EM). However, traditional manufacturing strategies still encounter limitations in creating personalized bio-piezoelectric scaffolds, hindering their clinical applications. Three-dimensional (3D)/four-dimensional (4D) printing technology based on the principle of layer-by-layer forming and stacking of discrete materials has demonstrated outstanding advantages in fabricating bio-piezoelectric scaffolds in a more complex-shaped structure. Notably, 4D printing functionality-shifting bio-piezoelectric scaffolds can provide a time-dependent programmable tissue EM in response to external stimuli for bone regeneration. In this review, we first summarize the physicochemical properties of commonly used bio-piezoelectric materials (including polymers, ceramics, and their composites) and representative biological findings for bone regeneration. Then, we discuss the latest research advances in the 3D printing of bio-piezoelectric scaffolds in terms of feedstock selection, printing process, induction strategies, and potential applications. Besides,
some related challenges such as feedstock scalability, printing resolution, stress-to-polarization conversion efficiency, and non-invasive induction ability after implantation have been put forward. Finally, we highlight the potential of shape/property/functionality-shifting smart 4D bio-piezoelectric scaffolds in bone tissue engineering (BTE). Taken together, this review emphasizes the appealing utility of 3D/4D printed biological piezoelectric scaffolds as next-generation BTE implants.

Keywords: 3D/4D printing, bio-piezoelectric materials, biomimetic scaffolds, electrical microenvironment, bone regeneration

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>EM</td>
<td>Electrical microenvironment</td>
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<tr>
<td>AM</td>
<td>Additive manufacturing</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>4D</td>
<td>Four-dimensional</td>
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<tr>
<td>BTE</td>
<td>Bone tissue engineering</td>
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<tr>
<td>PBF</td>
<td>Powder bed fusion</td>
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<td>DIW</td>
<td>Direct ink writing</td>
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<td>FDM</td>
<td>Fused deposition modeling</td>
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<td>BJ</td>
<td>Binder jetting</td>
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<td>VPP</td>
<td>Vat photopolymerization</td>
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<tr>
<td>SL</td>
<td>Stereolithography</td>
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<td>DLP</td>
<td>Digital light processing</td>
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<td>VAM</td>
<td>Volumetric additive manufacturing</td>
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<td>TiSL</td>
<td>Projection micro-stereolithography</td>
</tr>
<tr>
<td>TPP</td>
<td>Two-photon polymerization</td>
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<tr>
<td>PVDF</td>
<td>Poly (vinylidene fluoride)</td>
</tr>
<tr>
<td>PVDF-TrFE</td>
<td>Poly (vinylidene fluoride-trifluoroethylene)</td>
</tr>
<tr>
<td>PVDF-HFP</td>
<td>Poly (vinylidene fluoride-hexafluoropropylene)</td>
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<tr>
<td>PLLA</td>
<td>Poly (l-lactic acid)</td>
</tr>
<tr>
<td>PHA</td>
<td>Polyhydroxyalkanoates</td>
</tr>
<tr>
<td>PHB</td>
<td>Poly (3-hydroxybutyrate)</td>
</tr>
<tr>
<td>PHBV</td>
<td>Poly (3-hydroxybutyrate-3-hydroxyvalerate)</td>
</tr>
<tr>
<td>$d_{33}$</td>
<td>Piezoelectric coefficient</td>
</tr>
<tr>
<td>hASCs</td>
<td>Human adipose stem cells</td>
</tr>
<tr>
<td>HAp</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>BT</td>
<td>Barium titanate</td>
</tr>
<tr>
<td>KNN</td>
<td>Potassium sodium niobate</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal stem/stromal cells</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolactone</td>
</tr>
<tr>
<td>PLA</td>
<td>Polylactic acid</td>
</tr>
<tr>
<td>BST</td>
<td>Strontium-doped barium titanate</td>
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<tr>
<td>β-TCP</td>
<td>β-tricalcium phosphate</td>
</tr>
<tr>
<td>SBF</td>
<td>Simulated body fluids</td>
</tr>
<tr>
<td>TPMS</td>
<td>Triply periodic minimal surface</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>$\mu$CT</td>
<td>Micro-computed tomography</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>DMD</td>
<td>Digital micromirror microscope</td>
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<tr>
<td>SME</td>
<td>Shape memory effect</td>
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1. Introduction

Bone as one of the most important tissues in the body supports and protects various organs. Bone tissues are well known for their innate self-healing capacities [1, 2]. As for large segmental bone defects (>4–5 cm) caused by car accidents, gunshot wounds, and resection of malignant tumors, etc, however, the self-healing capacity would be extensively delayed or completely lost that requires external intervention to restore normal function [3, 4]. At present, autografting or allografting remains the gold standard for bone defect repair. Nonetheless, autografts have been subjected to the restrictions set by harvest and donor morbidity. Allografts, on the other hand, are readily available but may cause immunogenic rejection of cells/tissues [1, 5]. Therefore, large segmental bone regeneration remains a significant challenge in the clinical.

Emerging BTE offers a promising alternative treatment for challenging bone defects. It has an excellent ability to induce new bone regeneration through a synergistic combination of functional biomaterials, cells, and growth factors. Among them, 3D scaffolds act as the key factor that determines the final structure and mechanical properties of biomaterials, cell attachment/proliferation, and biological microenvironment [6, 7]. To date, various manufacturing strategies have been developed to create implant scaffolds with bone-like topologies, such as direct foaming, freeze drying, and electrospinning [8–10]. Although these conventional techniques have been widely used to manufacture bone scaffolds, they still have well-recognized morphological limitations and poor scalability. AM has emerged as one of the most promising strategies to overcome these limitations and offers significant advantages in fabricating complex patient-customized scaffolds over conventional techniques [11, 12]. However, the current additive-manufactured scaffolds mainly focus on the reconstruction of bionic topological structure and mechanical microenvironment, while the crucial EM in bone regeneration is neglected.

The intrinsic electrophysiological feature of native bones including piezoelectricity, pyroelectricity, and ferroelectricity, has been reported to play a crucial role in modulating metabolic activities such as growth, structural remodeling, and
bone repairing [13, 14]. For instance, Fukada and Yasuda [15] first discovered the piezoelectric effect of native bones in 1957. Park et al [16] attempted to use piezoelectric materials as bone implants for the first time in 1981. Lipieca et al [17] used infrared spectroscopy to demonstrate this piezoelectric effect at the molecular level for the first time in 2012. Since then, the bone tissues’ piezoelectricity and their role in modulating cellular metabolic activities to promote bone regeneration and remodeling have been extensively studied [18, 19]. In recent, several typical theories developed for the origin of piezoelectricity in native bone have been summarized in some reviews [20, 21].

Bio-piezoelectric materials with inherent piezoelectric characteristics have demonstrated outstanding advantages in EM reconstruction and attracted tremendous interest in recent years [22, 23]. To date, there have been numerous bio-piezoelectric materials (such as polymers, ceramics, and their composites) developed for BTE, as well as their corresponding AM technologies to achieve the fabrication of complex patient-customized structures [24, 25]. Notably, the additive-manufactured bio-piezoelectric scaffolds can reconstruct desired tissue EM through a non-invasive ultrasonic stimulation. This time-dependent functionality-shifting behavior of additive-manufactured structure when exposed to external stimulus, also defined as 4D printing [26, 27], could provide a potential advanced manufacturing strategy for smart bio-piezoelectric scaffolds. An overview of the relationship between bio-piezoelectric scaffolds and AM (i.e. 3D/4D printing) is illustrated in figure 1.

Although 3D/4D printed bio-piezoelectric scaffolds may be a promising alternative substitute for next-generation BTE, there is a significant gap between the capabilities of the present 3D/4D printing techniques and the clinical application requirements of bio-piezoelectric scaffolds. Hence, this review aims
to bridge the gap by analyzing the current research status with existing limitations in 3D/4D printed bio-piezoelectric scaffold manufacturing and point out future needs. Finally, we set out to offer guidance and enlightenment toward a potential breakthrough in 3D/4D printing smart scaffolds for next-generation BTE.

2. Bio-piezoelectric materials for bone regeneration

Piezoelectricity was discovered in 1880 when the Curie brothers observed surface charges on quartz crystals by applying pressure on them [28]. The piezoelectricity generally originates from the non-centrosymmetric nature of materials, that is, the distortion of a crystal arising from the external forces leads to the separation of positive and negative charge centers, leading to the generation of surface charges [29], as schematically depicted in figure 2(a). This piezoelectricity has been demonstrated in human bones, which generate positive and negative charges when subjected to compression or tension. For instance, the human tibia can generate a piezoelectric potential of ~300 µV during walking [20]. Therefore, piezoelectric materials show unique advantages in simulating the EM of bone tissues, which can significantly promote the metabolism of cells and new bone formation. The schematic representation and histological evidence for electric charge generation on piezoelectric material surface facilitating bone formation are shown in figures 2(b)–(d). The surface charges of piezoelectric materials can attract ions to promote cell adhesion through ion or charge interaction, as well as activate growth factor expression to improve cell proliferation and osteogenic differentiation. For instance, the electrical stimulation can open the voltage-gated Ca$^{2+}$ channels on cell membranes, increasing intracellular Ca$^{2+}$ concentration, which can activate calmodulin and further trigger calcineurin activation [30, 31]. Notably, although there is some literature on conductive scaffolds for bone regeneration [32, 33], their process and mechanism for establishing an EM are completely different, i.e. bio-piezoelectric materials are capable of generating electrical cues in response to mechanical deformation, while conductive biomaterials commonly require an external power source to conduct the stimulation that will cause inconvenience for practical uses. To some extent, bio-piezoelectric scaffolds are therefore more attractive for cell culture and in vivo implantation than conductive scaffolds.

To date, a variety of bio-piezoelectric materials with good biocompatibility and biosafety have been explored to stimulate bone regeneration. Bio-piezoelectric materials could be broadly classified as (i) piezoelectric polymers (including natural and synthetic biopolymers), (ii) piezoelectric ceramics, and (iii) piezoelectric composites. Natural piezoelectric polymers, such as collagen, keratin, and polysaccharides are usually characterized by renewable, low toxicity, and good bioactivity; nonetheless, they also have the drawbacks such as potential immunogenicity, unpredictable biodegradation rate, and poor mechanical properties [35–37]. Synthetic piezoelectric polymers, such as PVDF and its copolymers, PLLA, and PHA, possess the advantage over natural piezoelectric polymers in terms of design flexibility, mechanical strength, and impact resistance; nonetheless, they have relatively low piezoelectric coefficients are thus restricted in their capacity to reconstruct the physiological electrophysiological microenvironment for bone repair [38, 39]. Piezoelectric ceramics, such as HAp (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAp), barium titanate (BaTiO$_3$, BT), and (K$_0.5$Na$_0.5$NbO$_3$, KNN), with outstanding piezoelectricity, high mechanical performance, and elastic modulus close to native bone tissue, have attracted fast-rising attention in the field of BTE; nonetheless, their high brittleness and low damage tolerance restrict their processing flexibility and BTE applications to some extent [40–42]. By mimicking the organic and inorganic components of native bones, piezoelectric composites consist of polymers and ceramics to reveal a higher piezoelectric coefficient, and superior mechanical flexibility and stability for bone regeneration [43, 44]. Some representative findings of typical bio-piezoelectric materials for bone regeneration are summarized in table 1. Some reviews have highlighted the most recent advancements in bio-piezoelectric materials for bone regeneration [45, 46].

3. AM strategies of bio-piezoelectric materials for BTE

The structural characteristics of scaffolds determine their mechanical properties and biological responses, which are key to the success or failure of bone defect repair. Therefore, the structure design and manufacture of bone-like scaffolds has been a research hotspot in the field of BTE and has attracted increasing attention. To date, various manufacturing strategies such as electrospinning, freeze-drying, and solvent casting have been developed to fabricate BTE scaffolds [60–62]. These conventional methods can create pore structures with high interconnectivity and porosity but are still severely constrained by the geometrical design and structural complexity. Emerging AM (known as 3D printing [63, 64]) technologies enable overcoming these limitations and offer significant advantages in fabricating patient-customized complex-shaped scaffolds over conventional techniques [65]. The recent progress in the 3D printing of bio-piezoelectric materials will be discussed as follow.

3.1. 3D printing technologies

Three-dimensional printing refers to a disruptive manufacturing process that builds 3D objects into freeform geometries in a bottom-up layer-by-layer fashion according to the data of 3D computer models [63, 66]. The 3D printing technique has demonstrated several advantages over traditional forming methods, such as high flexibility in manufacturing highly complex structures, rapid prototyping of multiscale hierarchical pore features, and usage of a wide variety of multiple biomaterials. These advantages can significantly shorten the manufacturing cycle and reduce material waste to save production costs [67, 68]. Besides, combined with computed
tomography/magnetic resonance imaging scanning technology, 3D printing allows for the rapid prototyping of complex patient-customized bone scaffolds, which can provide bone tissues with a physiological microenvironment similar to native bone [69–71]. Given the aforementioned benefits, numerous 3D printing techniques have been used to create implant scaffolds in various bio-piezoelectric materials (including polymers, ceramics, and their composites). According to the ASTM standards [72], these 3D printing techniques are divided into the following four groups: (i) materials extrusion [such as DIW, and FDM]; (ii) BJ; (iii) laser PBF [such as selective laser sintering (SLS)]; and (iv) VPP [such as DLP, TPP], and hybrid 3D printing. The following will discuss the latest research progress made in the field of 3D-printed bio-piezoelectric scaffolds in terms of feedstock selection, printing process, printing resolution, and induction strategies.

3.1.1. Materials extrusion. The material extrusion-based 3D printing techniques involve a selective deposition of material onto a stage using a movable nozzle head to prepare a continuous filament utilizing a mechanical or pneumatic system [73]. DIW, as one of the representative extrusion-based 3D printing techniques, emerged as the most adaptable 3D printing method for the fabrication of BTE scaffolds due to its easy operation, a wide range of feedstock options, and high scalability [74, 75]. A schematic illustration of the DIW process is shown.
Some representative findings from studies on the use of representative bio-piezoelectric materials for bone regeneration.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Manufacturing strategies</th>
<th>Electrical properties</th>
<th>Key findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVDF</td>
<td>Solvent casting</td>
<td>$d_{33} \sim -32 \text{ pC} \text{ N}^{-1}$</td>
<td>Negatively charges improve cell adhesion/proliferation</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>3D printing</td>
<td>Maximal output voltage (~8.2 V)</td>
<td>Enhanced electrical charges increase ALP activity</td>
<td>[48]</td>
</tr>
<tr>
<td>PVDF-TrFE</td>
<td>Electrospinning</td>
<td>Average current of 0.76 nA</td>
<td>Promote alignment of Schwann cells/fibroblasts</td>
<td>[49]</td>
</tr>
<tr>
<td>PLLA</td>
<td>Electrospinning</td>
<td>Output voltage of ~30 mV</td>
<td>Enhance osteogenic differentiation of different stem cells in vitro</td>
<td>[50]</td>
</tr>
<tr>
<td>HAp</td>
<td>Dry pressing</td>
<td>Output voltage of 12 mV</td>
<td>HAp has intrinsically a piezoelectric response</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Tape casting</td>
<td>$d_{33} \sim -36 \text{ pC} \text{ N}^{-1}$</td>
<td>Sr dopant increases $d_{33}$ of HAp by one order of magnitude</td>
<td>[52]</td>
</tr>
<tr>
<td>BT</td>
<td>Direct foaming</td>
<td>—</td>
<td>No inflammatory reaction and short-term cytotoxicity</td>
<td>[53]</td>
</tr>
<tr>
<td>KNN</td>
<td>Dry pressing</td>
<td>$d_{33} \sim 63 \text{ pC} \text{ N}^{-1}$</td>
<td>Li dopant improves the material strength and $d_{33}$ of KNN</td>
<td>[54]</td>
</tr>
<tr>
<td>PVDF/HAp</td>
<td>Dry pressing</td>
<td>Remnant polarization 0.04 μC cm$^{-2}$</td>
<td>Electric field as a catalyst for the bone growth process</td>
<td>[55]</td>
</tr>
<tr>
<td>PVDF/BT</td>
<td>3D printing</td>
<td>$d_{33} \sim -8.2 \text{ pC} \text{ N}^{-1}$</td>
<td>Promote MG-63 cell proliferation/differentiation, exhibit a robust antibacterial activity</td>
<td>[56]</td>
</tr>
<tr>
<td>PLLA/HAp</td>
<td>3D printing</td>
<td>—</td>
<td>Facilitate bone-like apatite deposition and blood vessel tissue growth</td>
<td>[57]</td>
</tr>
<tr>
<td>PVDF-TrFE/BNNTs</td>
<td>Cast-annealing</td>
<td>Output voltage of ~20–60 mV</td>
<td>Enhance differentiation of SaOS-2 osteoblast-like cells</td>
<td>[58]</td>
</tr>
<tr>
<td>PHBV/PLLA/KNN</td>
<td>Spin coating</td>
<td>Output voltage of 2.2–6 V</td>
<td>Sciatic nerve damage can be repaired and monitored in real-time</td>
<td>[59]</td>
</tr>
</tbody>
</table>

in figure 3(a). In this process, a viscoelastic ink is extruded and deposited layer by layer through a nozzle head to fabricate 3D objects. After extrusion, the viscoelastic ink solidifies to generate desired structures under post-treatments (such as heat, light, and chemistry) [76]. The resolution and structural integrity of the DIW printed objects depend on a variety of factors, i.e. ink performance, nozzle diameter, and nozzle movement rate [77]. In this case, DIW allows for the printing of virtually any material, as long as the precursor ink is modified to exhibit the proper rheological behavior. Therefore, DIW has demonstrated unique advantages in the preparation of bio-piezoelectric scaffolds with different materials (polymers, ceramics, and their composites).

Mancuso et al. [78] produced PCL/BT composite scaffolds with a high level of consistency and seamlessly integrated internal structure by DIW (shown in figure 3(b)). The printed PCL/BT scaffolds outperformed the PCL and PCL/HAp scaffolds in terms of mechanical performance (figure 3(c)). Moreover, the addition of electrodielectric BT ceramic particles (10 wt.%) enhanced the dielectric constant and decreased dielectric loss over the entire frequency spectrum. In vitro experiments with human osteoblasts, Saos-2 demonstrated that PCL/BT scaffolds could support cell growth and viability while retaining the typical morphology of the osteoblastic phenotype. Besides, the PCL/BT scaffolds outperformed the PCL and PCL/HAp scaffolds in terms of ALP activity and deposited bone-like extracellular matrix, indicating a promising candidate in load-bearing bone scaffolds (figures 3(d)–(f)). Tariverdian et al. [79] added strontium-doped BST into β-TCP and DIW printed BST/β-TCP scaffolds for bone regeneration (figure 3(g)). The composite scaffolds contained 60% BST and 40% β-TCP exhibited the highest mechanical, electrical, and biological properties. The composite scaffolds made of 60% BST and 40% -TCP exhibited the highest levels of mineral deposition and development of bone-like apatite after 28 d in SBF. (figure 3(h)). Besides, the 60% BST/40% β-TCP composites revealed higher ALP activity of osteosarcoma cells than that of other composites (figure 3(i)), implying that the composite scaffolds have the potential to be the next-generation BTE. Notably, DIW technology enables the integration and programming of different formulations into predesigned multi-materials with complex structures by using multiple extrusion nozzles [80, 81]. These DIW-printed multi-material scaffolds have the potential to provide a multi-functional coupling of anti-inflammatory/angiogenic/osteogenesis/tumor immunity [82], which will become one of the key research directions in the BTE field. However, when printing different combinations of materials in DIW, very different physical properties of the materials (such as thermal expansion, melting point, or
Figure 3. Direct ink writing (DIW). (a) Scheme of the DIW process; (b) SEM images of the DIW printed polycaprolactone (PCL), PCL/HAp, and PCL/BT scaffolds; (c) compressive modulus of the DIW printed scaffold; (d) immunostained images of Saos-2 cells at day 28 in tested 3D composite scaffolds. The nucleus stained is by DAPI (blue), and actin stained by Phalloidin Alexa fluor 488 (green) and imaged at different magnifications using an inverted microscope. Scale bars 200 μm; (e) quantification of Alizarin stain by absorbance readings at 405 nm; (f) Collagen and osteocalcin deposition by Saos-2 cells in the scaffolds after 28 d of culture. Scale bars: 50 μm. (g) An optical image of the DIW printed BST/β-TCP scaffold; (h) the alkaline phosphatase (ALP) activity of MC3T3-E1 cells cultured on the BST/β-TCP composites for 7, 14, and 21 d; (i) cell viability of BM-hMSC cells in contact with BST/β-TCP composites as a function of culture time by MTT assay (One-way ANOVA, ** p < 0.01). Reprinted from [79], Copyright (2019), with permission from Elsevier.

One-way ANOVA statistic is displayed in the figure, with significance levels \( p < 0.05 \) (∗). Reprinted from [78], Copyright (2021), with permission from Elsevier. (g) An optical image of the DIW printed BST/β-TCP scaffold; (h) the alkaline phosphatase (ALP) activity of MC3T3-E1 cells cultured on the BST/β-TCP composites for 7, 14, and 21 d; (i) cell viability of BM-hMSC cells in contact with BST/β-TCP composites as a function of culture time by MTT assay (One-way ANOVA, ** p < 0.01). Reprinted from [79], Copyright (2019), with permission from Elsevier.

Oxygen sensitivity) need to be considered to avoid cracks and defects caused by mismatches during post-treatment (drying, debinding, and consolidation). Besides, DIW has great difficulties in building bone-like complex features such as the most popular typical TPMS structures and negative Poisson’s ratio metamaterials [83, 84]) with high resolution of submicron and nanoscales, since its extruded features are restricted by the woodpile structures and its printing resolution is primarily determined by the nozzle diameter of >100 μm.

FDM is another typical material extrusion-based 3D printing technique that has gained increasing popularity in industry and academia due to its ease of implementation, high simplicity, and cost-effectiveness [85, 86]. Unlike DIW, FDM technology utilizes a heated nozzle head to melt filament thermoplastic material which was then extruded through a nozzle and deposited layer by layer to create 3D objects [87]. A schematic representation of the FDM process is depicted in figure 4(a). The basic principle of FDM is simply melting the
raw materials and forming them into pre-designed new shapes. Therefore, filament materials with excellent thermo-plasticity play a vital role in the FDM process, which determines the bonding ability between layers and the mechanical strength of final objects. The most well-known thermoplastic polymers used in FDM are acrylonitrile butadiene styrene and PLA, with PLA gaining appeal among researchers in the field of biology due to its good biocompatibility [88, 89]. Moreover, other medical-grade thermoplastic polyurethanes (TPUs) such as PCL also began to be noticed for development [90]. Therefore, doping piezoelectric fillers into biocompatible filament thermoplastic materials is an effective way to FDM print bio-piezoelectric scaffolds for bone regeneration.

Mystiridou et al [91] prepared multifunctional PLA/PCL-based bone scaffolds by FDM technology using bioactive HAp and piezoelectric BT as fillers. They produced PLA and PLA/PCL composite filaments in a single screw extruder using flakes molded by the solvent casting method. Compared to pure PLA filament, the PLA/PCL composites exhibited a significantly enhanced plastic deformation, and lower glass transition and melting point temperatures (figures 4(b) and (c)), which was conducive to thermoplastic FDM printing. The FDM-printed composite scaffolds showed a good distribution of HAp and BT fillers, resulting in a $d_{33}$ value close to the human bone (figures 4(d) and (e)). Sikder et al [92] developed high-quality bioactive PCL-BT filaments and FDM-printed
design-specific piezoelectric scaffolds for the treatment of bone deformities (figure 4(f)). Cell experiments proved that ultrasonic treatment combined with piezoelectric scaffolds could accelerate bone healing and help shorten treatment time in individuals with serious orthopedic injuries (figure 4(g)). These FDM-printed design-specific piezoelectric and regenerative scaffolds have broad application prospects in maxillofacial, cranial, and dental restoration and regeneration. Similar to DIW, FDM technology enables the multi-feedstock structure printability by integrating multiple nozzles, especially when combined with a flexible multi-axis manipulator, FDM can further realize the free forming of random surfaces. For instance, our group has developed a six-jaw manipulator containing three pairs of filament-delivering/cutting systems for FDM printing diverse materials including polymers, ceramics, and metals [93]. This manipulator-assisted multi-degree-of-freedom FDM technology will be one of the most promising methods for the preparation of multifunctional coupled multi-material scaffolds for bone regeneration. In addition, the researchers have made some attempts to achieve significant mechanical properties improvement of FDM-printed objects by adding continuous fiber materials to the filament matrix [94, 95]. However, the preparation of thermoplastic filaments has been a major restriction on FDM printing. Besides, similar to DIW, FDM technology also has limitations in building complex bone-like structures and high-resolution features.

3.1.2. BJ. BJ is a powder-bed-based AM method that jets a liquid binding agent (a polymeric liquid) on powder layers and selectively joined into patterned shapes [97, 98]. BJ technology holds distinctive promise due to its compatibility with virtually any powder materials, rapid production of large-scale objects, and highly cost-effective mass production [99]. A schematic illustration of the BJ process is depicted in figure 5(a). In this process, the binders with good rheology and wettability, stable chemistry, and high binding strength play a crucial role in the preparation of high-performance objects by
BJ. The powder bed in the BJ process can offer support for the printed parts, and thus some complex structures (like hollow and overhanging) can be directly formed without additional support structures [100]. Besides, BJ technology can significantly improve its forming efficiency by simply increasing the number of printer nozzles. For instance, a printing head with 100 nozzles can build objects at a rate up to approximately 200 cm³ min⁻¹ [101]. Due to the utilization of multiple nozzles, BJ can perform color printing using multiple materials (including metals, polymers, and ceramics) [102, 103], which renders it more critical and competitive compared to other AM technologies. This outstanding competitiveness has also been fully reflected in the BJ-printed bio-piezoelectric scaffolds for bone regeneration.

Polley et al [104] prepared interconnected BT/HAp composite scaffolds by BJ technology (figure 5(b)), and systematically investigated their mechanical, piezoelectric properties, and cytocompatibility. The μCT analysis revealed that the composite scaffolds exhibited an open porosity of 50% with a pore size distribution of 100–200 μm that is favorable for osteogenesis (figure 5(c)). By optimizing the electric field strength and time of polarization, the BT/HAp composite scaffolds exhibited a piezoelectric coefficient of $d_{33}$ in a comparable range to dry bones. After seeding MC3T3-E1 cells on the BT/HAp scaffolds for 24 h, the spread cell morphologies and good adhesion to the BT/HAp surface could be observed (figures 5(d) and (e)). LIVE/DEAD fluorescent staining revealed that the BJ-printed BT/HAp scaffolds showed a high cytocompatibility, indicating implant scaffolds with improved bone regenerating potential (figure 5(f)). Recently, BJ technology has been applied to fabricate various porous piezoelectric ceramics such as BT and KNN [105, 106], but lacks the follow-up research content related to bone repair. Therefore, BJ technology has demonstrated significant advantages in forming bio-piezoelectric scaffolds, especially for ceramic materials. Recently, Clares et al [107] proposed a bimodal particle size distribution strategy that can significantly enhance the density and mechanical properties of BJ-printed objects, providing an effective solution for creating high-performance bio-piezoelectric scaffolds using the BJ technique. However, there are still several restrictions on piezoelectric scaffold manufacturing by BJ: (i) the post-treatment (sintering or infiltration) is typically required to improve densification, (ii) the printed objects show a high surface roughness and low printing resolution.

### 3.1.3. Laser PBF (LPBF)

LPBF is one of the most widely used AM techniques that uses a laser or electron beam as a heat energy source for irradiation, fusion, and melting of powders to form the intended shapes [108, 109]. LPBF technology is mainly subdivided into the following types according to ASTM, i.e. SLS [110], selective laser melting [111], direct metal laser sintering [112], and electron beam melting [113]. Among them, SLS is the most dominant LPBF-based AM technology used for the formation of piezoelectric materials including polymers, ceramics, and their composites. A schematic representation of the SLS process is depicted in figure 6(a). In this process, a high-intensity laser beam (e.g. CO₂ laser beam) is generally utilized to selectively sinter powders layer by layer according to computer-aided design data to form 3D parts [114]. SLS technology was originally developed for the fabrication of wax models for investment casting of metallic prototypes and has since been extensively researched for the processing of polymer materials such as acrylonitrile butadiene styrene, nylon, and polyether ether ketone, and later extended to metal and ceramic powders with higher melting points [115–117]. It is worth pointing out that SLS has developed into one of the most popular indirect AM technology for ceramic materials, which first bonds ceramic particles using the polymers and then burned them out at high temperatures [63]. In our previous reports, we have emphasized the inherent advantages of SLS technology in forming porous ceramic components [118, 119]. In particular, highly porous ceramics with high mechanical performance are capable of being prepared by SLS utilizing special raw powder materials (like hollow spheres and double core–shell structures) [120, 121]. Inspired by the above ideas, SLS technology has been gradually applied to the fabrication of bio-piezoelectric ceramic/polymeric scaffolds for bone regeneration in recent years.

Shuai et al [122] hydroxylated BT nanoparticles with polydopamine to achieve their uniform dispersion in PVDF scaffolds prepared by SLS. On the one hand, the polydopamine addition enhanced the interfacial adhesion between BT and PVDF, resulting in increased tensile strength of the scaffolds by ~14% (figures 6(b) and (c)). On the other hand, the polydopamine amino groups combined with the CeF group of PVDF increased its β-phase content from 46% to 59%, resulting in a significant increase of output voltage by 356%. Cell tests revealed that this improved output voltage could significantly promote cell adhesion and the generation of a more mature extracellular matrix (figures 6(d)–(f)), which indicated the potential application of bio-piezoelectric scaffolds in bone regeneration. Yang et al [123] introduced graphene and BT into PLLA scaffolds fabricated by SLS, in which the graphene served as a superior conductive filler and BT acted as the piezoelectric source (figure 6(g)). The electric field strength used to polarize the BT nanoparticles was strengthened by the inclusion of conductive graphene. In this instance, BT underwent a reorganization along the direction of the poling field that created a more electric domain, resulting in an increased piezoelectric response of the PLLA/BT/graphene scaffolds. Such enhanced piezoelectric signal plays a crucial role in promoting cell proliferation and differentiation (figure 6(h)). Similar SLS work on piezoelectric scaffolds has also been reported in other literature [56, 124]. The main advantages of SLS-printed bone scaffolds lie in their broad feedstock selection and inherent high porosity. The high porosity of SLS-printed objects mainly comes from the lower packing density of the powder bed and the gap left by the burning of the sacrificial binder [118], which is beneficial to cell adsorption, proliferation, and material transport. However, for indirect SLS of ceramics, the binder removal process at high temperatures will easily cause large shrinkages and cracks, which will reduce the mechanical properties of the scaffold. Besides,
the SLS-printed objects exhibit a low resolution and poor finish, which limit the fabrication of fine-structured scaffolds for bone regeneration.

3.1.4. VPP. VPP technologies have become the well-known and widely used 3D printing technique that gained extensive attention in a diverse range of fields such as microelectronics, semiconductors, biomedical engineering, and jewelry [125–127]. These technologies utilize light sources of a certain wavelength to selectively polymerize and cure photocurable resins to create complex 3D micro-lattices. With the advance of 3D printing, multiple technologies and techniques have been developed such as SL [128], DLP [129], VAM [130], PMSL [131], and TPP [132], all can produce prints with resolutions that are comparable to or better than 10–100 m. The schematic diagram and printing resolution of various VPP technologies are shown in figure 7(a). SL uses an UV light beam emitted from a laser that is deflected by the scanning galvanometers and concentrated in a single spot to line-wise
Figure 7. Vat photopolymerization (VPP). (a) Printing resolution comparison and schematic diagram of vat photopolymerization (stereolithography (SL), digital light processing (DLP), volumetric additive manufacturing (VAM), projection micro-stereolithography (P$_\mu$SL), and two-photon polymerization (TPP)). (b) Photograph of DLP-printed porous BT ceramics with different porosity; (c) trend line of piezoelectric coefficient $d_{33}$ and compressive strength of BT ceramics with variable porosity; (d) potential distribution of BT ceramics with variable porosity under 100 MPa pressure. Reprinted from [137], Copyright (2022), with permission from Elsevier. (e) XY optical sections from a z-stack and confocal laser scanning microscopy (CLSM) imaging of TPP-printed nanocomposite piezoelectric scaffolds; (f) nanocomposite bioinspired Osteo-Prints for the promotion of the osteogenic differentiation, and confocal laser scanning microscopy images of Ki-67 and fluorescence microscopy images of hydroxyapatite (HA) in SaOS-2 cultures. (US represents the absence of ultrasonic irradiation, average ± standard deviation, * represents significant difference $p < 0.01$). Reprinted with permission from [138]. Copyright (2015) American Chemical Society.

scan the surface of a photosensitive resin [128]. DLP, on the other hand, uses a DMD as a dynamic mask to flash a 2D image on the entire platform, and cure the resin in a layer-wise manner at a higher forming efficiency than SL [129]. VAM uses orthogonal or tomographic techniques to control three orthogonal beams to concurrently light on the resin and form the predefined 3D objects on a time scale of seconds [130]. Although SL, DLP, and VAM vary widely in forming efficiency, they have similar printing resolutions in the range of 10–100 $\mu$m due to the same light source and forming principle. P$_\mu$SL employs a 3D grayscale DMD as a dynamic mask and a demagnifying lens as the spatial light modulator to create feature size with a printing resolution of 2–8 $\mu$m [131]. In particular, TPP can print with a high resolution of 80–200 nm by simultaneously absorbing two photons from a near-infrared (780 nm) or green (515 nm) laser. The most recent advances
in VPP technologies have been summarized in some reviews [133, 134]. These VPP technologies have a significant potential for creating bone scaffolds with ultra-fine features and extremely high structural controllability.

To date, VPP technologies have been widely utilized for manufacturing piezoelectric components with complex lattice structures [135, 136]. Some representative findings of studies involving the VPP-printed bio-piezoelectric scaffolds have been carried out. For instance, Jiang et al [137] formed a series of porous piezoelectric BT scaffolds with DLP technology and tried to adjust their mechanical strength and density by changing the porosity from 10% to 90% (figures 7(b) and (c)). The authors studied the potential and stress distribution of the printed BT scaffolds under a pressure of 100 MPa by finite element method and summarized the empirical formulas for the mechanical and piezoelectric performance of the printed BT scaffolds (figure 7(d)), which would provide the potential significance for implanting bio-piezoelectric scaffolds for bone regeneration. Marino et al [138] pioneered the fabrication of bioinspired 3D nanocomposite piezoelectric scaffolds resembling trabeculae of sponge bone for cell stimulation by TPP technology. The piezoelectric BT nanoparticles were doped into the matrix capable of producing electricity in response to mechanical deformations or remote ultrasound, which can mimic the intrinsic piezoelectricity of the bone to improve osteogenic differentiation (figures 7(e) and (f)). The VPP has the following advantages over other 3D printing technologies: (i) high printing resolution down to submicron size enables the precise fabrication of bone-like hierarchical gradient porous structures; (ii) high structural fidelity can accurately biomimetic the complex bone-like microstructures; (iii) versatile material scalability enables the fabrication of functional bone scaffolds with variable stiffness and modulus. However, their limited feedstock selection that must be used in combination with photosensitive resins, reproductive toxicity of commonly used commercial photoinitiators, and manual support are still barriers for VPP-printed implant scaffolds.

3.1.5. Hybrid 3D printing. A single 3D printing technique or material construction hardly fulfills the criteria for producing an optimal tissue scaffold, thus necessitating the advancement and integration of printing technologies as well as the creation of cutting-edge materials. Hybrid 3D printing, known as multi-step 3D printing or multi-process 3D printing, is a fresh field of research in which basic 3D printing is enhanced with complementary processes to create objects with multi-material and multifunctionality [139–141]. In comparison to a single 3D printing technique, hybrid 3D printing has demonstrated numerous outstanding advantages in developing novel tissue scaffolds: (i) programmable integration of multiple biomaterials, including spatial control of material (cell), geometry, scale, and dimension [142]; (ii) designable couplings of multiple biofunctions such as antibacterial, osteogenic, angiogenic, and anti-tumor [143]; (iii) process flexibility enhancement to eliminating the limitations of a single 3D printing technique while integrating the advantages of traditional manufacturing, such as a subtractive process machine interlocking root structures [144]. (iv) Expanded clinical applications for bone tissue repair, such as holistic osteochondral repair in joints with vastly different mechanical and biological requirements [145]. These emerging new features of hybrid 3D printing bring tremendous potential and challenges for developing next-generation BTE.

Liu et al [146] summarized hybrid 3D printing technologies for biological applications and categorized them as follows based on the specific mechanisms involved: (i) basic multi-head biomansufacturing system using multiple printing processes such as materials extrusion or BJ; (ii) semi-hybrid multi-head biomansuration system combining pneumatic, piston, and screw-assisted material extrusion processes; (iii) fully-hybrid biomansuration systems combining various 3D printing techniques, electrospinning, or post-treatment (shown in figure 8(a)). Recently, several researchers have attempted to utilize these hybrid 3D printing systems to create multi-functional multi-material scaffolds for advanced biological applications. Shim et al [147] developed a multi-head tissue/organ-building system that allows the creation of 3D tissues or organs by dispensing a wide range of relevant biomaterials including thermosplastic biomaterial PCL/alginate hydrogel (figure 8(b)). Khani et al [148] reported a novel hybrid and hierarchical multi-material bioprinting technique that enables the deposition-on-demand of multi-material hydrogels and biodegradable polymers into advanced tissue scaffolds with multifunctionality (figure 8(c)). Zhuang et al [149] developed a layer-by-layer UV-assisted bioprinting strategy that combined the materials extrusion with an in-built UV curing system capable of building soft yet stable cell-laden multi-material constructs with a high aspect ratio for soft tissue engineering (figure 8(d)). Similar works on hybrid 3D printing scaffolds for advanced biological applications have also been reported in other literature, as shown in figures 8(e) and (f) [143, 150]. Although hybrid 3D printing has demonstrated numerous outstanding advantages in building novel tissue scaffolds, few studies on bio-piezoelectric scaffolds have been reported. For hybrid 3D printing technology to become a mature tool to construct bio-piezoelectric scaffolds, the following issues must be addressed: (i) the diversity of printable bio-piezoelectric materials and multipath printing control software; (ii) enhanced material interfacial performance, especially the heterogeneous interface between bio-piezoelectric polymers and ceramics; (iii) matched sintering densification to ensure the reliability of structures composed of multiple bio-piezoelectric ceramics.

3.2. 4D printing technologies

The term 4D printing was first introduced by Tibbits at a 2013 TED talk, i.e. the 3D printed objects could change their shapes over time [151]. In the same year, the first paradigm of 4D printing was reported by using the concept of printed active composite materials with a time-dependent programmed SME.
Since then, 4D printing has gained increasing attention in research communities from various disciplines, including materials science [153, 154], mechanical engineering [155, 156], artificial intelligence [157, 158], and biomedical application [159, 160]. The timeline of representative events of 4D printing technology is shown in figure 9. In particular, Miao et al [161] synthesized novel shape memory polymers with excellent biocompatibility for 4D printing biomimetic hierarchical scaffolds for regenerative medicine. Compared to conventional PCL scaffolds, 4D printed smart scaffolds revealed outstanding SME and shape recovery at physiological temperature, as well as excellent attachment, proliferation, and differentiation of MSCs. Recent research has confirmed that the dynamic topography of 4D scaffolds can promote cell proliferation and osteogenic differentiation [162, 163]. However, this shape-changing 4D printing technology has great limitations when applied to brittle ceramic materials with high hardness and low toughness [164, 165]. In this case, our group developed silicone rubber matrix nanocomposites that can be printed, deformed, and then transformed into mechanically robust elastomer-derived ceramics [166]. This is the first report on origami and shape-changing 4D printing of elastomeric-derived ceramic structures, starting a new chapter in 4D printing of mechanically robust ceramics that will significantly advance a wide range of fields including aerospace propulsion and biomimetic ductile ceramics. With the advance of...
4D printing, it is worth noting that the concept of 4D printing has been extended and refined by the academic community. One widely recognized definition of 4D printing today is that the 3D printed objects can self-transform into a predefined shape/property or exert a predefined function when exposed to certain physical, chemical, or biological stimuli [167]. Since then, piezoelectric materials (including polymers, ceramics, and their composites) as a typical smart material have become an exciting branch of 4D printing and are currently attracting enormous interest [168, 169].

With the discovery of inherent piezoelectricity in native bones and the highlight of its role in promoting bone repair (mentioned in section 2), the 4D printed shape/functionality-changing bio-piezoelectric scaffolds are widely considered as the potential next-generation smart implants for bone repair. For instance, Grinberg et al. [169] 4D printed a bio-piezoelectric polyamide/BT knee prosthesis for high-fidelity assessment of the mechanical strain created on the knee joint (figure 10(a)). Based on the piezoelectric effect, the 4D printed knee prosthesis could inversely infer the mechanical strain and stress on the knee joint according to the induced electrical charge (figure 10(b)), which opens up a promising future of 4D printing smart bone implants for biological medicine. Camarero-Espinosa and Moroni [162] 4D printed Janus scaffolds composed of biodegradable PCL and PLA blends and behaved as ultrasound transducers, which present reversible mechanical nano-vibration that is transmitted to the surrounding cells upon ultrasound stimulation (figures 10(c) and (d)). The PCL and PLA were used as damping and deflection materials, respectively, which could activate shorter pulse width and smaller deflection under ultrasonic irradiation after combination. Consequently, these ultrasound-stimulated 4D scaffolds could enhance cell proliferation, matrix synthesis, and osteogenic differentiation of seeded human bone marrow-derived stromal cells by activating voltage-gated Ca$^{2+}$ ion channels (figures 10(e) and (f)). This ultrasonic-activated dynamic topography as a cell fate switch (ultrasonic transducer principle) provides an alternative and effective way for 4D printing smart bio-piezoelectric scaffolds. It is worth noting that the precise control of the reversible change in shape/property/functionality of piezoelectric materials when exposed to certain stimuli and their effects on cell behavior is the key to its definition as 4D printing technology, and also the main difference from conventional bio-piezoelectric scaffolds.

Emerging hybrid 3D printing techniques are considered to have tremendous potential in the development of 4D bio-piezoelectric scaffolds. Here, we summarize the following potential 4D printing strategies for bio-piezoelectric scaffolds
using a hybrid 3D printing technique: (i) integrating bio-piezoelectric materials, particularly ceramic materials with high fragility and low damage-tolerance, with conventional shape memory bio-hydrogels or biopolymers [171, 172], which can achieve a dual shape/functionality transformation providing both dynamic topography and crucial EM for promoting cell proliferation and osteogenic differentiation (figure 11(a)); (ii) combining materials based on magneto-electric effect to construct integrated 4D scaffolds with newly emerging piezoelectric effect [173, 174]. For instance, our group developed a novel nucleus pulposus substitute composed of NdFeB/TPU magnetic top, a porous TPU part in
the middle, and a polymer bottom containing four independently distributed conductive coils for monitoring tissue rehabilitation after discectomy (figure 11(b)). This significantly broadens the range of feedstock selections used for 4D printing bio-piezoelectric scaffolds; (iii) multi-freedom manipulator-assisted printing of multi-material components [93, 175], which can further broaden the range of feedstock selections (including metals, polymers, and ceramics) for printing bio-piezoelectric scaffolds with complex form-dependent surfaces (figure 11(c)). The progress thus far has demonstrated the potential for hybrid 3D printing for 4D bio-piezoelectric scaffold fabrication, however, substantial challenges mentioned in

Figure 11. Potential 4D printing strategies for bio-piezoelectric scaffolds. (a) Hybrid 3D printing of multiple materials (consisting of shape memory materials). Reprinted from [171], Copyright (2021), with permission from Elsevier. (b) A material combination based on magneto-electric effect with newly emerging piezoelectric effect. Reprinted from [174], Copyright (2022), with permission from Elsevier. (c) Multi-freedom manipulator-assisted 3D printing of intelligent multi-material magnetoelectric components. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Science China Technology Sciences [93], Copyright (2022).
section 3.1 must be addressed for the rapid development of 4D bio-piezoelectric scaffolds for next-generation BTE. Finally, an overview of 3D/4D printed bio-piezoelectric scaffolds for bone regeneration is summarized in Table 2.

4. Induction strategies for EM reconstitution

For 4D printing bio-piezoelectric scaffolds, the induction strategies for shape/property/functionality changes and EM remodeling at the cellular level are particularly important. So far, three major EM reconstruction strategies for bio-piezoelectric materials have been reported as follows: (i) some tissue activities or human body movements; (ii) low-intensity pulsed ultrasonic irradiation; (iii) magnetostrictive-driven by alternating electric fields. For instance, Liu et al [181] used biodegradable PLLA scaffolds that served as a battery-free stimulator to form beneficial surface charges under joint load for cartilage repair. The applied joint pressure exerted by rabbit hopping could induce a controllable piezoelectric charge, which promotes cell migration/recruitment and cartilage regeneration in vivo (figures 12(a) and (b)). Chen et al [182] prepared a biodegradable 3D piezoelectric PLA/KNN@polydopamine scaffold with ultrasound-driven wireless electrical stimulation capability for spine repair in rats. Upon programmable ultrasound treatment as a remote mechanical stimulus, the on-demand in vivo electrical stimulation with an adjustable timeline, duration, and strength can be delivered, promoting endogenous neurogenesis and angiogenesis in the lesion and improving the repair of spinal cord injury as a result (figures 12(c) and (d)). Ge et al [183] proposed a novel magnetic-driven and piezoelectric-catalyzed therapy modality based on core–shell-structured CoFe2O4-BiFeO3 magnetostriective-piezoelectric composites. Under an alternating magnetic field, the BiFeO3 shell exhibited von Mises stress and volume strain and corresponding induced potential due to the magnetostriective effect, which resulted in some chemical reactions for biological medicine.

<table>
<thead>
<tr>
<th>Tech.</th>
<th>Materials</th>
<th>Electrical properties</th>
<th>Key findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIW</td>
<td>PCL/BT</td>
<td>$d_{33} &lt; 0.1 \text{ pC N}^{-1}$</td>
<td>High ALP activity and deposited bone-like extracellular matrix</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td>BST/β-TCP</td>
<td>Dielectric constant = $\sim 10$</td>
<td>High quantities of mineral deposition and ALP activity of osteosarcoma cells</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>Chitosan/ nanocellulose</td>
<td>Surface charge $-47–42.8 \text{ mV}$</td>
<td>Excellent biocompatibility with human monocyte/macrophages</td>
<td>[176]</td>
</tr>
<tr>
<td>FDM</td>
<td>PCL/BT</td>
<td>$d_{33} = 1.2–2.6 \text{ pC N}^{-1}$</td>
<td>1 Hz US enhanced osteoblast proliferation, 3 Hz US benefited osteoblast differentiation</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>PLLA</td>
<td>Output voltage of $\sim 25 \text{ mV}$</td>
<td>Fibrinogen coating benefited</td>
<td>[177]</td>
</tr>
<tr>
<td></td>
<td>Whitloloite/PCL</td>
<td>Output voltage of $0.8 \text{ V}$</td>
<td>Osteoblast-like cell adhesion</td>
<td>[178]</td>
</tr>
<tr>
<td></td>
<td>BJ</td>
<td>$d_{33} \sim 3 \text{ pC N}^{-1}$</td>
<td>Excellent pre-osteoblast adhesion and high cytocompatibility</td>
<td>[104]</td>
</tr>
<tr>
<td>LPBF</td>
<td>PVDF/BT/polydopamine</td>
<td>Output voltage $1.8–7.0 \text{ V}$</td>
<td>Enhanced electric cues greatly promoted cell adhesion, proliferation, and differentiation</td>
<td>[122]</td>
</tr>
<tr>
<td></td>
<td>PLLA/BT/graphene</td>
<td>Output voltage of $1.4 \text{ V}$</td>
<td>Electrical signals considerably promoted cell proliferation and differentiation</td>
<td>[123]</td>
</tr>
<tr>
<td></td>
<td>PVDF/BT/Ag</td>
<td>$d_{33} \sim 8 \text{ pC N}^{-1}$</td>
<td>Promote cell proliferation and differentiation, and robust antibacterial properties</td>
<td>[56]</td>
</tr>
<tr>
<td>VPP</td>
<td>BT</td>
<td>$d_{33} \sim 240 \text{ pC N}^{-1}$</td>
<td>Increased porosity resulted in a more uniform potential distribution of scaffolds</td>
<td>[137]</td>
</tr>
<tr>
<td></td>
<td>Nano BT/ormocomp</td>
<td>$d_{33} \sim 0.6 \text{ pm V}^{-1}$</td>
<td>Enhanced osteogenic differentiation of SaOS-2 bone-like cells</td>
<td>[138]</td>
</tr>
<tr>
<td>Hybrid 3D printing</td>
<td>PHBV/SiHA</td>
<td>$d_{33} \sim 1.6 \text{ pC N}^{-1}$</td>
<td>Excellent hMSCs adhesion and differentiation abilities and calcium deposition potential</td>
<td>[179]</td>
</tr>
<tr>
<td></td>
<td>HA/PLLA/deECM</td>
<td>—</td>
<td>Significant cell attachment, proliferation, and osteogenic differentiation</td>
<td>[180]</td>
</tr>
<tr>
<td>4D printing</td>
<td>PLA/PCL</td>
<td>Output voltage $-2.0–2.0 \text{ V}$</td>
<td>Enhanced hBMSCs osteogenic differentiation via formation and activation of voltage-gated calcium ion channels</td>
<td>[162]</td>
</tr>
</tbody>
</table>
Among the above three induction strategies, the EM reconstitution of bio-piezoelectric materials induced by low-intensity pulsed ultrasonic irradiation is one of the most widely used methods at the present stage due to its outstanding advantages such as simple operation, high penetration, strong directivity, and minimally/non-invasive. However, the interaction between ultrasonic irradiation, piezoelectric materials, and 3D structures, and its effect (figures 12(e) and (f)).
of stress-to-polarization conversion efficiency on the regulation mechanism of induced surface potential remain unclear, which limits the wide application of 4D printing smart bio-piezoelectric scaffolds in the biomedical field.

5. Challenges and perspectives

Bio-piezoelectric materials have demonstrated outstanding advantages in promoting bone repair by mimicking the inherent piezoelectricity in native bone and are rapidly attracting increasing attention. In this review, we have first discussed the physicochemical properties of several commonly used bio-piezoelectric materials and their representative biological findings in bone regeneration. Then, we pointed out the advantages of 3D-printed bio-piezoelectric scaffolds in the BTE field in terms of feedstock selection, printing process, induction strategies, and potential applications (anti-inflammatory, osteogenesis, and vascularization). We emphasized the potential and viability of producing bio-piezoelectric scaffolds using shape/property/functionality-changing 4D printing to get around technical challenges and raise the intelligence level of BTE technology. Finally, we highlighted several current issues relating to the 3D/4D printed bio-piezoelectric scaffolds and their corresponding outlooks for overcoming those issues more effectively, to provide guidance that would encourage the advancement of 3D/4D printed bio-piezoelectric scaffolds more closely oriented towards BTE applications. Taken together, the constant driving force behind the evolution of BTE has always been the ever-evolving and improving manufacturing techniques.

Given the benefits of high flexibility in manufacturing highly complex structures, high matching features for personalization, and a wide range of feedstock options, the 3D/4D printing technology has shown great promise in the fabrication of bio-piezoelectric scaffolds. However, the following issues must still be addressed: (i) the challenges in manufacturing multifunctional coupling bio-piezoelectric scaffolds. For example, the integration of anti-inflammatory, osteogenic, angiogenic, and antitumor functions is usually required for the

Figure 13. Perspectives of 3D/4D printed bio-piezoelectric smart scaffolds for next-generation bone tissue engineering.
repair of large bone defects after bone tumor surgery [184]. (ii) The challenges in controlling the stress-polarization conversion capability of the scaffolds. For example, the stress absorption and transformation ability of various bone tissues, such as articular cartilage, skull, and vertebrae, have great differences. (iii) The limitations in non-invasive inducing EM of the scaffolds. For example, the existing non-invasive induction strategies mainly include ultrasonic irradiation and magnetostriiction. (iv) The challenges in synergistic regulating multi-cellular behavior in different microregions of the scaffolds. For example, cartilage and rigid bone repair in joints require cell proliferation and differentiation in different ways [185].

To overcome the aforementioned challenges, additional efforts are strongly advised in the following areas (figure 13): (i) integrate the multi-material AM technology [81] and innovative sintering densification technology [186] to promote the multifunctional coupling bio-piezoelectric scaffolds, (ii) push the precise regulation of stress-polarization conversion ability in various bone tissue with novel biomimetic metamaterial technology [187], (iii) develop novel non-invasive induction strategy for EM reconstitution with advanced stimulation methods such as heat and optics [160], (iv) combined with intelligent machine learning technology [188] to promote the collaborative regulation of micro-region multi-cellular behavior in the scaffolds. In addition, some special post-treatment (infiltration, debinding, and sintering [189]) are typically required for the 3D/4D printed bio-piezoelectric ceramic scaffolds to avoid internal stresses, cracks, and side reactions.

6. Conclusions

This review has shown the enormous potential of 3D/4D printing of bio-piezoelectric scaffolds for next-generation BTE, but more efforts are needed to completely close the gap between the 3D/4D printing technology’s present capabilities and future clinical requirements. The 3D/4D printing of bio-piezoelectric scaffolds fully integrates the benefits of multiple disciplines such as materials science, mechanical engineering, and bioengineering, and its great development requires a multidisciplinary joint effort. From a perspective of materials science, further experimental and computational studies are required to thoroughly investigate the bio-piezoelectric scaffold’s composition-structure-property-functionality relations, allowing for a wider selection of bio-piezoelectric materials. From the mechanical engineering perspective, a deeper combination of advanced AM technology and metamaterial bionic strategy is required to realize the precise regulation of stress-polarization conversion ability. From the perspective of bioengineering, the development of novel non-invasive induction strategies for EM reconstitution and advanced machine learning techniques are needed to promote the intelligent level of next-generation smart bio-piezoelectric scaffolds for bone regeneration. Over the years, 3D/4D printing technology has incorporated many advantages observed in traditional production techniques. With the collaborative efforts of multidisciplinary studies, 3D/4D printing is expected to soon reach its full potential in creating smart bio-piezoelectric scaffolds for next-generation BTE applications. We believe that the widespread adoption of 3D/4D printing technology should also draw inspiration from some cutting-edge technologies such as intelligent manufacturing, bionic medicine, and machine learning.

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Conflict of 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.

ORCID iD

Jian Lu https://orcid.org/0000-0001-5362-0316

References

foaming agent with freeze-drying to obtain hybrid highly macroporous bone scaffolds \textit{J. Mater. Sci. Technol.} \textbf{43} 52–63


[22] Liu Y et al 2017 Built-in electric fields dramatically induce enhancement of osseointegration \textit{Adv. Funct. Mater.} \textbf{27} 1703771


[67] Chen A N, Qu C H, Shi Y S and Shi F F 2020 Manufacturing strategies for solid electrolyte in batteries *Front. Energy Res.* **8** 571440


[70] Wang H Z et al 2022 Comparative evaluation of printability and compression properties of poly-ether-ether-ketone triply periodic minimal surface scaffolds fabricated by laser powder bed fusion *Addit. Manuf.* **57** 102961


[81] Han D and Lee H 2020 Recent advances in multi-material additive manufacturing: methods and applications Curr. Opin. Chem. Eng. 28 158–66


[92] Sidker P, Nagaraju P and Nagabanoyobi H P S 2022 3D-printed piezoelectric porous bioactive scaffolds and clinical ultrasonic stimulation can help in enhanced bone regeneration Bioengineering 9 679


[109] Sing S L and Yeong W Y 2020 Laser powder bed fusion for metal additive manufacturing: perspectives on recent developments Virtual Phsy. Prototyp. 15 359–70


Mg0.02Sn3PO4 solid electrolyte for solid-state sodium batteries Rare Met. 37 480–7


[129] Su J et al 2022 Three-dimensional printing of gyroid-structured composite bioceramic scaffolds with tuneable degradability Biomater. Adv. 133 112595


[136] Cui H et al 2022 Design and printing of proprioceptive three-dimensional architected robotic metamaterials Science 376 1287–93


[139] MacDonald E and Wicker R 2016 Multiprocess 3D printing for increasing component functionality Science 353 aa2093


[146] Liu F Y, Quan R X, Vyas C and Aslan E 2023 Hybrid biomedical systems applied in tissue regeneration Int. Bioprinting 9 646

promotes the spinal cord injury repair ACS Nano 16 16513–28
[184] Liao J F, Han R X, Wu Y Z and Qian Z Y 2021 Review of a new bone tumor therapy strategy based on bifunctional biomaterials Bone Res. 9 18