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PAPER

Recent Biomaterial Developments for Bone Tissue Engineering and Potential Clinical Application: Narrative Review of the Literature

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ABSTRACT

Over the course of time, there has been a progression in the materials utilized for implants, transitioning from inert substances to those that replicate the structural characteristics of bone. Consequently, there has been a development of bioabsorbable, biocompatible, and bioactive materials. This article presents a comprehensive survey of diverse biomaterials with the potential to serve as scaffolds for bone tissue engineering. The objective of this study is to present an in-depth review of the predominant biomaterials utilized in the fabrication of scaffolds. This review encompasses the origins, classifications, characteristics, and methodologies involved in the development of these biomaterials. The review also highlights the incorporation of additives in biomaterial scaffolds. This study ultimately underscores the potential advantages and challenges associated with the utilization of biomaterials in scaffolds for bone tissue engineering. Additionally, it critically examines the integration of state-of-the-art technology with biomaterials.

KEYWORDS

bone tissue engineering, biomaterials-based scaffolds, metallic-based scaffold materials, nanomaterials

1 INTRODUCTION

About nine million fractures occur annually around the world due to bone disorders and associated consequences, and these conditions represent the majority of health problems in those aged fifty and up [1, 2]. However, bone has the inherent ability to heal and regenerate, so even a minor fracture can be fixed. Large bone deficiencies, such as those caused by trauma, the removal of a tumor, or an accident, are difficult for the body to repair on its own [3, 4].

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As a result, it is up to the orthopaedic surgeon to figure out what options they have. Autologous bone grafting [5] has been shown to be more promising and effective than other methods, earning it the nickname "gold standard." [6] Inadequate medical achievements can be seen with the autograft approach due to issues such a lack of grafts, persistent discomfort, a high donor site morbidity, secondary injury, and infections [6–8]. About 34% of bone substitutes are allografts, which use bone supplies from donors that come in a range of sizes and do not cause donor site morbidity compared with autografts [9]. Immunological rejection and the spread of infectious diseases are two of the main obstacles to using allografts, and current demand considerably exceeds supply. Aside from using biological grafts, bone surgery has also made use of bioinert materials [7, 10]. Despite their accessibility and repeatability, it is possible that following transplantation, these materials would get wrapped in fibrous connective tissue since they do not merge effectively into the natural bone. There is a limitation on the usage of bioinert grafts due to the stiffness mismatch between the load-bearing implant and the neighboring bone [11, 12]. All in all, the constraints of conventional clinical therapies call for cutting-edge strategies for simplifying surgery and speeding up bone regeneration. Accordingly, it was important to discover new approaches that can help us get around the drawbacks of the ones we now have. It was discovered that any sizeable hole in a bone can be repaired using bone tissue engineering. This subject of tissue engineering approach has, as a direct result of ongoing research and development, become an increasingly relevant and important area of concern for the field of medicine's foreseeable future [13–15]. The term "scaffold" refers to a three-dimensional matrix component that may be fabricated from a wide variety of materials that are all biocompatible, bioactive, and biodegradable. Scaffolds are used to improve the physical qualities of defective bone by creating an environment that promotes cell proliferation, differentiation, and adhesion in a manner similar to that of the original bone's extracellular matrix (ECM) [16, 17].

Tissue scaffolds, thanks to their porous nature, allow cells to spread throughout their surroundings and contribute to the scaffold's increased mechanical stability. Therefore, it helps with tissue regeneration in both the laboratory and the body. Scaffolds for bone defects have been fabricated using a mixture of biomaterials [18, 19].

In the field of bone tissue engineering, biomaterials play a fundamental and crucial role in the construction of a scaffold. Tissue engineering, a technique used to treat, enhance, and replace damaged organ tissue, makes use of biomaterials. A biomaterial should be biodegradable, printable, non-cytotoxic, and osteoconductive in vivo [20]. To keep up with the rising need for scaffolds, tissue engineers have begun experimenting with composite compositions that include biomaterials and other materials that match the characteristics of bone [21, 22]. Scaffolds have also been made out of bioactive glass, calcium phosphate, and calcium carbonate, all of which are synthetic and among the several polymers used. A bone tissue repairs transdisciplinary study used several scaffold materials. Scaffolds made out of a variety of composite materials and other fabrication methods, with new bone tissue, can be formed on these scaffolds [22, 23]. This article provides a comprehensive overview of the various biomaterials that have the potential to create scaffolds for bone tissue engineering. It aims to provide an overview of the most prevalent biomaterials used to create scaffolds, including their origins, classifications, qualities, and development processes. The review also emphasizes the biomaterial scaffolds with additives. Finally, it highlights the opportunities and problems of biomaterials in bone tissue engineering scaffolds and analyses the combination of cutting-edge technology and biomaterials.

2 BIOMATERIALS-BASED SCAFFOLDS FOR BONE TISSUE ENGINEERING

Bone tissue engineering provides a 3D framework for cell survival, tissue growth, and vascularization, allowing for the eventual healing of bone abnormalities. Osteoconductive, osteogenicity, osseointegration, and osteoinductivity are all necessary for a bone tissue engineering scaffold to effectively mimic bone development [24]. When it comes to maximizing cell differentiation and new tissue development, the scaffold's porosity and pore size are two of the most important regulators of degradation and mechanical properties [25]. While a new bone tissue is being formed, several biomaterials and compositions are proposed for use in creating scaffolds that resemble the ECM, the new bone tissue (Figure 1).

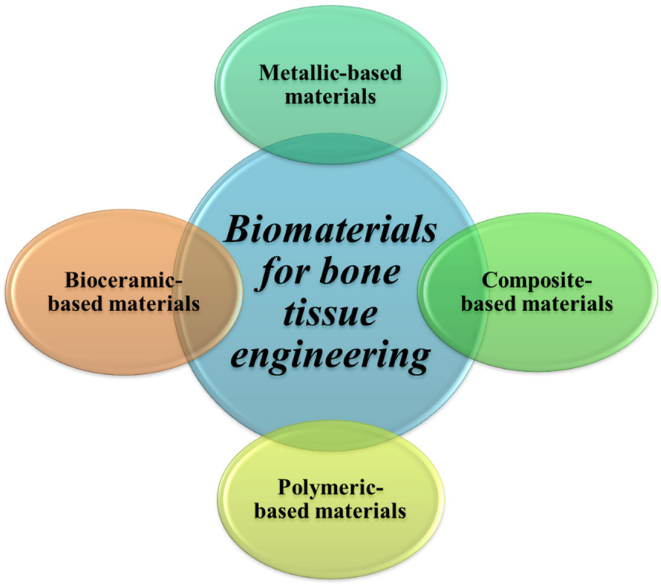

Fig. 1. Biomaterials-based scaffolds for bone tissue engineering

3 METAL-BASED SCAFFOLD MATERIALS

Metals are implemented as metallic biomaterials in the disciplines of orthopaedics and dentistry to enhance bone repair [26]. This is mostly due to the exceptional fracture toughness and mechanical strength that metals possess. Since bioactive metal matrix composites provide superior mechanical qualities, biocompatibility, thermal stability, and corrosion resistance, they find extensive application in clinical medical settings.

As tantalum is a non-reactive, corrosion-resistant metal, its high modulus of elasticity greatly outstrips that of cancellous and cortical bone [27]. The elastic modulus of tantalum scaffolds is often decreased by fabricating a porous structure, making them more analogous to autologous bone. As of now, porous tantalum stents have been implemented in the treatment of femoral head necrosis, as well as arthroplasty, spinal fusion, foot and ankle surgery, and necrosis of the talus. The results validated the porous tantalum scaffold's superior biocompatibility and osteoinductivity in bone tissue engineering [28], as it increased bone trabecular structure. A porous tantalum scaffold merged tightly with the host bone in canine femoral shaft

bone defect models, and new bone growth was detected at the scaffold-host bone interface 3- and 6-months post-implantation. Clinical applications of tantalum are limited, however, by the difficulty of its production and the sluggish rate at which it promotes osteogenesis.

Titanium and its alloys are frequently utilized in bone tissue engineering scaffolds because of their superior antibacterial qualities and biocompatibility [29]. However, because of their high elastic modulus, titanium implants may loosen the scaffold by bone absorption at the interface where the implant and bone meet. That is why porous titanium scaffolds are so common. Scientific research has established that titanium possesses osteogenic characteristics. Titanium-modified scaffolds were shown to stimulate ASC proliferation and osteogenic differentiation in the absence of GFs [30]; this was discovered by Frachet et al. Nonetheless, as long-term efficacy evaluations are lacking, follow-up clinical investigations are required for additional verification.

Zhu et al. fabricated D printed multi-morphology porous Ti6Al4V scaffolds with spatially changing pore patterns for bone tissue engineering application (Figure 2). Ti6Al4V is an excellent example of a metal matrix composite. Appropriate porous Ti6Al4V scaffolds can have a Young's modulus close to that of real bone, enhancing the mechanical protection afforded to live tissue [31]. The tantalum coating, adding simvastatin/hydrogel, or coating the Ti6Al4V scaffolds with polydopamine-assisted hydroxyapatite (HA/pDA) can significantly improve bone ingrowth, osteointegration, and osteogenesis. The results demonstrated that the multi-morphology structure has both low elastic moduli and strong yield strength. Reduced bone damage and improved implant stability are both possible with this combination. As a result, the multi-morphology porous Ti6AlV scaffold that was 3D printed showed promising results for use in orthopaedics.

Metal matrix composites, such as Ti6Al4V, offer numerous remarkable advantages; yet their potential as perfect materials are fundamentally limited by the non-biodegradable features of metal matrix composites [32–34].

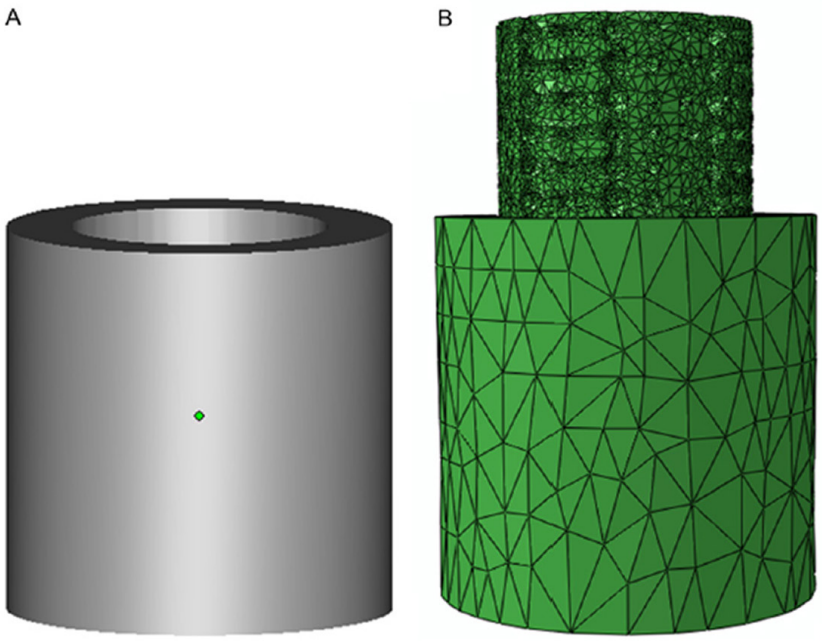

Fig. 2. Schematic illustrating bone modeling and meshing. (A) simplified 3D model of femur bone, (B) mesh of scaffold bone assembly [31]

4 BIOCERAMIC-BASED SCAFFOLD MATERIALS

Ceramics have a long history of usage in the medical field for the repair and rebuilding of broken bones and other body parts. Bioceramic is the collective name for these materials, which can be further subdivided into two major groups: bioactive and bioinert [35]. Scaffolding materials that have chemical characteristics and crystallinity that are analogous to those of bone minerals are desirable. Furthermore, these have excellent bioactivity and biocompatibility. Ceramic materials like bioglass, calcium phosphate, and corals have all found application in the field of bone repair and regeneration. Controlling deterioration, being bioactive, being able to distribute cells, and having strong osteoconductivity [36] are all advantages of bioglass, but its hardness, low strength, and unreliability [37] are drawbacks.

Bone flaws can be repaired with calcium phosphate material, which is available in CPC (self-hardening calcium phosphate), ceramic HA granules, and TCP, which are all forms that this material can take. [38], because of its unique chemical make-up, pore size, and crystal structure, it is bioactive, resorbable, and osteoconductive [39]. Adding magnetic nanoparticles to ceramic scaffolds stimulates bone formation. Corals have a high porosity like trabecular bone and a pore size distribution that is precisely regulated [40]. Coral scaffolds, made from adipose-derived mesenchymal stem cells (AMSCs), have been shown in a canine model to be biocompatible and effective at repairing defects in cranial bone [41]. Repairs were made to the flaws in both the experimental group (where ASC-coral constructs were used) and the control group (where coral alone was used). At 12 weeks post-implantation, 3D CT scans revealed that new bone had grown in the experimental group, while coral scaffolds had partially disintegrated in the control group. At 24 weeks post-transplant, radiographic study revealed that in the experimental side, 84.196.45% of each defect volume had been healed, but in the control side, this number was just 25.0418.82%. Histological analysis showed that in the experimental group, normal bone tissue grew to fill the gap, but in the control group, only a few bone cells formed, connected by fibrous tissue. The new method has been shown to successfully heal critical-sized bone lesions, demonstrating the promise of combining ASCs with coral scaffolds for bone regeneration [41].

5 NATURAL POLYMERIC-BASED SCAFFOLD MATERIALS

Scaffolds made from natural polymers are ideal for use in bone tissue engineering due to their high biodegradability and biocompatibility. Scaffolds' porosity, mechanical strength, and charge are all susceptible to changes in polymer characteristics, such as concentration and environment [42]. In addition, additives can be used to enhance the properties of polymer-fabricated scaffolds. Polymeric natural materials are frequently employed in bone tissue engineering [43].

Given its importance as a building block of bone, it should prove valuable in the development of scaffolds. Poor mechanical qualities are offset by its biodegradability and biocompatibility. Scaffolds made from collagen material have been employed in certain research, with the osteoinductivity enhanced by the addition of other chemicals [44]. To overcome this barrier in mechanical characteristics, Ogawa, and Huang [45] used a robot dispensing technology to mix poly-e-caprolactone (PCL) with collagen. There was a marked rise in the number of cell divisions, which was clinically meaningful when employing PCL/collagen combination scaffolds compared to using static conditions of cells. The ability of mesenchymal stem cells (MSCs) from a variety

of origins to perform osteoinductive growth and, as a result, overcoming collagen's superior mechanical properties has been evaluated using collagen hydrogels [46].

Collagen has been combined with other materials in certain research to increase the strength of the bone-matrix interface. One study [47] used a CaO-P2O5-SiO2 bioactive glass (BA) as an addition to collagen solution to produce composite scaffolds. The scaffold's compressive strength diminished, and its porosity value increased as PS ratios were raised.

Chitosan was discovered to be an ideal material for scaffold development due to its biocompatibility, biodegradability, wound healing, antimicrobial, and bioadhesive properties [48]. Scaffolds made from chitosan have had their osteogenic characteristics altered in a number of experiments. One study added chitosan and poly(butylene succinate) (CH-PBS) to an already existing scaffold, and then seeded it with human mesenchymal stem cells (MSC) to see if it could stimulate bone growth [49]. Likewise, bone marrow stem cells have been used to create hydrogels with varying chitosan/collagen ratios in order to evaluate the matrix mechanical properties of the combined materials [50].

Certain researchers used chitosan, silk fibroin, and hydroxyapatite (HA) to create the porous scaffolds [51]. The lack of flexibility and formability of silk fibroin/hydroxyapatite (SF/HA) scaffolds was cited as the main cause for their unsuitability for bone tissue creation [52]. Some research has considered injecting chitosan as a biomaterial that, when combined with tri-calcium phosphate and protein plasma, can be used to construct a structure [53].

The natural polymer found in silk fibroin has many uses in the field of tissue engineering [54]. It is biocompatible, flexible in its shape, and has excellent mechanical qualities. Studies on composite scaffolds have focused on the incorporation of pertaining to the ingredients found in silk particles that enhance porosity and other necessary qualities of tissue engineering scaffold. Research study [55] found that adding CaP/silk powders to silk scaffolds improved the distribution of CaP powders throughout the composite scaffolds. Additional study has focused on the mechanical properties of composite scaffolds made from silk sponge matrices reinforced with silk microparticles [56].

It has been discovered that alginate is soluble in water and that, in the field of tissue engineering, it has many uses for the production of scaffolds. At room temperature, alginate takes the shape of a gel. A scaffold was built using macroporous alginate in one of the studies, after which a biocompatible inorganic coating was applied [57]. Oxidation was used to make alginate more biodegradable, and then fibrin was mixed in to make it less biodegradable after it had already been made more biodegradable. In another work, alginate was mixed with a three-dimensional matrix of calcium phosphate cement (CPC), with the goal of improving the characteristics of the scaffold [58]. In addition, scaffolds made of alginate have also been employed in conjunction with combinations of peptides and proteins in order to boost osteogenesis [59].

In order to maximize the osteogenic potential of hyaluronic acid polymer, it is typically combined with a number of other additives. It was discovered to be a possible material for use as a bone scaffold [60]. In one of the studies, a hydrogel containing simvastatin and a medication was produced with the intention of improving the qualities of the scaffolds [61]. This hydrogel was used in the study. The osteogenesis of the material has also been improved through the fabrication of hyaluronic acid scaffolds. A great number of further researches have been carried out in an effort to enhance the qualities of the scaffold by the inclusion of various other additives.

6 SYNTHETIC POLYMERIC-BASED SCAFFOLD MATERIALS

The most common type of substance found in bone tissue engineering was a polymer composed of polylactic acid [62]. There are many synthetic polymers available, some of which have been approved by the Food and Drug Administration, such as polyglycolic acid (PGA), polylactic acid (PLA), and their copolymer (PLGA) [63]. Because of its degradability, mechanical characteristics, and cellular viability, PGA has been utilized in the process of internal bone fixation, and the nonwoven polyglycolide scaffold has been shown to work as tissue regeneration substrates [64]. The fact that PLA degrades slowly, that it is hydrophobic, and that it has a poor impact hardness work against its use in clinical settings. As a result, the techniques of particle leaching and electrospinning have been chosen in order to enhance the scaffolds by combining PLA with various other polymers. For example, Zhang et al. [65] developed PLA/octadecylamine functionalized nano-diamond composites for tissue engineering, which increased the mechanical properties of PLA (Figure 3).

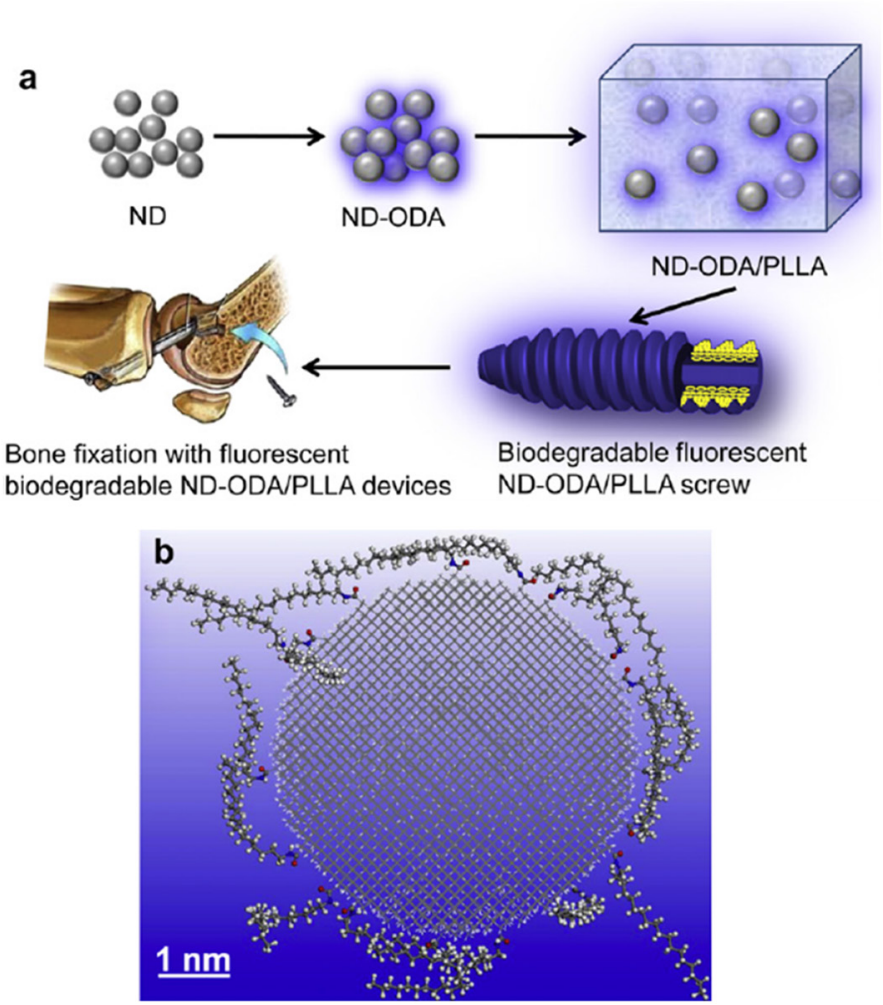

Fig. 3. a) Schematic illustrating manufacturing and use of ND-OD/PLLA composites; b) Molecular model of an ND-ODA particle

These nano-diamond composites were used for tissue engineering. Because the addition of 10% by weight of composites resulted in an increase of more than 200%

in Young's modulus and an increase of 800% in hardness [65], the nanocomposite possessed properties that were comparable to those of human cortical bone. The culture of mouse smooth muscle cells on the modified PLGA scaffold revealed that the cells were in healthy condition [66]. As the temperature of the surrounding environment rose, it was seen that the scaffold's exterior wall thickened visibly and the microtubules in its vicinity were disorganized. As the temperature gradient or polymer solution concentration increased, the microtubules in the vessel scaffolds shrank. Scaffolds with distinct morphologies and microtubule diameters were produced by adjusting TIPS parameters. However, by altering the size of the polyethylene mold, both the inner and outer diameters of the vessel scaffolds could be managed. The scaffolds' ability to support cells was evaluated in vitro using A10 cells as test subjects. The results demonstrated that cells expanded healthily in vascular scaffolds treated with ammonia plasma and fastened with collagen. The cells might form an array following the microtubules' line of travel [66].

In addition, the PLGA/gelatin scaffolds that were used for the culture of mouse sciatic nerve cells resulted in good adhesion and proliferation [67]. However, PLA, PGA, and PLGA all have a number of drawbacks, including low hydrophilicity, a limited capacity for cell adsorption, a tendency toward aseptic inflammation, and inadequate mechanical characteristics.

Calcite phosphate, bioglasses, and glass ceramics make up the bulk of the synthetic inorganic materials employed in bone tissue engineering. These polymers are highly biocompatible, biodegradable, osteo-conductive, and osteoinductive [68]. Since it is the main component of bones and teeth, in the field of bone tissue regeneration, HA is widely regarded as a potential scaffold material. Because it is a synthetic created in a lab, HA has little immunological rejection and high biosecurity, bioactivity, and affinity [69]. It also has the capacity for bone conduction and chemical stability, making it an ideal environment for seed cells to develop into osteoblasts. In addition, HA's mineral components like calcium and phosphorus can play a part in other metabolic functions. Several investigations have coupled HA with other materials that have osteoinductivity or osteogenesis capabilities to construct viable composite scaffolds [70], despite the fact that pure HA scaffolds have limited osteoinductivity. Induced gingival fibroblasts' proliferation and differentiation were both promoted by a nano-HA/chitosan/gelatin matrix [71]. All of the scaffold's mechanical properties were enhanced by this matrix. The Young's modulus and compressive strength of the scaffolds were found to rise in the presence of non-sintered or sintered hydroxyapatit or hydroxyapatite, and these values were found to be remarkably close to those of human spongy bone. Results from MTS experiments, confocal microscopy, and scanning electron microscopy all pointed to the superiority of sintered hydroxyapatite composite scaffolds for cell adhesion and proliferation. Incorporating sintered hydroxyapatite into chitosan, gelatin, and HA scaffolds demonstrated their suitability as cell carriers for bone tissue creation.

Chemical synthesis of HA has so far made use of precipitation, hydrothermal, electro-chemical deposition, emulsion, and ultrasonic spray freeze drying [72]. These methods may inhibit bone healing and perhaps cause inflammation [73].

7 COMPOSITE BASED SCAFFOLD MATERIALS

Calcium phosphate coatings on metals, HA/poly-(D, L-lactide), HA/chitosan-gelation [4, and those including bioceramics] are just some of the common composite materials utilized to make bone tissue engineering scaffolds. Metals coated with calcium phosphate are another type of composite material. They have desirable

properties such as biodegradability, osteoconductivity, compressive strength, and osteointegration [74]. The excellent biocompatibility of bioactive glass (BG) in bone and soft tissues is due to its SiO2-CaO-P2O5 network [75]. In the case of bioceramics containing calcium silicate, this holds true. Degradation of BG results in the release of sodium and calcium ions, as well as soluble silica, which stimulate cell proliferation and osteogenesis [76]. The angiogenic activity and in vivo stiffness of a collagen scaffold may be enhanced by the implantation of BG, as suggested by Zhang et al. research [77]. Integration of BG into collagen scaffolds has been shown to increase new bone formation in rats. Collagen matrix features including porosity, structural stability, osteoinductivity, and osteogenicity can be greatly enhanced by integrating various biomaterials. Bioceramic, carbon, and polymer materials are all discussed in this review as potential building blocks for collagen-based composite scaffolds utilized in bone regeneration. Also discussed are potential future research and development avenues for this area.

Bone will adapt its structure in the wild to the mechanical stresses to which it is exposed. By adjusting the force, time, and mechanical stirring frequency, a composite material with tunable modulus has been manufactured [78, 79]. Because of this, the material can change shape in response to mechanical stress. The piezoelectric ZnO helps the composite material to respond to different conditions. This versatility regulates the crosslinking process between mercaptan and olefin in the polymer composite gel, which modifies the driving modulus of the mechanical system. Organo-gel remodelling is aided by mechanothiolene polymerization, and piezoelectric ZnO is selectively polymerized upon mechanical activation, thereby reinforcing certain segments of the organo-gel matrix [79]. Furthermore, the organo-gel was formed due to mechano-thiol-ene polymerization. As a result, the material might redesign its modulus and stress distribution in response to loading, much like bone does naturally, and the bone tissue engineering scaffold could benefit from mechanically adaptive biomaterials if the right combination of materials were used. It is due to the unique way bone remodels itself.

8 BIOCOMPATIBILITY AND BIOINTERACTIONS OF BONE TISSUE ENGINEERING SCAFFOLDS

8.1 Biomaterials cytotoxicity assay

Biomaterials intended for implantation must be relatively non-cytotoxic. ISO10993-5:2009 (Biological evaluation of medical devices-Part5: Tests for in vitro cytotoxicity) provides guidance for in vitro cytotoxicity testing of biomaterials but proposes only a small number of test techniques. Indirect testing using fluid extracts of the components is the standard method.

The regulation of extract preparation is of significant importance and is governed by ISO10993-12:2012, which pertains to the biological evaluation of medical devices. This standard provides guidelines on various aspects of sample preparation, including the recommended ratio between sample weight/surface area and the volume of the extraction vehicle, the utilizations of appropriate reference materials as negative and positive controls, and the selection of the optimal extraction media. Nevertheless, the methodologies outlined in ISO10993-5:2009, particularly those of an indirect nature, exhibit certain limitations [80, 81].

Several biomaterials based on calcium phosphate exhibit significant reactivity with ions, leading to alterations in the ionic composition of the adjacent

microenvironment, such as the culture medium [82, 83]. The ceramic-based materials discussed in this study exhibit a high level of reactivity. They have the ability to alter the concentration of ions in the culture medium through processes such as ion uptake, which is often associated with the spontaneous formation of appetite. Additionally, these materials can undergo ionic substitutions or release ions through the dissolution of calcium phosphates [82–85].

To evaluate cytotoxicity of highly reactive biomaterials while avoiding the confounding effects of variations in the ionic composition of the culture medium, an indirect agar diffusion test in accordance with ISO 10993-5:2009 is recommended. Samples of the tested material and controls (ISO 10993-12:2012 recommends appropriate positive and negative reference materials) are placed on the solidified agar (note that any absorbent material needs to be pre-soaked with the medium before being placed on the agar) and allowed to diffuse through the monolayer of cells. Cell lysis under and surrounding the specimens, as well as changes in general shape, vacuolization, detachment, and membrane integrity, are observed microscopically after 24–72 hours of incubation [80, 86].

The attachment of cells and proliferation assay

Bone scaffolds for regenerative medicine applications should encourage osteoblast adhesion and spreading, as this is essential for fast cell proliferation on the implant's surface. Anchorage-dependent cells like osteoblasts, osteoprogenitor cells, and mesenchymal stem cells require a biomaterial implant to retain their high viability, quick division rates, and high osteogenic potential, allowing for a rapid bone regeneration process [80, 87].

Protein adsorption mediates osteoblast/osteoprogenitor cell adherence to the scaffold, which in turn is influenced by the wettability [88, 89], surface chemistry and charge [88, 90], or topography (microstructure) [80, 91] of the implant surface. Proteins can be adsorbed to a scaffold most efficiently when its surface is positively charged and polar [92].

In addition, scaffold surfaces that exhibit greater wettability, specifically hydrophilicity, have the potential to enhance the adsorption of adhesive proteins, thereby facilitating the adhesion of osteoblasts [80]. Ultimately, it has been noted that the surface topography and roughness significantly influence the adhesion and proliferation of osteoblasts [80, 93].

9 IMMUNE RESPONSE TO BIOMATERIALS

The assessment of biocompatibility for novel bone scaffolds often involves evaluating their cytotoxicity as well as their ability to stimulate osteoblast adhesion, proliferation, and differentiation [94, 95]. Nevertheless, it is important to note that when biomaterials are inserted into a bone with a deficiency, they should not result in the development of long-term inflammation [96]. When biomaterials are surgically implanted, they induce injury to bone tissue, subsequently initiating an inflammatory response [97]. The initial acute inflammatory phase plays a critical role in normal bone healing, as it involves the release of cytokines that recruit osteoprogenitor cells and mesenchymal stem cells, stimulating their osteogenic differentiation and ultimately resulting in the formation of new bone [98].

Nevertheless, the occurrence of fibrous encapsulation and the formation of granuloma tissue could potentially hinder the successful integration of biomaterials with the host bone, particularly if the inflammatory response persists beyond a few weeks [99, 100].

The scaffold surface undergoes protein coating, derived from blood plasma or surrounding tissue, within a short period of time ranging from seconds to minutes following implantation. The host cells, primarily leukocytes and fibroblasts, subsequently identify the proteins bound to the implant's adsorbate [99, 101]. Following implantation surgery, cells migrate to the wound site within a time limit of 4–8 hours. These cells possess the ability to identify and interact with proteins that have been absorbed onto the surface of the biomaterial. Consequently, the cells adhere to the biomaterial [80]. The formation of a multinucleated foreign body giant cell can occur when monocytes adhere to the surface of a biomaterial and undergo maturation into macrophages [100, 101]. Following the adhesion of fibroblasts and monocytes to the surface of the implant, a fibrotic tissue consisting of fibroblasts and collagen is observed to develop on the scaffold within a few days [80]. The scaffold is also enveloped by macrophages and foreign body giant cells.

10 SCAFFOLD FABRICATION APPROACHES

10.1 Solvent casting and particulate leaching

The solvent casting and particulate leaching technique is a widely used and straightforward method in which the manipulation of pore size and porosity can be achieved by adjusting the salt/polymer ratio. The technique involves the dissolution of a polymer in an organic solvent, followed by the addition of a water-soluble porogen, such as salt (e.g., sodium chloride, sodium citrate) [102]. The resulting mixture is then cast into a mould. The solvent undergoes evaporation or lyophilization, resulting in the leaching of the polymer/porogen composite into water [103].

The porosity of the scaffold is contingent upon the quantity of porogen utilized, while the dimensions of the pores are determined by the size of the crystals. Alternative pyrogens include waxy hydrocarbons [102] and gelatin particles [104]. The study revealed that a significant level of interconnectedness among pores was attainable when the porogen concentration reached 70 wt. %. The solvent-casting method is capable of producing flat sheets and tubes without the need for specialized equipment [102]. The presence of residual toxic solvents can result in the denaturation of molecules that have been incorporated, leading to a reduction in the activity of bioinductive molecules [103]. Additionally, it can prevent the addition of pharmacological agents [102]. The 3D scaffolds derived from polymer-ceramic materials exhibit controlled pore interconnectivity and porosity at low levels of porogen [102].

10.2 Emulsion freeze-drying method

The emulsion freeze-drying technique is founded on the principle of phase separation, which involves the processes of emulsification and freeze-drying [105]. This method yields scaffolds with a high degree of porosity. The initial stage involves the preparation of the emulsion through the process of homogenization, wherein a polymer is combined with both an organic solvent and water. The emulsion undergoes a rapid cooling process, leading to the separation of its liquid phases (water and solvent) through freeze-drying. The resulting pores exhibit close proximity to each other, with a porosity exceeding 90%. The pore size falls within the range of 20 to 200 μm [106]. The emulsion freeze-drying technique has the potential to be integrated with particulate leaching. This involves the addition of sucrose or sodium

chloride to the emulsion, resulting in the formation of porosity. Following the process of freeze-drying, it has been observed that particles have the ability to be effectively removed through the process of washing [107–108].

10.3 Electrospinning techniques

The technique of electrospinning shows great promise as a versatile method for producing submicrometer fibres or nanofibers through the application of a high electric field, which effectively reduces the surface tension within polymer fluids. An electrical potential is applied to a solution or melt of a synthetic or natural polymer [105] in order to induce a charge imbalance [109]. This charge imbalance facilitates the stable and continuous deposition of electrospun fibres onto various substrates [105]. Various biopolymers, such as PCL, PU, and collagen, have been demonstrated to be suitable for electrospinning. The utilization of a combination of natural and synthetic polymers is commonly employed due to the inherent instability of natural polymers and the detrimental effects caused by the degradation of products of synthetic polymers [107].

The process of electrospinning has the capability to produce non-woven matrices that possess nanoscale characteristics. The control of fibre thickness and orientation can be achieved through the manipulation of polymer type and concentration, as well as the adjustment of the electrospinning device settings. The polymeric non-woven nanofiber scaffolds exhibit a notable degree of porosity and possess a substantial surface area [110].

10.4 3D bioprinting

The utilizations of three-dimensional (3D) printing techniques are becoming more prevalent in the field of tissue engineering. In order to facilitate cell migration and proliferation, it is imperative for 3D porous scaffolds to possess a uniform and appropriate size of interconnected pores. A number of frequently employed methodologies for fabricating these three-dimensional scaffolds have been previously discussed, yet they all share certain drawbacks, including insufficient regulation of scaffold architecture, pore network and size, and suboptimal quality of the resulting three-dimensional scaffolds [111]. The processes employed by these methods lack versatility [112]. The utilizations of 3D-printing techniques have the potential to address these limitations by means of rapid prototyping, solid free-form fabrication, biofabrication, bioprinting, and additive manufacturing [101]. The fundamental principle underlying bioprinting involves the fabrication of a well-defined architecture that houses cellular entities, achieved through the utilization of advanced 3D bioprinting methodologies [113]. Over forty distinct techniques for 3D printing have been developed, with fused-deposition modelling (FDM), stereolithography, inkjet printing, selective laser sintering (SLS), and colour jet printing being the most widely utilized methods for processing plastics [111].

One notable advantage of this technique is its ability to create 3D structures at both microscale and nanoscale without the need for physical masks or moulds. Moreover, it offers cost-effective, flexible, and highly efficient transfer of these structures. According to the cited source [103], all models are individually tailored and highly specialized. The application of 3D bioprinting has emerged as a novel and highly prospective method for fabricating three-dimensional tissue structures.

However, it is imperative to assess various bio-ink materials in order to meet specific property requirements [80]. Additional objectives include reducing cell loss, enhancing cell interactions, and promoting tissue vascularization [113]. However, it is currently feasible to generate perusable vascular constructs containing cells through the use of 3D bioprinting. These constructs are anticipated to have applications in the development of prevascularized tissue constructs [114].

11 THE POTENTIAL AND CONSTRAINTS OF MEDICAL TRANSLATION BONE TISSUE ENGINEERING

It is feasible that a 3D layout of cultured cells and the constituents of an adequate extracellular matrix could alter the performance of tissue-like engineered bone. Different pore-sized, permeable, and long-lasting scaffold materials have been produced. Tissue-engineered bone has been able to modify its function as bone tissue engineering has become increasingly popular and explored. It has become possible to isolate and stimulate osteogenic potential in bone progenitor cells from multiple human body sites, and the effect and application of growth factors on osteogenesis and angiogenesis have been clarified and greatly enhanced in vitro and in vivo. Because of this, researchers in both fields have been able to make great strides. As of now, bone tissue engineering is being utilized clinically to treat a wide variety of bone abnormalities, including traumatic calvarial defects, mandibular ridge resorption, anterior mandibular defects, and spinal stenosis, with a number of encouraging therapeutic results appearing. Stem cells, scaffolds, and growth factors all come together in bone tissue engineering [115, 116].

Bone tissue engineering success is highly dependent on a number of factors, including the biomaterial's design, the patient's age and health, the severity of the bone defects, and the patient's lifestyle and activity level after surgery. Examination of these factors can improve the bone tissue engineering system's chances of being approved by regulators and brought to market [117]. Since osteoporosis mostly affects elderly individuals, the development of bone tissue engineering as an alternative to autografts is very crucial in the treatment of this disease. These individuals may have a diminished ability to repair new bone using autologous tissues, such as adipose stem cells (ASCs) [118]. The possibility exists that this is correct.

Important difficulties in treating bone abnormalities include osteogenesis and implant integration with surrounding tissues [119, 120]. Inflammation caused by an infection in the bone can lead to additional complications, such as bone fractures or the need for surgery to remove malignant tumors. The process of integrating the implants with the native tissues can be aided by developing bone tissue engineering systems that can deliver multiple medications at once, such as those that fight inflammation, germs, and cancer [121–123]. Versatile implants that can prevent biomaterial-associated infection and boost osteointegration would be ideal. In particular, "statically-versatile" implants that don't call for external stimulations are preferred for the sake of convenience in application. Methicillin-resistant Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and S. aureus were all killed by a "statically versatile" titanium implant with an antibacterial activity of over 96.8% [124]. This was achieved by permanently encoding a novel fusion peptide (FP) consisting of an HHC36 antibacterial motif and antigenic sequence. The FP-engineered implant may stimulate cellular proliferation, vascularization, and osteogenesis all at once. In addition, the combination of doxorubicin drug release and thermal ablation showed excellent ability to load and release drugsin a porous scaffold. Scaffolds

contain components that activate the BMP-2/Smad/Runx2 signaling pathway, which promotes osteogenic differentiation of BMSCs and cell proliferation [125]. In addition, there is a lack of diversity among the identified scaffolds in their application to peripheral bone tissue engineering because of delayed vascularization implantation. To reduce dependency based on pre-existing internal and exterior attachments, it is also important to simultaneously achieve mechanical support and mass regeneration.

12 NANOMATERIALS AS A POTENTIAL TOOL FOR BONE TISSUE ENGINEERING

Bone tissue engineering initiatives necessitate the usage of scaffold materials. Porous structures that promote cell adhesion, growth, and migration to boost cell scaffold interaction; adequate elasticity and mechanical properties; a controllable degradation rate; uniform distribution of new bone formation to prevent bone necrosis; minimal inflammation and toxicity; these are all necessary for a scaffold to be effective in mimicking the three-dimensional structure of the extracellular matrix [126]. The tremendous progress in nanomaterials has enabled the construction of composite scaffolds with biological activity and reabsorption. These scaffolds promote cell adhesion and proliferation in addition to their remarkable mechanical qualities.

Mechanical characteristics, bone conductivity and biocompatibility, protein adsorption, cell adhesion, tissue proliferation, and differentiation are only a few of the areas where composite nanomaterials may be superior to more conventional materials. Researchers discovered that bone regeneration was enhanced by the use of HAp nanocomposite scaffolds (HAp/MoS2NSs) reinforced with molybdenum disulfide nanoflakes (MoS2NSs) [127]. ALP activity, adhesion, and cell proliferation were all dramatically enhanced in cells treated with HAp@MoS2NSs compared to those incubated with HAp alone. A higher amount of ALP was seen in in vivo and in vitro experiments, suggesting that HAp@MoS2NSs may stimulate bone formation.

The nanocomposite hydrogel has a rich interconnected hydrophilic network porosity structure, very much like the extracellular matrix. This structure provides more room for cells to attach themselves and interact with one another. As revealed by Hou et al. [128], microparticle annealed nanofibrous (MANF) hydrogels are a new form of injectable hydrogels that are produced by the self-assembly and crosslinking of gelatin nanofibrous microparticles (NF-MPs). In the bath of liquid nitrogen, temperature-induced nanoscale phase separation enables the gelatin solution to transform into NF-MPs after being sprayed from the nozzle. The gelatin solution is a mixture of ethanol and water as the solvent.

In order to maintain the stability of the gelatin NF-MPs, we utilized both EDC crosslinking and photocrosslinkable methacrylamide groups. With the help of photocuring, modified NF-MPs could be used to make hydrogel scaffolds with high cell viability. These scaffolds could then be used to enclose cells before the crosslinking process took place. In vitro, the hydrogels promoted cell proliferation and osteogenesis, whereas in vivo, the hydrogels assisted neovascularization and bone defect healing due to their hierarchically structured 3D design (Figure 4), and this was the case even though in vitro the hydrogels were used [129].

The nanocomposite fibrous scaffold in BTE, which has a porosity of 95% and replicates the fibrous structure of the natural extracellular matrix, is likely to be responsible for the increased cell adhesion, migration, proliferation, and differentiation shown in this model. Yahia et al. (2019) [130] developed a new family of electrospun

nanofiber scaffolds (NFS) with a sandwich structure. This was accomplished by mixing poly(caprolactone) (PCL) and chitosan/polyethylene oxide (CS/PEO) composites. These nanocomposite scaffolds may be able to influence the biological processes of angiogenesis and osteogenesis due to their bionic structure, programmable porosity, and porous network. Additionally, it was discovered that the modified scaffolds promoted fracture repair and bone regeneration in an in vivo rabbit model of a mandibular bone defect.

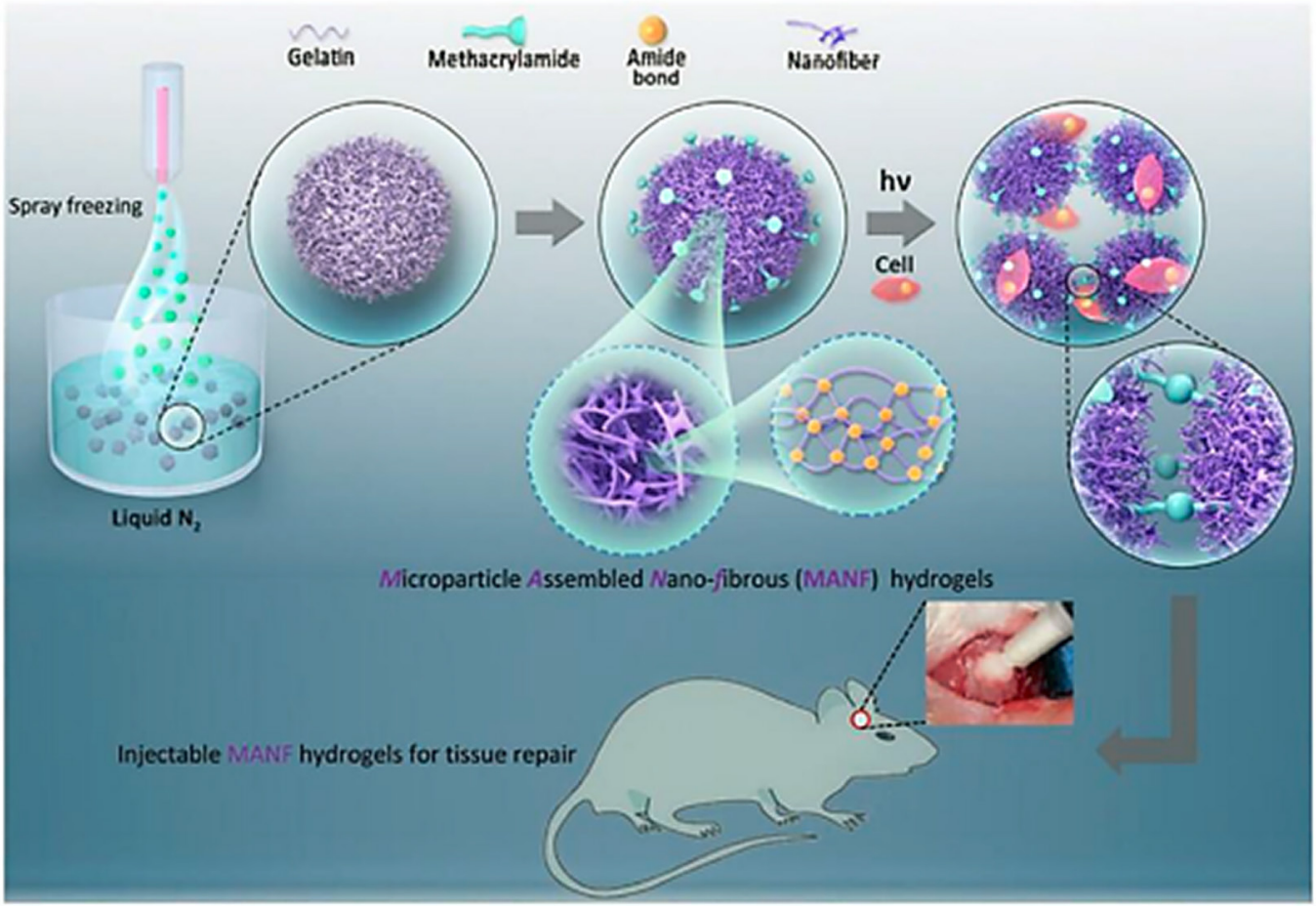

Fig. 4. Methodology for synthesizing gelatin nanofibrous MANF microparticles hydrogels

Biocompatibility of nanomaterials is determined by their capacity to exhibit non-toxicity towards living tissues and effectively elicit an appropriate host response within the human biological system [131].

The satisfactory performance of bone tissue engineering scaffolds necessitates the inclusion of biocompatibility as a fundamental requirement in materials design. The cytotoxicity of nanomaterials is assessed based on the ability of living cells to adhere, proliferate, and integrate with host tissues. The studies consist of an in vivo investigation, which involves the examination of living biological entities within an organism, and an in vitro analysis, wherein living cells derived from humans or animals are utilized in a laboratory setting [132].

Shaheen et al. [133] used MG63 osteoblast cells in a cell culture MTT experiment for cellulose nanocrystals-filled chitosan and alginate scaffolds. The cells have begun to proliferate inside the scaffold holes at an early stage in the culture process. After 72 hours, cells are firmly linked inside the holes of a 3D framework via filopodium and lamellipodium. The cells formed in clusters with a highly interconnected 3D

network structure, both inside and outside the pores. This finding demonstrated that the scaffold was safe to use and optimal for the development and attachment of MG63 osteoblasts. Whereas Luo et al. [134] conducted a study on the feasibility of utilizing M058K cells on PLA and PLA incorporated with nanocomposite. The Alamar blue assay is employed to measure cellular activity, which is subsequently documented on days 3, 6, and 12. The experimental findings indicate that the nanocomposite-filled PLA exhibits a greater fluorescence intensity when compared to pure PLA. The living cells were stained in green, while the dead cells were stained in red. The incorporation of nanocomposite within the scaffold promotes cell adhesion and growth due to its low cytotoxicity and favourable cytocompatibility.

Additionally, Zhou et al. [135] conducted a study in which human adipose stem cells (hASCs) were cultured on a scaffold made of PLA nanofibers and MPLA/nanofibers, with a constant cellulose nanocrystals content of five wt %, for a duration of 7 days. The findings pertain to cell cultures. According to the report, a higher number of live cells (stained green) were observed in the MPLA/ cellulose nanocrystals-5 group compared to the PLA/cellulose nanocrystals group. A minimal number of deceased cells were observed on the MPLA nanofibrous scaffolds. The present discovery suggests that the cytotoxicity of the composites was diminished during the cultivation of human adipose-derived stem cells (hASCs). In addition, the study employed an Alamar blue proliferation viability assay. The experimental analysis revealed that the inclusion of NCCs in the scaffold did not have any cytotoxic effects on hASCs over a period of 7 days.

The characterization of surface properties of nanomaterials implemented in nanomedicines poses a significant challenge. The zeta potential refers to the electrical potential at the interface between a nanomaterial's surface and a layer of solvent molecules and ions that are strongly associated with the nanomaterial surface, as well as the solvent molecules and ions in the surrounding bulk. The determination of the zeta potential is significantly influenced by the composition and concentration of salts present in the dispersing medium utilized for nanomaterial measurements. This feature encompasses the entirety of the system, including nanomaterials and other components [136]. Nevertheless, the utilizations of zeta potential can be applied in the evaluation of quality control if there is sufficient documentation of the sample preparation for measurement, including detailed information regarding the composition of the dispersing media. This method allows for the prediction of the in vivo behavior and subsequent biological performances of nanomedicines [137]. The evaluation of zeta potential is a crucial criterion mandated by health regulatory bodies for the classification of nanomedicine [138].

Due to their diminutive size, nanomaterials possess a significantly elevated surface-to-volume ratio, resulting in a comparatively augmented surface area per unit mass in comparison to particles of micrometre dimensions. The measurement and quantification of the specific surface area of porous and non-porous materials, as well as nanoparticle materials, can be effectively achieved through the application of the Brunauer-Emmett-Teller (BET) analysis method [139]. This involves the utilization of physical gas adsorption, specifically nitrogen gas, at a temperature of 77 K during the analysis process. The specific surface area obtained through this analysis is commonly known as the BET surface area, and it is typically expressed in units of square meters per gramme (m^2/g). In order to account for potential variations in density values among nanomaterials and nanoparticles, the true density is incorporated into the calculation of the specific surface area. This results in the derivation of the Volume Specific Surface Area (VSSA), which is subsequently expressed in units of square meters per cubic metre (m^2/m^3) [140, 141].

13 BONE TISSUE ENGINEERING ADVANCEMENT

The employment of bone bioreactor technology, which is supposed to offer the ideal environment for the co-culture of seed cells, growth factors, and scaffolds, was employed in the preparation of tissue-engineered bone that was then isolated and purified [142]. In recent years, there has also been a significant amount of focus placed on the creation of a new generation of overlay manufacturing technologies. Utilizing 3D printing technology, it is possible to create porous scaffolds that are bone-conducting and biocompatible. These scaffolds also have excellent mechanical qualities [143].

Qiao et al. (2020) [144] constructed a hydrogel scaffold that may be used for a wide variety of purposes by combining sodium tetraborate (Na2B4O7), polyvinyl alcohol (PVA), silver nanoparticles (NPs), and tetraethyl orthosilicate (TEOS). These 3D composite scaffolds with adequate pore size and matching bone porosity showed good antibacterial and biological activity. They demonstrated this activity by encouraging BMSC proliferation and osteogenic differentiation while suppressing bacteria. The implant has demonstrated significant potential as an antibacterial agent through trials conducted in vivo, in addition to encouraging bone healing. Using a technology for additive manufacturing that is based on extrusion, Sallstrom et al. (2020) [145] were able to build a composite material with exact geometrical control and the ability to modify the material's mechanical properties. In this zwitterionic sulfobetaine hydrogel system, the cells proliferated well on the hydrogel surface because the printed structures supported their own weight without becoming rigid during the printing process. This allowed the system to function properly.

Using unique combinatorial 3D printing and freeze-drying technologies on gelatin (Gel), nano-hydroxyapatite (n-HA), and poly(lactide-co-glycolide, Kankala et al. (2018) [146] were able to regenerate bone with the assistance of a 3D porous scaffold that they had developed (PLGA). The biocompatibility, biodegradability, and mechanical properties of the generated Gel/n-HA/PLGA scaffolds were all beneficial to the enhancement of cell adhesion, proliferation, and differentiation regarding the expression of certain biomarkers during the ossification process. Scaffolds with controlled structure, porosity, and quality can be printed using a combination of 3D imaging and CT data processing. The scaffolds are then modified to precisely fit the patient's specific areas of bone loss.

Nanocomposites have benefited greatly from the recent advances in nanomaterial preparation processes such as sol-gel synthesis, hydrothermal synthesis, molecular self-assembly, freeze-drying, and phase separation [147]. Using tissue-engineered bone as an example, genetic engineering has revealed unimaginable benefits. Bone regeneration and repair can be aided by genetic engineering's ability to regulate transgene expression and lengthen the time for producing proteins [148]. In the years afterwards, however, medications based on modifying gene expression have emerged as a promising new approach to orthopedic disease treatment.

By targeting genes that inhibit bone formation, RNA interference-based therapy has been effective in the treatment of osteoporosis, for instance. Mayo Clinic researchers conceptualized this method. Mesoporous silica nanoparticles (MSNs@PEI) with polyethyleneimine functional groups were synthesized by Mora-Raimundo et al. (2019) [149]. Mesoporous MSNs@PEI nanoparticles with SOST siRNA and human parathyroid hormone-related peptide may stimulate osteoblast development and differentiation, which could be useful in the treatment of osteoporosis [149].

The key determining factor for implant treatment in patients with osteoporosis and others is the deteriorating ability of the bone implant combination. Specifically,

and efficiently transporting the right cells and genes to the right places is the key to fixing these issues. Through the application of bio-based polymer materials and LBL self-assembly technology, Xing et al. (2020) assembled Au NPs modified by siRNA-CTSK onto the surface of a titanium implant. Cathepsin K regulation was improved by the release of siRNA, which also increased bone-implant interfacial contact. Since siRNA-CTSK could be secreted and taken up by neighboring macrophages, this provided evidence of a synergistic impact that promoted bone regeneration and vessel repair when used as part of osteointegration therapy in vitro and in vivo. Inhibiting osteoclastic differentiation, altering the cell secretion properties, and promoting bone and vascular tissue regeneration around the titanium implant are all possible outcomes of this coating [150]. Although safety was still a major challenge of gene engineering for bone regeneration, recent studies had focused on developing highly efficient delivery vectors and transfection methods. In light of the promising results obtained through gene engineering in BTE, it is hoped that novel genes or regulatory RNAs will be discovered and used to control the expression of proteins and transgenes via gene transfer and to regulate the host immune system in order to prevent adverse effects on bone healing. Future research efforts by scientists and clinicians in related domains will continue to concentrate on questions like the security of clinical applications and the efficacy of evidence-based medicine, among other related challenges. So, it is expected that progress in related technologies would propel tissue-engineered bone to new heights [151].

14 CONCLUSION AND OUTLOOK

This article provides a synopsis of the research literature on the topic, with a particular emphasis on the several different biomaterials capable of being employed as templates for bone tissue creation. It has been shown that different kinds of biomaterials serve different purposes and have different properties. Numerous researches have addressed and analyzed the addition of various additives to the base materials with the aim of improving their attributes. The goal was to enhance the functionality of the raw materials.

In order to ensure a complete recuperation, it is imperative that the constituent material of the implant possesses specific attributes. The latest research suggests that sanctified metal alloys continue to be widely employed. One of the primary benefits associated with metals is their inherent strength and ability to withstand fatigue degradation. These objects possess shape memory and can be readily sterilized prior to utilization. One primary drawback is the susceptibility of metal to corrosion as a result of chemical reactions with enzymes and acids present in the body. Furthermore, it has the potential to induce metal ion toxicity within the human body.

Biocompatible polymers, which are a type of material, offer a range of capabilities in replicating anatomical structures, undergoing gradual reabsorption, and eliciting targeted responses from the biological environment. These polymers belong to the most recent generation of materials. Biocompatible polymers are extensively employed due to their ability to be tailored for specific applications through manufacturing processes. The manufacturing and modification processes of these items are relatively straightforward. The biodegradability of these items presents both advantages and disadvantages. As a result of their extensive interaction with the human body, these substances have the potential to leach, resulting in degradation and deterioration. Additionally, they possess the ability to assimilate crucial nutrients and water from the bloodstream.

Ceramic materials are commonly considered suitable for bone replacement bearings due to their biocompatibility, high hardness, and high resistance to wear. The primary objective of incorporating ceramics in bone repair bearings is to mitigate clinical wear, thereby minimizing the potential for debris-induced osteolysis. The primary drawback lies in the challenges associated with the manufacturing process. Additionally, they have the potential to reduce bone ingrowth. Occasionally, implants may experience a gradual loosening phenomenon and subsequently become displaced.

A composite material refers to the amalgamation of multiple materials, each possessing unique characteristics in terms of shape and composition. Composites are fabricated by combining two or more elements, resulting in a final product that exhibits a blend of properties derived from all the constituent materials. The primary advantage provided by composites is their combination of strength and lightness. In addition to possessing a low density, these materials also exhibit resistance to corrosion. One limitation associated with the manufacturing of composites is the comparatively elevated expenditure involved. Furthermore, modifying their overall shape presents a challenging task.

Given the increasing need for scaffolds in bone tissue engineering, it is likely that many more novel approaches and technological advancements will be required in this field to improve the performance of scaffolds. It will need still more study before tissue engineering can be used to successfully mend other human organs. A major step forward will be made in the creation of novel methodologies and biomaterials, especially at nanoscale, with research into stem cells and similar subjects for the sake of bone regeneration and restoration. Improving the performance of biomaterials is necessary if they are to have a beneficial influence on host tissues. Significant improvements in bone regeneration procedures are possible through the use of novel biomaterials and approaches, in particular the in-depth integration of nanotechnology, stem cell science, and other domains. As a result of these advancements, we will make tremendous progress.

Table 1 presents advantages and disadvantage of different biomaterials used for bone tissue engineering scaffolds.

Table 1. Summary of different biomaterials used for bone tissue engineering scaffolds

Biomaterials for Bone Tissue Engineering	Benefits	Drawbacks	References
Metal	High mechanical performance	Potential for Toxicities; Corrosion; Non-biodegradable	26–34
Bioceramic	Biocompatibility; Bioactivity	Low mechanical properties; Uncertainty in degradation rates	35–41
Natural polymeric	Biocompatibility; Bioactivity; Biocompatibility;	Low mechanical properties; Intense rates of biodegradation; Large variation from batch to batch	42–61
Synthetic polymeric	Biocompatibility; Bioactivity Versatility	Exhibit tissue adverse reactions; Low mechanical properties; Uncertainty in degradation rates	62–73
Composite	Biocompatibility; Bioactivity Versatility; good mechanical performance	Potential for Toxicities; Uncertainty in degradation rates	74–79

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