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Functional scaffolds and methods for bone tissue engineering applications

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Highlights:

- Functional scaffolds offer better tissue regeneration besides structural support.
- The bone tissue engineering with 3D printing has advanced the regeneration potential.

Abstract: Bone tissue engineering is an evolving area of tissue engineering using conventional and state-of-the art 3D printing methods. The present review focuses on introducing different methods used in developing scaffolds from biocompatible and biodegradable materials using several tissue engineering techniques including 3D printing. In addition, surface modification methods were also discussed to ensure the functionality of the scaffolds that facilitate a differentiation response in human mesenchymal stem cells *in vitro* or *in vivo* bone mineralization.

Keywords: bone tissue engineering; surface modification; 3D printing; polydopamine; functional scaffolds

1. Introduction

Tissue engineering aim to create functional biological tissues for replacement or repair applications [1]. In this process, scaffolds, cells, and signaling induction play crucial roles. Scaffolds mimic the natural extracellular matrix (ECM), providing a suitable environment for cellular growth and proliferation [2]. They have a vast surface area and three-dimensional structures, which are important for their functionality in tissue engineering. Scaffolds are widely used as implants to deliver cells [3], drugs [4,5], and genes [6,7] into the body. Their porous structure supports cell attachment, proliferation, differentiation of functions, and migration. Scaffolds can also be used to deliver drugs and genetic materials in a controlled manner, potentially preventing infection and treating chronic diseases. Synthetic functional grafts are considered alternatives to conventional grafts provides physical support and precise signaling for tissue healing [8]. In the context of bone tissue engineering, 3D-printed

scaffolds have gained attention as an emerging technology. Functional scaffolds that actively interact with stem cells can help overcome these limitations and improve the outcomes of stem cell-based therapies [9].

An ongoing challenge in tissue engineering is the lack of oxygen supply in engineered constructs. After implantation, spontaneous blood vessel formation does not occur rapidly, resulting in limited oxygen availability in engineered tissues [10]. Addressing this challenge is important for the success of tissue engineering and regenerative medicine. In the field of vascular transplantation, the fusion of endothelial monolayers is crucial for rapid reendothelialization, particularly in cases of long-distance vascular injury. This process helps remodel the injured blood vessels over a distance of at least 10 mm and reduces the overall repair period [10]. Furthermore, bioactive functional scaffolds can be used to improve bone healing processes where the body's natural regenerative capacity is insufficient. These scaffolds provide a supportive environment for bone tissue growth and regeneration [11]. In summary, the design and development of functional scaffolds play a vital role in tissue engineering applications. They mimic the natural ECM, support cellular functions, deliver therapeutic agents, and address challenges such as oxygen supply and tissue integration. By advancing scaffold technology, researchers aim to enhance tissue regeneration and improve the outcomes of regenerative medicine approaches. This review highlights the recent advancements and fabrication methods in the development of bone tissue engineering scaffolds for their adaptability with unique functionality facilitating the differentiation of the human mesenchymal stem cells (hMSC) towards the osteoblasts *in vitro* and *in vivo*.

2. Fabrication techniques

This section discusses the techniques used to prepare the scaffolds (Figure 1).

2.1. Supercritical fluid technology

In the field of tissue engineering, scaffolds play a crucial role as advanced devices that aim to replicate the characteristics of the extracellular matrix. Conjugated materials are special materials with unique optical and electronic properties have gained significance in the production of therapeutic scaffold foams, offering unique properties that are absent in other commonly used polymers. Supercritical fluid technology utilizes the supercritical temperature and pressure conditions to create porous scaffold avoiding the use of toxic solvents (Figure 1A). Conjugated polymers are artificially synthesized polymers containing delocalized electrons on the pi-bond whose backbone is made up of alternating single and double bonds. These conjugated polymers are also conductive in nature. Several studies have focused on the development of functional porous scaffolds using conjugated and/or non-conjugated polymers through supercritical CO_2 foaming/impregnation processes [12,13]. One study used polyvinyl acetate and polypyrrole as conjugated nonconjugated polymers, impregnated with gallic acid as a model drug. The resulting scaffolds exhibited approximately 35% porosity and achieved controlled drug release for up to 10 hours, with an initial rapid release phase. Optimal operating conditions were identified as 353K temperature, 30MPa pressure, 0.5MPa/min depressurization rate and 1 hour contact time. These scaffolds showed promising properties for tissue engineering applications [12]. Another investigation focused on a supercritical CO_2 foaming process to create porous polymeric devices with improved conductive properties, specifically using a PLGA-PEDOT:PSS system. The resulting scaffolds demonstrated close to 40% porosity, uniform polymer distribution, expansion factors up to 10 orders,

and a wide range of conductivity and mechanical properties [13]. A separate study combined polycaprolactone (PCL) and polyaniline (PANI) through supercritical CO_2 foaming techniques to develop three-dimensional scaffolds. The optimized operating conditions included a temperature of 70 ℃, pressure of 100 bar, a ratio of (PCL:PANI), 5:1, and a depressurization rate of 20 bar/min, including a contact time of 1 hour [14]. Furthermore, a novel approach of simultaneous manufacturing and sterilization method for scaffolds. This integration of processes aimed to overcome technological limitations and advance scaffold development for clinical use. The medicated scaffolds exhibited a similar release pattern for infection prophylaxis and treatment. Moreover, these scaffolds supported the cell attachment and growth of mesenchymal stem cells without inducing any differentiation towards a specific cell line [15]. The use of supercritical CO_2 foaming/impregnation processes in conjunction with conjugated materials shows promise in producing functional scaffolds with desired properties such as controlled drug release, enhanced conductivity, and suitable mechanical characteristics.

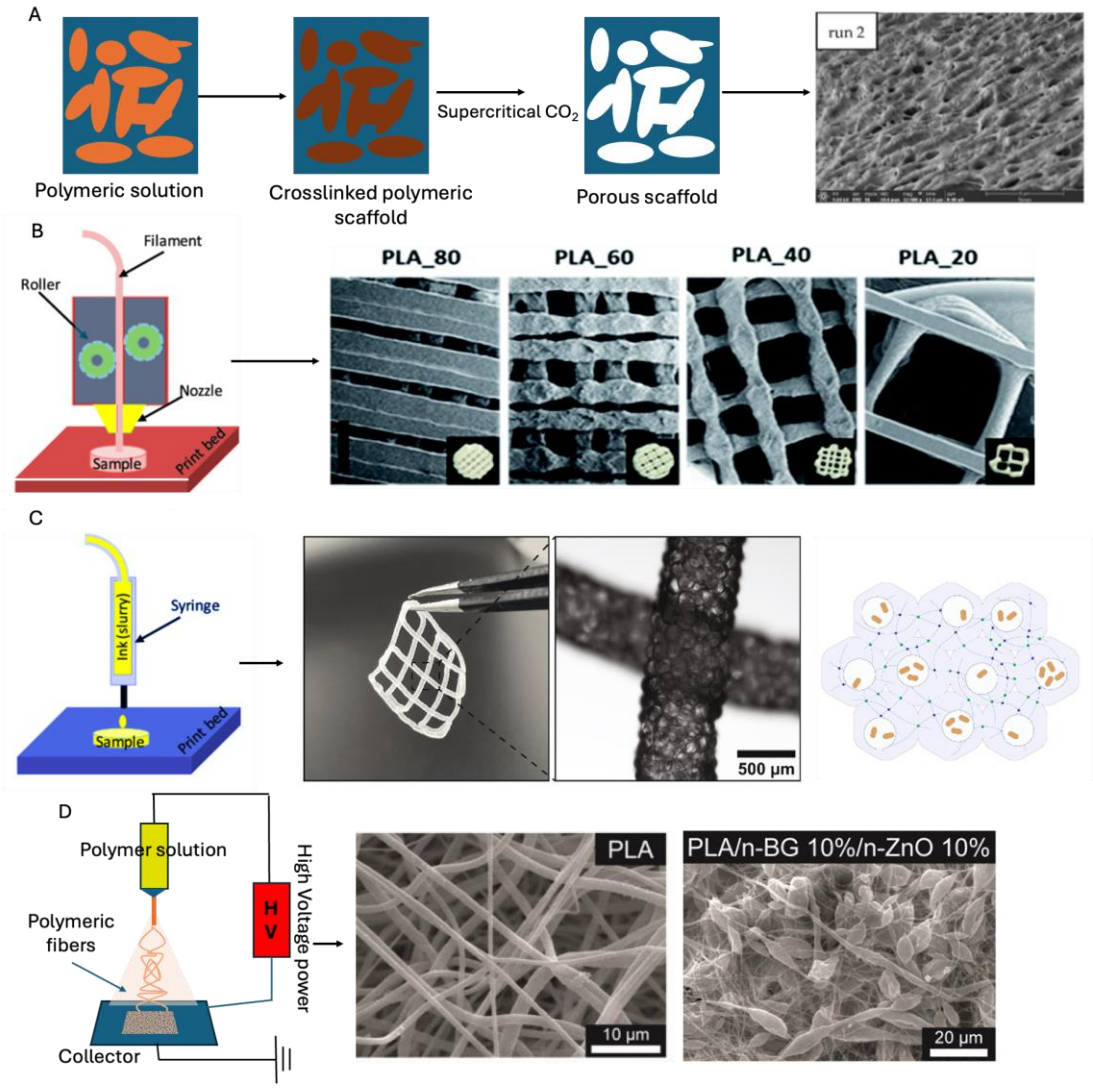

Figure 1. The cartoon detailing the fabrication techniques used to develop the scaffolds **(A)** Supercritical fluid technology [12]. Reprinted with permission. Copyright 2022, MDPI; **(B)** FDM 3D printing [43]; **(C)** Semi-solid extrusion 3D printing using bio-ink [18]; and **(D)** Electrospinning technique [19]. Reprinted with permission. Copyright 2023, Elsevier.

2.2. Three-dimensional (3D) printing

The 3D printing technology is widely regarded as a superior technique due to its ability to design functional scaffolds with customizable properties (Figure 1B & C). This approach involves the precise deposition of bio-inks, composed of biomaterials and cells, layer by layer to fabricate three-dimensional functional scaffolds [16]. Decellularized extracellular matrix (dECM) has emerged as a novel bio-ink with immense potential. It can serve as a suitable substrate, providing essential biological cues for cellular interactions. By utilizing dECM as a bio-ink, the 3D-printed scaffolds can better mimic the natural cellular environment and enhance tissue regeneration outcomes [17]. The convergence of material science and biology has given rise to the concept of living materials, which allow for the engineering and enhancement of living systems with new functionalities. Bioprinting techniques offer precise control over the deposition of cells in soft materials, enabling the creation of complex living materials. However, challenges remain in fine-tuning the microenvironments of cells while maintaining robust macroscopic structures. A core-shell strategy has shown promising results by mitigating cell leakage and providing a favorable cell culture environment [18]. For craniomaxillofacial (CMF) bone and periodontal regeneration, electrospinning and 3D printing technologies are key technologies. This review paper provides a comprehensive analysis of the latest innovations in 3D printing strategies for bone regeneration along with future directions. The topic discusses the principles of 3D printing technologies based on electrospinning and extrusion, highlighting their potential for creating scaffolds with improved regenerative capacity. The article also emphasizes the convergence of multiple technologies to enable reconstruction of bones considering complementary biomaterials and fabrication techniques. The use of personalized and functional scaffolds in clinical settings is a key focus for future advancements. 3D printing of bioinstructive materials has significant potential to guide cell behavior for enhanced regeneration purposes.

2.3. Electrospinning

Electrospinning is a variety of spinning strategy that utilizes electrostatic forces to create fibrous platforms/scaffolds from biocompatible polymers (Figure 1D). A straightforward gear arrangement makes electrospinning a flexible method for handling every one of the different biocompatible polymers into fibrous scaffolds. It has been used for several decades for the development of extracellular matrix mimicking scaffolds. The recent developments in electrospun scaffold modification and their applications in tissue engineering are well explored for fabrication of functional scaffolds. Various techniques and materials have been employed to enhance the surface area and functionality of electrospun scaffolds, with a focus on nanofiber-based structures. One modification method involves the flushing of nitrogen gas through the collector's mesh holes in the opposite direction of the jet movement during electrospinning. This technique leads to the formation of thin nanofibrous layers at nitrogen flush intervals, facilitated by the cooling effect of the sweeping nitrogen. Furthermore, electrospun scaffolds based on poly(l-lactic acid) (PLLA) have been developed incorporating bioglass (n-BG) and zinc oxide (n-ZnO), individually or as a mixture, to create bifunctional biomaterials with enhanced bioactive and biocidal properties [19]. The potential of electrospun nanofibers for soft tissue regeneration is emphasized, highlighting the evolution of materials used, incorporated microorganisms (such as cells, proteins, and nucleic acids), and manufacturing processes to control adhesion, proliferation, and differentiation [20]. However,

challenges remain in scaffold design and properties, including hydrophilicity, biodegradability, and biocompatibility. The translation of biological nanofiber products into practical industrial use also poses a significant hurdle [21]. Poly(caprolactone) (PCL) has emerged as a promising biopolymer for electrospinning, exhibiting favorable outcomes for tissue regeneration applications due to its similarity to the natural extracellular matrix [22]. Another biopolymer, polyvinyl alcohol (PVA), was investigated for scaffold production in tissue engineering due to its biocompatibility, biodegradability, and mechanical performance [23]. Overall, the advancements in electrospun scaffold modification techniques and the use of different biopolymers have significantly contributed to the field of tissue engineering and regenerative medicine, for improved functional scaffolds with diverse applications.

2.4. Origami

The unique potential of hybrid RNA: DNA origami structures was explored for various biomedical applications, including mRNA delivery, tertiary structure of long RNAs, and fabrication of artificial ribozymes. Despite being explored to a limited extent, the ability of long RNA scaffold strands to fold into target nanostructures *via* thermal annealing with complementary DNA oligos holds great promise [24]. The research presents the computational design, fabrication, and characterization of diverse polyhedral shapes, including tetrahedra folded EGFP-encoding messenger RNA, and octahedron and pentagonal bipyramids folded with 23S ribosomal RNA. These examples demonstrate the capability to create distinct structural and functional RNA scaffolds for various applications. Nucleic acid networks, conjugated to native enzymes or integrated into supramolecular DNA nanostructures serve as functional reaction modules for guiding dynamic catalytic transformations. The assembly of constitutional dynamic networks composed of nucleic acid-functionalized enzymes leads to dynamically switched biocatalytic cascades, demonstrating the potential of these systems [25]. In addition to catalytic applications, the review paper also discusses gene delivery from scaffolds as a versatile approach to manipulate cell function. The foam replica method fabricated bredigite porous scaffolds loaded with vancomycin hydrochloride were encapsulated in poly lactic-co-glycolic acid (PLGA) coatings. The biodegradable PLGA coatings modified the drug release kinetics, buffered physiological pH, and significantly improved cell viability [26]. Overall, hybrid RNA:DNA origami structures show great potential in biomedical applications, and further exploration of their capabilities is warranted. The controlled positioning of enzymes, switchable catalytic transformations, and gene delivery approaches offer promising avenues for advancing various fields, including mRNA delivery, structural characterization of RNAs, and bio-catalysis.

3. Bone regeneration

The development of functional scaffolds for bone tissue engineering (BTE) aims to create bioengineered bone structures with intricate architecture by utilizing biomaterials to support cells and deliver signaling molecules necessary for bone rejuvenation. The restoration of bone structure and function involves a complex signaling cascade that includes the secretion of cytokines, growth factors, and pro-inflammatory factors by cells in the defect site. These signaling molecules recruit surrounding stem cells, which then migrate, proliferate, and differentiate into bone-forming cells. Functional scaffolds play a crucial role in enhancing bone healing processes when the natural regeneration of lost tissue is insufficient [11]. The

development of bioactive functional scaffolds that act as osteoconductors and osteoinducers is crucial for successful bone tissue engineering. Osteoconductive scaffolds provide physical support for bone-forming cells, while also facilitating nutrient and blood vessel delivery. Osteoinductive scaffolds release signaling molecules that stimulate the differentiation of mesenchymal stem cells into bone-forming cells and promote the deposition of mineralized bone matrix [11]. Functionalization techniques, like surface modification with RGD peptides, have been employed to improve the interaction between scaffolds and cells, thereby enhancing adhesion and proliferation of hMScs [27].

Various approaches for developing functional scaffolds are explored in the reviewed literature (Table 1). These include the use of 3D printing technology to fabricate drug delivery scaffolds with antibacterial properties, such as the HA@TA-CS/SA scaffold loaded with curcumin-loaded dendritic mesoporous organic silica nanoparticles [28]. Functionalization of scaffolds with RGD peptides promotes cell adhesion and proliferation, as demonstrated in RGD peptide end-functionalized poly(ethylene glycol)-b-poly(lactic acid)-b-poly(globalide)-b-poly(lactic acid)-b-poly(ethylene glycol) scaffolds [27]. Incorporation of nanoparticles, such as bioglass (n-BG) and zinc oxide (n-ZnO), in scaffolds can enhance their biocidal capacity and structural properties [19]. Additionally, the incorporation of specific materials, such as CaSiO3/Ca2SiO4 and metformin, in scaffolds can enhance bone growth and osteogenic differentiation [29,30]. Functional scaffolds were fabricated to address the challenge of bone repair and bone tumor treatment. Poly (L-lactic acid) (PLLA)/nanoscale hydroxyapatite (nHA)/metformin nanocomposite scaffolds show potential for enhancing cell adhesion, biocompatibility, and osteogenic differentiation [30]. Chemotactic functional scaffolds, which release VEGF to attract circulating mesenchymal stem cells, offer a promising approach for the treatment of large-scale bone defects [31]. Dendriplexes of generation 4 polyamidoamine (G4-PAMAM)/BMP-2 plasmid incorporated in PLLA/PEO scaffolds promote osteogenic activity [32]. Furthermore, biomimetic intrafibrillar mineralized scaffolds exhibit superior osteogenic abilities compared to conventional scaffolds [33]. The potential of combining functional scaffolds with exosome vesicles (EVs) for bone regeneration was studied by developing mineral-doped PLA scaffolds. These scaffolds enriched with EVs demonstrate improved osteogenic commitment of adipose stem cells, indicating their potential for enhancing bone tissue regeneration [34].

Intelligent scaffolds can exhibit responsive behavior, such as controlled drug release, in order to enhance the therapeutic outcomes. One approach involves the use of 3D-printed scaffolds loaded with drugs, such as the multifunctional PCL/HA/DOX scaffolds, which not only enhance bone repair but also have the potential to inhibit tumor cells after malignant bone tumor resection [35]. Natural polymers, such as chitosan (CS), are considered promising candidates for fabricating reliable tissue constructs for bone tissue engineering [16].

4. Surface modifications

Surface modifications are essential techniques to enhance the effectiveness of biomaterials used in bone and tissue regeneration. These alterations aim to enhance biocompatibility, promote bone growth, and establish a harmonious connection between implants and host tissues. This section provides an overview of the most recent progressions in techniques for modifying surfaces to facilitate the regeneration of bone and tissue. Various techniques have been developed to alter surfaces to tailor biomaterial properties

in the field of tissue engineering. Categorically, these methods can be divided into physical, chemical, and biological alterations (Table 2).

Table 1. The materials and applications developed using different 3D printing methods.

3D printer	Material/Component	Description	Applications	Benefits	Ref
Melting Deposition Forming 3D Printer	Doxorubicin (DOX) Multifunctional PCL and Hydroxyapatite (HA) Nanoparticles scaffolds	Used for fabricating PCL/HA and PCL/HA/DOX scaffolds.	-Produces cylindrical and circular scaffolds with specific dimensions. -Provides multifunctionality to scaffolds, combining bone repair with potential tumor inhibition.	Allows precise control over scaffold architecture and composition, enabling the creation of complex structures.	[35]
FabX3.0 material extrusion-based 3D printer And extrusion Nozzle (E3Dlite extruder, 0.4mm	PLA, PEI based scaffolds and calcium-deficient hydroxyapatite (HaP)	used to fabricate 3D porous scaffolds for bone tissue engineering	-Mimics bone mineral, enhancing osteogenic activity and supporting bone tissue regeneration. -Improves cell adhesion and proliferation by modifying surface properties.	Enhances the scaffold's ability to support bone regeneration by promoting mineral deposition. Provides mechanical support and is biodegradable, making it suitable for medical applications.	[42]
PrinterBot 3D printing system	PLA- acetylated collagen linked on 3D printed bi-phasic key scaffold to mimic the osteochondral region of joints.	MSCs are seeded onto the scaffolds and cultured in chondrogenic media to promote differentiation and tissue formation. This modification mimics the natural extracellular matrix, enhancing the scaffold's integration with host tissue.	-Ensures mechanical stability and support for cell growth -Enhances cell proliferation and distribution	-Facilitation of Chondrogenic Differentiation, -Potential for Customization to match specific architecture for developing targeted tissue scaffolds. -The incorporation of acetylated collagen on the scaffold surfaces improves their biocompatibility, making them more conducive to cell growth and differentiation.	[55]
Cryogenic 3D Printing	3D printing of β-tricalcium phosphate and osteogenic peptide (OP) containing water/poly (lactic-co-glycolic acid)/dichloromethane emulsion inks. Angiogenic peptide (AP) containing collagen I hydrogel was then coated on scaffold surface to further provide scaffolds with angiogenic capability.	A technique that involves printing at low temperatures. Allows for the fabrication of scaffolds with hierarchical porous structures and suitable mechanical properties.	The scaffolds are engineered to deliver both osteogenic and angiogenic peptides. This dual-delivery system supports the sequential release of biomolecules, which is crucial for promoting both angiogenesis (formation of new blood vessels) and osteogenesis (bone formation).	-Helps in forming a stable emulsion for printing. -Enhances angiogenesis, improving nutrient and oxygen supply. - Enhances osteogenic differentiation and bone regeneration.	[56]

Table 2. Surface modification employed for developing functional scaffolds.

Biological Modification	Benefits	Application	Ref
pVEGF plasmid	Polydopamine, PLA, PEI and VEGF plasmid activation of the scaffold surface	Bone Regeneration, Sustained Release of Growth Factors, Gene Delivery, Angiogenesis	[43]
Nano-Hydroxyapatite (nHA) Particles	Hydroxyapatite, Collagen, Plasmid DNA, Cross-linking Agents such as EDAC and NHS, ascorbic acid, β-glycerophosphate, and dexamethasone, Staining Agents.	Gene and stem cell-based therapies, Gene Delivery, Bone repair, gene activated matrix, non-viral gene delivery.	[44]
Incorporation of Bioactive Ions	Substitution of calcium ions in hydroxyapatite with foreign ions to promote osteoblast adhesion and enhance biomaterial capabilities.	Medical implants where enhanced bone integration is required	[45]
Chitosan/Cefazolin Composites	- Initial burst release followed by sustained release of antibiotics. - Nearly 100% antibacterial activity against Escherichia coli.	- Prevents local infection and bacterial recolonization after implantation.	
Graphene-Coated Titanium	- Extended drug-release period and greater corrosion resistance. - Strong antibacterial activity against Escherichia coli.	- Dental implants to prevent bacterial infections and enhance biocompatibility.	
Chitosan/ Melittin/ Vancomycin/ Oxacillin	- Strong bactericidal and anti-biofilm properties.	- Effective against MRSA and VRSA bacteria.	
Smart Coatings with Quaternary Ammonium Salts (QAs)	- Surface charge reversal in response to pH changes. - Antibacterial, anti-inflammatory, and osteointegration properties.	- Effective against Escherichia coli and Staphylococcus aureus *in vitro*.	
Iodine-Supported Coatings	- Broad-spectrum antibacterial action and biocidal effects against viruses and fungi. - Higher antibacterial effectiveness compared to anodized titanium surfaces.	- Prevents post-operative infections, effective against various bacteria and fungi.	
Hydroxyapatite-Based Coatings	-Utilizes hydroxyapatite for its biocompatibility and ability to bond with hard tissues. Bioactive metals and metal oxides are used as dopants to enhance properties.	Used in orthopedic and dental implants for enhanced osseointegration	
Selenium-Doped Coatings	- Enhanced antibacterial, antioncogenic, and osteogenic properties. - Maximum osteogenic activity and anti-oncogenic properties.	- Optimal concentration eradicates 97% of Escherichia coli and Staphylococcus aureus.	
Smart Coatings for Drug Release	Develops coatings that release antibacterial agents in response to stimuli like enzymes or pH changes, aiming for controlled drug release and reduced antibiotic resistance.	Reduces risk of antibiotic resistance in medical implants.	
Antimicrobial Peptide-Based Coatings	Involves covalent immobilization of antimicrobial peptides on titanium surfaces, such as RRP9W4N with elastin-like polypeptide, to provide bactericidal action and support cell adhesion.	Effective against various bacteria, supporting osteogenic cell adhesion in implants.	
Osteo-Immunomodulation	Designs coatings to interact with the immune system, creating an environment that inhibits bacterial colonization and enhances antibacterial efficacy without antibiotics.	Enhances antibacterial efficacy without relying on antibiotics.	

4.1. Physical modifications

Techniques involving physical changes impact the topography and roughness of biomaterials, thus affecting the behavior of cells. Common procedures include laser treatment, plasma spraying, and mechanical polishing, all of which generate patterns at the micro- and nano-scale levels on the surfaces of scaffolds. These patterns imitate the natural extracellular matrix (ECM), augmenting cell adhesion, proliferation, and differentiation. Physical surface modifications significantly enhance cell-material interactions, leading to improved bone tissue regeneration. Physical modifications of surfaces can be made using fabrication techniques such as selective laser sintering, particulate leaching, gas foaming, extrusion, and Cryo-milling to improve mechanical properties, porosity, and scaffold architecture which is crucial for allowing blood vessels to grow into the scaffold and supporting tissue growth, ensuring efficient nutrients transport. The inclusion of metallic-doped polymers such as magnesium, strontium, and zinc to polymer scaffolds, and improving scaffold stiffness by changing the orientation of polymer filaments during fabrication, significantly boost interactions between cells and materials, ultimately resulting in enhanced regeneration of bone tissue [36]. Research has demonstrated the effectiveness of antibacterial metallic ions such as silver, cerium, copper, zinc, and iron, both individually and in combination, compared to bioactive glass for tissue engineering applications [37,38]. In examining various surface modification methods for inorganic nanoparticles, condensation reactions, and grafting are extensively studied to improve their properties and compatibility [39].

4.2. Chemical modifications

Chemical surface modifications involve the incorporation of functional groups or coatings onto the material surface to enhance its bioactivity. Various methodologies such as acid etching, alkali treatment, and the grafting of bioactive molecules (e.g., peptides, and growth factors) are commonly utilized for this purpose. The materials used for chemical modifications include Ceramics such as Tricalcium phosphate coatings and hydroxyapatite (HA), bioactive glass coatings, polymeric coatings, organic-inorganic hybrids, Calcium silicate Bioceramics, PDMS hybrids, and PGS in combination with silica-based bioactive glass. These materials ensure the effective development of bone regeneration offering a combination of strength, bioactivity, and compatibility with natural bone. In another study the application of calcium phosphate coatings on titanium implants enhances osteointegration by facilitating bone cell attachment and mineralization. Furthermore, the implementation of bioactive glass coatings has been proven to enhance the osteoconductivity of scaffolds, ultimately making them more effective for bone regeneration [40,41].

The chemical modification of the scaffold can stimulate it towards bone regeneration. The reactivity of the hydroxyl groups of the biodegradable and biocompatible polymeric 3D porous scaffold can facilitate a reactive surface by allowing the covalent bonding of the polymer and polyethyleneimine (PEI). The PEI surface is further reacted with citric acid which reported to have a mineralization property facilitating an osteoconductive property [42]. Polydopamine (PDA) is another chemical modification that was widely explored by us for developing functional scaffolds. The gene delivery of vascular endothelial growth factor plasmid through complexation with cationic branched polyethyleneimine has facilitated a significant mineralization in scaffolds compared to scaffolds alone. The PDA coating has facilitated the binding of the transfection factors to the thermoplastic polymer surface of the 3D printed scaffold increasing the hydrophilic nature of the scaffold facilitating an improved cell adhesion and cell

proliferation. The VEGF expression has facilitated the angiogenesis and also stimulate the bone regeneration pathways *in vitro* [43].

4.3. Biological modifications

Biological surface alterations encompass the immobilization of biological entities such as proteins, peptides, and DNA onto the surface of a scaffold. These alterations aim to replicate the natural biological environment, thereby augmenting cellular recognition and response. Some of the common materials used in biological modifications for bone regeneration include nano-hydroxyapatite (nHA) particles, collagen, and Plasmid DNA (pDNA) to encode specific genes like BMP2 and methods such as Gene delivery using nHA particles to deliver genes into cells without using viruses, combining nHA with collagen scaffolds to support bone growth and repair, calcium quantification and mineralization and transfection of mesenchymal stem cells using nHA particles to deliver a specific gene BMP2 into MSCs. The application of collagen and hydroxyapatite is prevalent in coating scaffold surfaces, creating a favorable setting for the attachment and proliferation of bone cells. The efficacy of collagen nano-hydroxyapatite scaffolds as proficient non-viral vectors for gene delivery, leading to a significant enhancement in bone formation facilitated by stem cells [44].

5. Applications in bone tissue regeneration

Surface modifications play a crucial role in the advancement of scaffolds designed for bone and tissue regeneration. These adaptations effectively address concerns such as limited cell adhesion, inadequate mechanical robustness, and insufficient assimilation with the surrounding tissue. The process of bone regeneration necessitates scaffolds with excellent osteoconductivity, osteoinductivity, and mechanical properties. To meet these requirements, surface modifications are essential. A comprehensive study on various surface modification techniques for bone grafts and biomaterial substitutes, emphasizing the importance of surface-modified scaffolds. They found that scaffolds coated with materials such as calcium phosphate or bioactive glass demonstrated improved effectiveness in promoting bone regeneration [45]. Titanium and its alloys are commonly used for bone implants because they are strong and compatible with the body [46]. However, since they don't naturally bond well with bone, their surfaces need to be modified. The latest progress in magnesium-based biomaterials (MBs) involves enhancing corrosion resistance, creating magnesium alloys with superior implant properties, doping materials such as calcium phosphate with magnesium to aid bone growth, and enhancing bioceramics and cements for quicker healing. Innovative production methods like microwave-assisted techniques and a deeper understanding of immunomodulation have also boosted biocompatibility and osteogenesis, making MBs suitable for different surgeries because they are lightweight and safely degrade within the body [47]. Implant-associated infections (IAIs) caused by biofilm formation are challenging due to inflammation and poor tissue integration, and current antibiotic treatments are often ineffective due to bacterial resistance. Nanocatalytic therapy, using nanomaterials like CeO_2 NRs and Mn_3O_4 to regulate oxidative stress and produce ROS, shows potential in combating drug-resistant bacteria. Preventing biofilm formation is crucial, with anti-adhesive coatings such as super hydrophilic PEG inhibiting bacterial adhesion. Combining antibacterial and anti-adhesive strategies offers a promising approach to preventing IAIs caused by resistant bacteria [48].

PLGA is a popular synthetic material for making fibrous scaffolds in tissue engineering. Enhancing PLGA scaffolds with various polymers and ECM components greatly benefits tissue engineering. Polymers like PEG, PDA, polypyrrole, poly-lysine, and PEI make surfaces more hydrophilic and help cells stick better. ECM elements like hyaluronic acid, laminin, fibronectin, and collagen improve cell attachment and growth. These modifications add extra features, such as increased strength and electrical properties, useful for bone and cartilage repair, 3D-printed scaffolds, and nerve repair. By combining PLGA with these materials, we overcome its limitations and widen its use in medicine, including tooth tissue regeneration and potential diabetes treatment [49,50]. Orthopedic implants with materials such as Li/BGs and polyetheretherketone (PEEK) improved their compatibility with bone tissue, reduced inflammation, and promoted better bone healing, making them more effective for clinical use [51]. PEEK, despite its mechanical benefits, often triggers inflammatory and immune responses, leading to poor osseointegration. To address this, researchers coated PEEK with lithium-doped bioglass nanospheres (Li/BGs), enhancing its bioactivity and immune response modulation. Laboratory and animal studies showed that this modification improved cell adhesion, reduced inflammation, and promoted better bone growth, making biofunctionalized PEEK a promising candidate for orthopedic implants [52]. Angiogenesis is critical for tissue vascularization and nutrient supply in regenerative processes. A study investigated bioactive coatings loaded with vascular endothelial growth factor (VEGF) for enhanced bone regeneration [53,54]. Their study demonstrated increased vascularization and improved tissue regeneration outcomes, emphasizing the potential of bioactive coatings in angiogenic modulation for regenerative medicine applications.

Recent advances in the manufacturing of scaffolds include 3D-printing techniques which ensure personalization with enhanced mechanical properties [55]. A study focuses on making 3D-printed scaffolds for bones that release two types of helpful substances: one helps grow blood vessels, and the other helps bones grow. These scaffolds copy how real bones are structured and strong. They release these helpful substances slowly and in the right order—quickly for blood vessels and slowly for bones. They are made using a special 3D printing method at very low temperatures. After making them, they are coated with a gel that contains another helpful substance to grow blood vessels, ensuring bones heal better [56]. Another promising technique for surface modification is plasma immersion ion implantation (PIII). This method involves the introduction of ions into the surface of biomaterials to enhance their osteogenic activity. Recent studies have shown that PIII-treated titanium implants exhibit improved bone integration due to the formation of bioactive surfaces that promote osteoblast adhesion and proliferation [57]. The functional scaffolds for BTE play a crucial role in promoting bone healing and regeneration. Advances in scaffold design, fabrication techniques, and incorporation of specific materials and signaling molecules offer promising opportunities for improving bone tissue engineering strategies.

6. Conclusion & outlook

In conclusion, the evolution of surface modification techniques and material innovations in bone tissue engineering (BTE) has led to significant advancements in scaffold design, offering substantial improvements in osteoconductivity, mechanical properties, and biocompatibility. The application of surface treatments such as coating with calcium phosphate, bioactive glass, and biofunctionalized materials has shown great promise. These advancements pave the way for more effective, safe, and

sustainable solutions in the field of bone tissue engineering, marking a promising future for regenerative medicine.

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Conflicts of interests

All authors have no potential conflict(s) of interest like employment, consulting fees, research contracts, stock ownership, patent licenses, honoraria, advisory affiliations *etc*.

Authors' contribution

Conceptualization, J.C. and M.M.; writing—original draft preparation, J.C., S.C. and S.B.; writing—review and editing, J.C. and M.M. All authors have read and agreed to the published version of the manuscript.

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