Review

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Posted Date: 18 October 2023

doi: 10.20944/preprints202310.1082.v1

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Application of Multiscale Hierarchical Porous Structure Design in Bone Tissue Engineering Scaffolds: A Review

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Abstract: In recent decades, porous scaffolds for bone tissue engineering (BTE) have gained significant attention. Considerable research efforts have been dedicated to investigating the impact of scaffold pore size on a diverse array of biological processes, including cell-scaffold interactions, substance transportation, and vascularization. However, the multi-scale hierarchical porosity, a key structural characteristic of natural bone, has rarely been replicated in BTE scaffolds due to the challenges of controlling the scaffold structure across multiple length scales. With the advancement of manufacturing technology, there has been increasing research on biomimetic multi-scale hierarchical materials, which are also gaining favor in the field of BTE. Therefore, there is an urgent need to review multi-scale hierarchical porous BTE scaffolds. This paper aims to review the role and fabrication methods of multi-scale porous BTE scaffolds at different length scales, the design and manufacturing of new BTE scaffolds for bone regeneration.

Keywords: multi-scale; hierarchical structure; porous scaffold; bone tissue engineering; bone regeneration

1. Introduction

Bone defects are a serious public health concern associated with trauma, infections, tumors, metabolic diseases and aging. These conditions can eventually lead to incomplete bone healing or non-union fractures [1]. Although bones possess inherent regenerative capacity, allowing for the repair of small damages, the spontaneous self-healing of large bone defects that exceed critical size thresholds is challenging without clinical intervention [2]. The current widely accepted standard approach for treating large bone defects involves the utilization of bioinert metal devices allografts or and autografts to fill the defects. These techniques are utilized in millions of surgical procedures annually [3-5]. However, the utilization of metal devices often necessitates supplementary surgical interventions for their retrieval post-implantation, and the utilization of allografts poses the potential risk of disease transmission. Furthermore, the limited availability of autografts introduces further complexities regarding the healing process at the donor site. The risks associated with skeletal grafts are particularly heightened in elderly individuals [6]. Given the aforementioned challenges, there is a growing demand for alternatives to traditional skeletal grafts in order to facilitate bone regeneration within the scope of BTE. Significant progress has been made in the field of BTE, particularly in the development of materials that can facilitate the process of bone regeneration at defective sites without the aforementioned risks [7]. Despite notable advancements in the area of BTE regarding material composition, diverse shapes, pore structures, nano-surfaces, and the incorporation of bioactive factors or cell loading to enhance vascularization and ossification [8-13], the clinical translation and commercialization strategies in the field have predominantly relied on pure materials-based approaches, without the inclusion of bioactive ingredients. This is primarily due to cost and

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regulatory complexities associated with biomaterial systems containing bioactive ingredients [14,15]. Indeed, one promising approach in BTE is the utilization of biomimetic materials that replicate the natural hierarchical structure found in organisms [16,17]. In fact, natural bone tissue is characterized by a precisely organized extracellular matrix (ECM) at the micro-scale, and there is a growing need to incorporate multi-scale structures into BTE scaffolds [18-20].

Through billions of years of evolution, natural materials have achieved an optimal combination of constituents, arrangement, and function [21]. It can be argued that early human history is divided based on advancements in structural materials. Although the categories of materials have significantly expanded with the development of chemistry, the production of structural materials still occupies a crucial position. The progress in developing high-performance artificial materials by imitating the structural properties of natural materials has been an ongoing pursuit in materials science [22]. Biological materials, despite their limitations in terms of raw resources, possess remarkable multifunctional performance, including strength, toughness, fatigue resistance, wear resistance due to their exquisitely hierarchical structural design [23-26]. An excellent example of a material with a typical coaxial hierarchical arrangement structure is banboo, which demonstrates excellent mechanical properties and mass transfer capabilities [27]. These properties can be primarily attributed to the embedding of primary fibers within the matrix lignin in different layers of the lignified cell walls, resulting in the formation of microfibrils. These microfibrils are further organized into compact components through physical and chemical bonding, creating structures that span diverse scales. To optimizes the mechanical efficiency for bending, bamboo additionally refines the distribution of fiber density based on the coaxial hierarchical structure, presenting a radial gradient distribution from the inner to outer layers [28]. Mammalian bones is an organ possessing intricate multi-scale hierarchical structures comprised of collagen fibers and hydroxyapatite crystals [29-31]. These collagen fibers are formed through the self-assembly of a few sub-nanometer collagen molecules, resulting in the generation of collagen fiber bundles with a diameter of approximately 3-7 μm and oriented in a specific direction. The fiber bundles are organized in a concentric manner around a Haversian canal, typically ranging in thickness from 150 to 300 μm (Figure 1). Cortical bone is characterized by densely arranged concentric layers, which form a compact outer surface that provides structural support. In contrast, trabecular bone is composed of a more porous network with interconnected spaces that accommodate blood vessels and bone marrow (Figure 2). Due to its well-organized hierarchical structure, natural bone exhibits the best mechanical properties, including rigidity and fracture resistance [32]. In addition, the nano-surface of bone also determines the adhesion, growth and orientation of cells [33]. Consequently, optimal bone tissue engineering (BTE) scaffolds should possess adaptable multi-scale structures that can conform to the irregular shape and internal porous structure of the damaged site. This allows for efficient cell ingrowth, vascularization, nerve invasion, and metabolite transport [34-37]. In addition, nano-surface, which can influence cellular behaviors, should also be considered in BTE scaffolds design [38]. As a result, the biomimetic multi-scale hierarchical structures of BTE scaffolds have attracted much attention in clinical and biomaterials fields [39].

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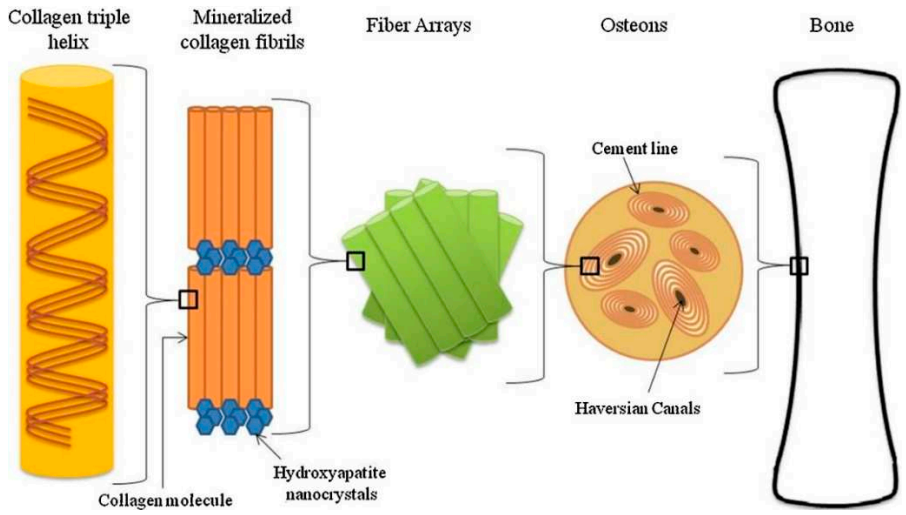

Figure 1. Hierarchical organization of bone at multiple scales. [40].

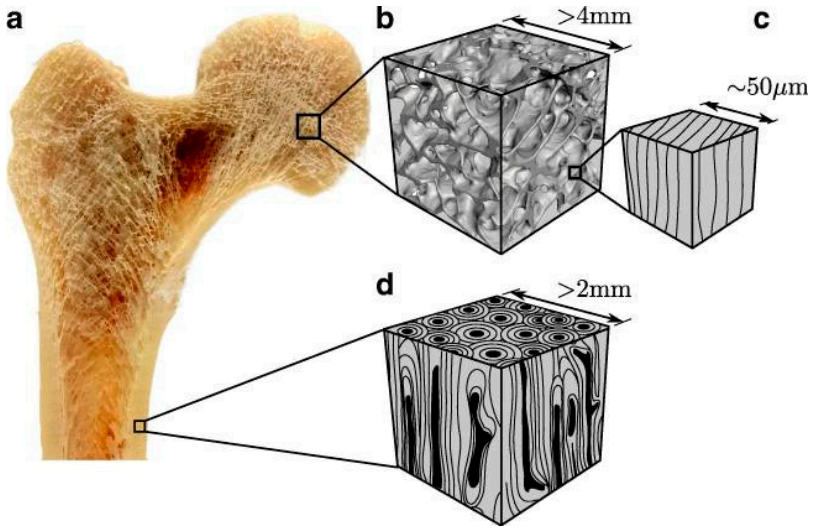

Figure 2. The multi-scale structure of human bone. a, Cross section of the human proximal femur. b, Representative volume element (RVE) of the trabecular network and c, RVE of single trabeculae. d, Representative volume element of cortical bone. [41].

During the current period, the significance of developing structural materials has been widely recognized in the field of biomedicine [42-44]. When reviewing the history of BTE scaffolds, it is evident that every progress in this field is built upon the discovery, combination, and utilization of various new components [45-47]. Recently, there has been a growing interest in the structural design of scaffolds [48-51]. However, there is still a relatively limited amount of research in the field of multi-scale structural design over an extended period. By reviewing current studies, it is evident that there are abundant design strategies for existing BTE scaffolds with various compositions [52], heterogeneous shapes [53], porosity [54], nano-surface [55-57], and incorporating bioactive ingredients [58-61]. The design strategies aim to optimize a suitable cellular microenvironment to achieve more efficient vascularization and ossification processes [62-67], as shown in Figure 3. Extensive research has demonstrate that the porosity and mechanical properties of scaffolds significantly influence their biomedical functionality in various bone defect treatments [68]. Hierarchical porous scaffolds, characterized by open and interconnected networks, play a vital role in promoting cell penetration, facilitating nutrient transport, enhancing angiogenesis, supporting extracellular matrix (ECM) deposition, and facilitating the formation of new bone tissue [69,70]. In addition, cellular behavior is directly influenced by the nano-surface and the stiffness or

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viscoelasticity of the matrix, because the ECM provides clues to regulate the specific integrin-ligand interactions between cells and surrounding materials [71]. Although there is no precise evidence of the role of multi-scale hierarchical porous scaffold, the general consensus is that nano-pores affect cellular response, while micro-pores facilitate the transport of nutrients, metabolites, and the formation of blood vessel [72]. The macroscopic shape of local heterogeneity provides mechanical performance and support [73]. However, traditional BTE scaffolds pay little attention to multi-scale hierarchical structures, which may be attribute to the complexity of multi-scale structures far exceeding the capabilities traditional design and manufacturing techniques. As a result, there are significant challenges in applying multi-scale hierarchical materials in the field of BTE [74,75]. The development of biomimetic technology has opened new avenues for inspiration by imitating the intricate structures found in natural tissues [76]. There is a growing recognition that designing multi-scale hierarchical structures in BTE scaffolds that mimic natural tissue may provide remedies for problems ranging from promoting vascularization to controlling cellular behavior [19,77]. Earlier studies have concentrated on designing scaffolds with interconnected pores at a micro-scale. However, with the emerging of new nanomaterials and processing technology, there has been a shift in focus towards understanding the role of scaffold nanostructures in tissue regeneration [78]. This is also due to the realization that the cellular environment in natural tissues is nanostructured. For an ideal scaffold, firstly, it should possess a porous and interconnected structure that allows for the penetration of cells and nutrients [79]. Secondly, it should exhibit the appropriate surface structure and chemical cues that facilitate cell adhesion and proliferation [8]. Moreover, it is essential for the scaffold to exhibit mechanical properties comparable to those of native tissue, ensuring optimal compatibility [80]. Additionally, the scaffold should be biodegradable, allowing it to undergo degradation and eventually transform into a soluble and non-toxic substance [81]. Indeed, the ultimate success of scaffolds in the field of BTE relies on the intricate interplay between multiple biological, mechanical, and physicochemical properties [3,82]. Based on the extensive research on the effects of nano-scale to macro-scale structures on cell biological behavior and tissue repair and regeneration, it can speculate that the next breakthrough in BTE scaffolds is likely to be the realization of the controllability of multi-scale structure assembly and the creation of scaffolds with excellent properties.

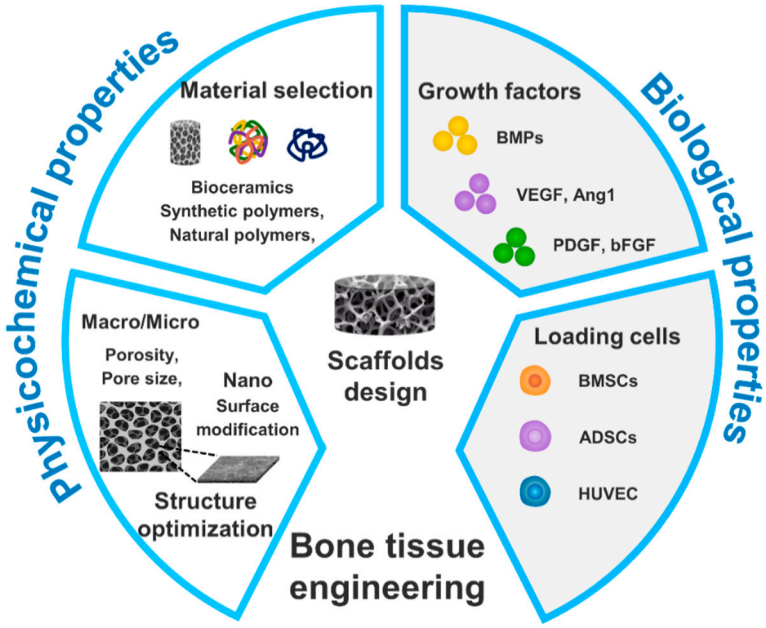

Figure 3. Overview of the strategies to design BTE scaffolds.

Although the recognition of the significance of multi-scale structure design and regulation by researchers, and the availability of numerous biological structural materials as references, the preparation of artificial biomimetic multi-scale structure scaffolds for BTE remains a challenging task.

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So far, there have been some preliminary findings regarding multi-scale scaffolds with relatively simple structure, but it is difficult to generate ordered multi-scale pores in different directions with current technology. In addition, it is difficult to ensure interconnections between multi-scale pores. Moreover, the complex design of both interior and exterior features of BTE scaffolds presents a significant challenge to the traditional techniques of isolation and combination. Based on the preceding discussion, it can be inferred that there are both boundless opportunities and significant challenges in the realm of biomimetic multi-scale structural BTE scaffolds, which hold immense potential in advancing human health. Therefore, it is both pertinent and appropriate to comprehensively outline the diverse functions exhibited by multi-scale structures and highlight the latest advancements in multi-scale construction methods, as well as their applications in the field of biomimetic BTE scaffolds. The present review predominately elucidate the significance of hierarchical porous structures in bone regeneration, as well as the current preparation methods utilized for the preparation of multi-scale porous scaffolds, and briefly addresses some of the challenges encountered in the current preparation techniiques and presents future directions of the current preparation of multi-scale porous BTE scaffolds.

2. Multi-scale hierarchical scaffolds and functions

To successfully prepare and develop biomimetic BTE scaffolds with multi-scale hierarchical structures, it is essential to explore and draw insights from different length scales. Drawing inspiration from nature can provide innovative approaches to overcome the technological bottlenecks in BTE scaffold manufacturing. Natural bone exhibits a complex and sophisticated hierarchical structure, characterized by the presence of interconnected hard, orderly phases of inorganic calcium phosphate and soft, orderly phases of organic collagen fibers. The multi-scale structural model of bone represents an inherent functional characteristic that has evolved to meet the survival demands of organisms. Therefore, the multi-scale precise control of scaffold structure is of decisive significance for the development of biomimetic artificial scaffold for BTE [19]. Numerous studies have demonstrated that the composition, morphology and surface topography of scaffolds can provide pivotal cues to direct cellular responses and promote tissue regeneration in BTE. Among these factors, the presence of a micro-/nano-scale hierarchical porous structure is particularly crucial as it contributes to shaping the topological characteristics necessary for the development of functional interfaces between cells and materials [83]. By modulating pore structure of scaffold, it is possible to mediate cellular responses in situ by inducing alignment of cytoskeletal in cells. Membrane receptors interact with multi-scale topography of the scaffolds to induce cytoskeletal deformation and assembly, thereby controlling cell adhesion, proliferation, cell morphology, and gene expression [84]. Additionally, the macro-scale shape of scaffolds plays a vital role by providing mechanical cues, support, and the spatial environment that influences tissue growth. As a result, researchers have developed effective methods to achieve bone grafts with controllable diameters at multiple length scales, ensuring the scaffold's macroscopic structure aligns with the desired tissue requirements (Fig 4). With the aim of replicating the intricate hierarchical structure observed in living systems, novel strategies have emerged for the fabrication of scaffolds featuring multi-scale porous networks [85]. These strategies endeavor to replicate the intricate complexity of the natural structure and provide an environment that closely resembles the microenvironment found in living tissues (Table 1). Currently, there are several consensus views that suggest pore size has different effects on the host response: nano-pores (size < 100 nm) increase surface area for cell adhesion, promoting cell-scaffold interactions; micro-pores (size: 100 nm - 100 μm) enhance permeability and promote cell migration within the scaffold, while macro-pores (size > 100 μm) provide sufficient ample room for vascularization and inward tissue growth, facilitating gas diffusion, substance transport [86-88]. The impact of hierarchical porous scaffolds on bone regeneration has been visually depicted in the schematic diagram presented in Figure 5. At the macro-scale, natural bone is characterized by the presence of cortical bone and an inner cancellous bone. Many BTE scaffolds strive to reconstruct this intricate hierarchical structure found in native bone [17]. Cortical bone is composed of high-density components that provide the primary mechanical characteristics of bone tissue. Cortical bone-

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mimicking materials provide the main mechanical support for the treatment of mechanical instability defects. Previous studies have designed more complex BTE scaffolds, such as Harversian and Volkmann canals, that allow blood vessels and nerves to grow inward and facilitate nutrient transport [80]. In fact, apart from promoting effective gas diffusion and nutrient supply, rapid vascular infiltration is required to maintain the sustaining inward growth of tissues within the body. Simulated porous cancellous bone is another micro-scale structure commonly employed in BTE scaffolds. Porous structures offer a controllable degradation rate, which is more conducive to new bone formation and remodeling compared to non-porous structures [89]. In addition, micro-pores within the scaffolds provide increased surface area, favorable protein adhesion and improved cell attachment compared to macro-pores [90]. Previous research indicates that smaller pore size (e.g., 95μm) are mare suitable for initial osteoblast adhesion compared to larger pore size (>150μm) [91]. The ability of scaffolds to promote bone regeneration is associated with the capture and adsorption of specific types of proteins surrounding the biomaterials. Cell recognition of certain peptide domains within these proteins leads to initial attachment and subsequent control of cellular fate. Consequently, the presence of micro-pores in biomaterials becomes as important as the competitive "Vroman effect," governing which serum proteins bind to the implanted material and thereby determining cell adhesion [92]. Increasing the number of micro-pores enhances interaction with serum proteins, which may prove to be an effective strategy for promoting bone induction and conduction properties of scaffolds [93]. In addition, micro-pores also induce capillary forces that anchor cells to the surface and drive their migration within the 3D structure [94,95]. Various techniques, including salt leaching, gas foaming, freeze-drying, electrospinning, and additive manufacturing, have been employed to create micro-scale porous structures mimicking the natural bone structure[74,96,97]. Additive manufacturing, though popular in BTE scaffold fabrication, is limited to the laboratory level due to resolution and manufacturing cost constraints. Additionally, nano-scale multiple forms hydroxyapatite (HA) deposits exist on the surface of natural bone, influencing cell fate and mineral deposition. Therefore, nano-surface modification emerges as a appropriate strategy to enhance the osteogenic potential of BTE scaffolds by closely replicating the intricate spatial structure of the ECM in natural bone [83]. Several techniques, such as sandblasting, acid etching, and micro-arc oxidation, have been employed to modify the surface morphology of BTE scaffolds at the nanoscale. Nano-surface modification can facilitate osteogenic differentiation of stem cells [98], regulate macrophage-mediated inflammatory response, and promote bone integration [99].

Table 1. The describes of the pore size distribution, processes, and characteristics previously used to produce multi-scale BTE scaffolds.

Materials	Multiscale pores	Process	Characteristic	Ref
Geltin, alginate, PVA	~800μm, ~40μm	3-D printing, electrospinning, vacuum freeze-drying	Enhance initial cells attachment; enhence the mechanical interlocking between the scaffold and the tissue; promote the substances transportation and cell infiltration; facilitate vascularization.	[100]
Silk fibroin	100-390μm, 1-30μm,	Paraffin sphere leaching, phase separation	Provide certain mechanical property, promote cell attachment and proliferation.	[101]
Poly (butylene succinate), cellulose nanocrystals	~68.9 μm, ~11 μm	Supercritical carbon dioxide foaming process	Strengthen, increase hydrophilicity, and optimize the degradation rate.	[102]

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Poly (ε-caprolactone) (PCL), HA, β-TCP	~350 μm, ~105 μm	Freeze casting, sacrificial templating	Accelerate osteogenic differentiations, provide biomechanical support.	[103]
PCL, mesoporous bioactive glass (MBG)	5.6nm, <40μm	3D printing, porogen leaching	The early mineralization in the MBG led to an increased surface hardness, while the presence of micro-pores enhanced cell activity and stimulated the osteogenic differentiation.	[104]
PCL	500μm, 100μm	High-resolution EHD 3D printing	Promote cell orientation; exceptional permeability for cell infiltration, substances transportation.	[105]
PCL	>100μm, <30μm	3D printing based a multi-scale direct writing system	Provide sufficient strength for mechanical support; offer a cell-appropriate microenvironment.	[106]
PCL	300μm, 75μm, 50μm	3D printing integrated with FDM, SE, and MEW system	Exhibited optimal biocompatibility, facilitating cell adhesion, and demonstrating the potential to promote cell alignment.	[107]
PCL	~315μm, ~325μm, ~ 8μm	Emulsion templating, 3D printing	Enhance bioactivity, promote differentiation of precursors into mature bone cells, and induce angiogenesis.	[108]
PCL	300μm, ~20.2 μm	3D printing, electrospinning	Possess osteoinductive properties, stimulating the expression of osteogenic markers in MC-3T3 osteoblasts.	[109]
PCL	~400μm, ~10μm	3D plotting systems, non-solvent-induced phase separation	Demonstrated mechanical characteristics similar to cancellous bone, enhance cell attachment, proliferation, and differentiation.	[110]

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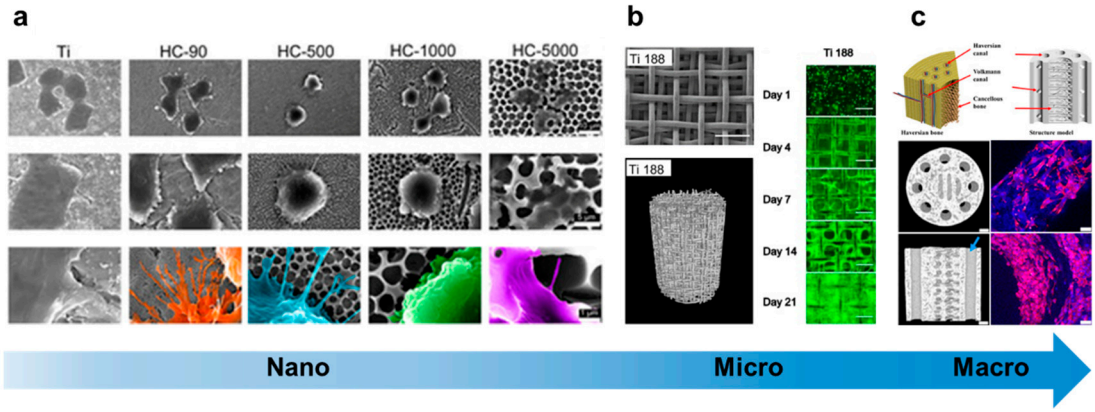

Figure 4. The bone implants with structure optimization from nano-scale to macro-scale. a, Cell morphologies of macrophages on different nano-surface samples [111]. b, The characterization of porous titanium samples and adhering cells fluorescence images [112]. c, 3D printing Haversian bone–mimicking scaffolds and adhering cells fluorescence images. [80].

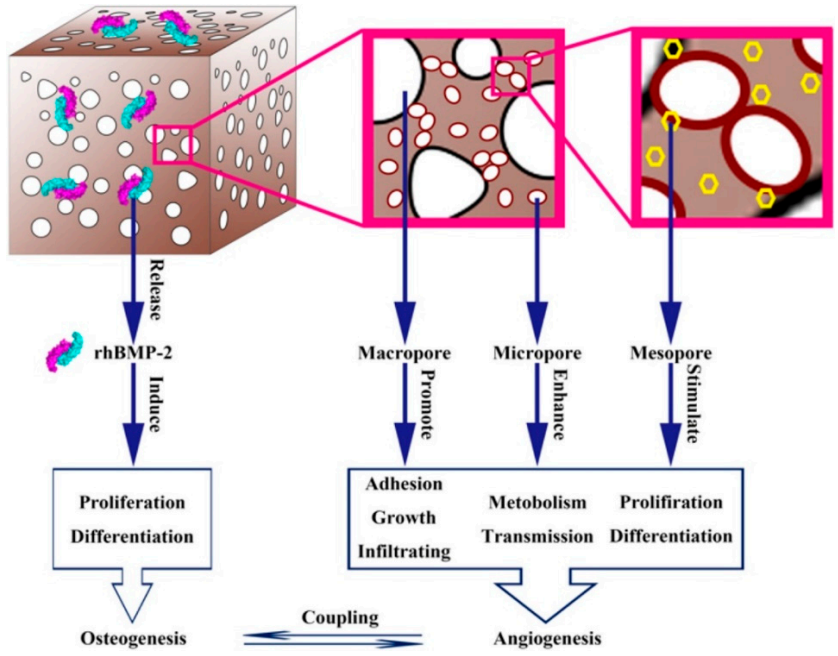

Figure 5. Schematic depiction illustrating the impact of a hierarchical scaffold on bone regeneration. [113].

The reason for the ongoing controversy surrounding the optimal pore size for BTE scaffolds is that different pore sizes can exhibit different characteristics and regulate bone formation from different perspectives. Therefore, researchers can only provide a suitable range of sizes rather than a specific optimal pore size [88]. Remarkably, multi-scale hierarchical porous scaffolds that include both macro- and micro-pores exhibit superior bone conductivity compared to scaffolds with only macro-pores. This is because the micro-pores in the scaffolds can retain a higher concentration of growth factors [114]. However, the influence of multi-scale hierarchical structures on regulating cell behavior has often been overlooked, mainly due to the technical challenges associated with designing and fabricating such scaffolds. Previous studies have compared cell inoculation differences between scaffolds with single scale (unimodal) and multiscale (bimodal) pore size distributions, highlighting the importance of spatial cells distribution within the scaffold [115]. Additionally, scaffolds with multi-scale pores (non-uniform pore distributions), such as pore gradients, have shown higher cell inoculation efficiency compared to single-scale pores (uniformly sized pores), attributed to increased

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encounters between cells and the scaffold [116]. Furthermore, customization of bulk mesoporous structures with size-matched bone morphogenetic protein-2 (BMP-2) embedded in meso-pores enables continuous release and preservation of biological activity, demonstrating their clinical potential in inducing local bone development (Figure 6) [117]. To fully mimic the complex and hierarchical structure of natural bone, it is essential to integrate multiple length-scale structures into scaffolds. However, the intricate interactions between different cell types and multi-scale structures in scaffolds are yet to be fully understood [118]. Additionally, the production of scaffolds with multi-scale hierarchical porous structures and high pore interconnectivity remains a challenge, requiring further advancements in existing technologies [101].

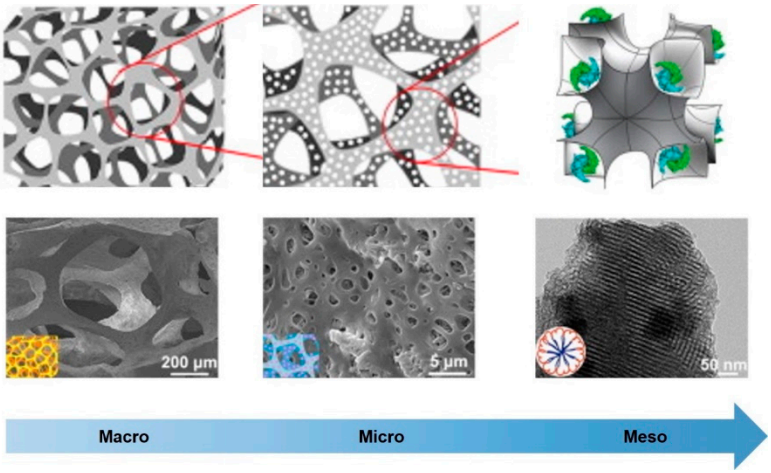

Figure 6. Mesoporous bioactive glass scaffolds presenting a multi-scale porous structure. [117].

3. The structures of living organisms can serve as biomimetic hierarchical structure design models for BTE scaffolds.

Despite the recognition of the crucial role played by cross-scale porosity in influencing the physicochemical and biological performance of BTE scaffolds, the design and fabrication of such structures spanning multi-scale pose immense challenges that surpass traditional design and manufacturing techniques [34]. Consequently, there is a growing interest in drawing inspiration from natural structural materials. In recent years, structural design strategies that utilize natural structural materials as templates have gained considerable attention, with the objective of achieving structural functionalization and diversification [119,120]. The consolidation of various biomimetic multi-scale structural templates provides valuable insights for the cross-scale structural design of BTE scaffolds [76,121]. Prominent models extensively studied in the field of biomimetic BTE scaffolds include bone, bamboo, wood and other similar materials. These materials, characterized by their multi-components, different structures and excellent mechanical properties, provide abundant inspiration for the design of material structures in biomimetic BTE scaffolds. The subsequent sections will introduce the design principles behind several frequently encountered biomimetic structural BTE scaffolds.

3.1. Natural bone as a multi-scale structural model

Bone, with its unique hierarchical structure, remarkable porosity, and exceptional mechanical properties, serves as a valuable model and source of inspiration for scaffold design in BTE. Bone is a complex composite material comprised primarily of collagen proteins and hydroxyapatite. It exhibits a meticulously organized hierarchical structure, spanning from the nano-scale to the macro-scale. In the field of BTE, there is significant importance placed on the development of biomimetic materials that can effectively replace autografts. These biomimetic materials aim to closely mimic the components, microstructure, and mechanical properties of natural bone. An excellent example of biomimetic scaffold development is the work of Wu's group, who successfully fabricated bone-like scaffolds with hierarchical Haversian canal and Volkmann canal using 3D printing (Figure 7) [80]. By

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precisely controlling the number, diameter, and arrangement of these channels, the macroscopic shape and performance of the scaffolds could be effectively regulated, resulting in controlled compressive strength and porosity. Furthermore, these micro-/macro-scale canals promoted vascularization and facilated new bone regeneration in vivo. This advanced scaffold, inspired by 3D hierarchical structure of natural bone, has been extensively developed and applied in bone regeneration, making it a highly promising strategy. In addition to considering the micro-/macro-scale structure and mechanical properties, mineralization is also a highly regarded biomimetic approach in BTE scaffolds [122]. It plays a crucial role as it is not only resembles an essential component of natural bone but also represents an effective strategy for enhancing both biological activity and mechanical performance. Sheng's group developed a biomimetic multi-scale hierarchical scaffold for bone regeneration through 3D printing, which featured dense outer structure and sponge-like inner structure at macro-scale. The sponge-like porous matrix of the biomimetic multi-scale hierarchical scaffold comprised of hydroxyapatite mineralized collagen protein and Poly(ε-caprolactone) (PCL) composites, with patterned single crystal silicon films embedded internally (Figure 8) [123]. The presence of this biomimetic structure has been found to facilitate cell adhesion, growth, and differentiation in both in vitro experiments and in vivo studies. Additionally, the light-simulated response from the silicon microstructures, when combined with the biomimetic structure, has shown a significant enhancement in bone regeneration. By closely simulating the micro/nano-scale structure of natural bone, this scaffold effectively creates a favorable microenvironment for tissue regeneration. While 3D printing is an effective approach for manufacturing complex biomimetic structure, challenges such as time consumption and cost remain significant obstacles for its clinical application.

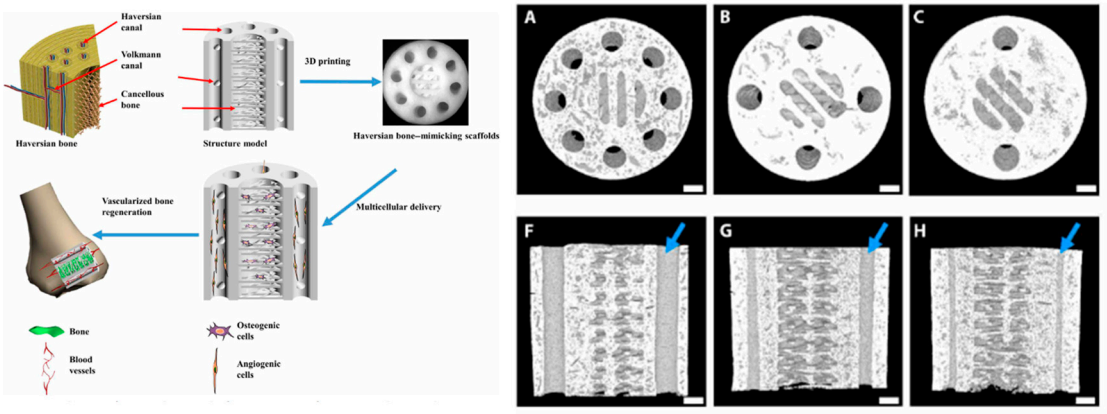

Figure 7. The schematic diagram and the characterization of 3D printing Haversian bone–mimicking scaffolds integrated with Haversian canals, Volkmann canals, and cancellous bone structure. **[80].**

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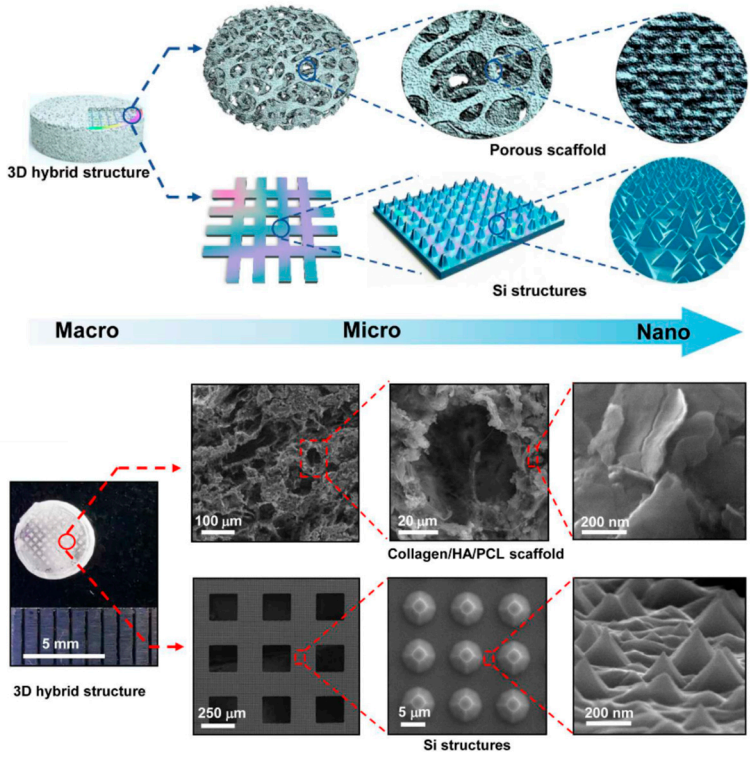

Figure 8. Biomimetic strategy to construct a 3D optoelectronic scaffold with Si nanostructures for bone regeneration. [123].

In addition, from a macroscopic perspective, natural bone tissue is composed of an outer cortical bone with higher density and an inner trabecular bone with lower density when viewed. The high-density cortical bone structure is capable of withstanding greater stress and transmitting it to the wider trabecular bone, while the lower-density trabecular bone further helps in distributing the stress. Drawing inspiration from this, Xie's team collaborated with Tang's team to leverage photocurable 3D printing in the fabrication of biomimetic bone scaffolds that replicate the microstructures found in natural jawbones [53]. This innovative approach offers an ideal solution for regenerating and repairing jawbone defects with intricate chewing functions, thereby opening up new avenues for function-oriented BTE strategies.

Nonetheless, it is important to note that, no BTE scaffold has achieved complete replication of the multi-scale structure of bone. In most studies, a combination of several factors such as chemical ingredient and nano/microstructure and mechanical properties are taken into consideration during the design of biomimetic materials. These efforts typically result in the production of macro-scale blocks or films that aim ro mimic certain aspects of natural bone structure and function [124]. When utilizing natural bone as a design model for the multi-scale structure of BTE scaffolds, there are three key scientific issues that must be carefully considered: i) Understanding the mechanisms of natural bone formation and its multi-scale structure. This involves studying the processes involved in the development of natural bone and gaining insight into its hierarchical structure; ii) Controlling the manufacturing processes involved in construction of multi-scale biomimetic BTE scaffolds. It is essential to develop precise fabrication techniques to reproduce the desired multi-dimensional structure of natural bone within the scaffolds; iii) Understanding the relationship between the structure and functionality of biomimetic BTE scaffolds produced. It is crucial to investigate how the structural features and properties of these scaffolds affect their functionality in promoting cell adhesion, growth, and differentiation. Addressing these three scientific questions is vital in ensuring the successful design and development of biomimetic BTE scaffolds that mimic multi-scale structure in natural bone and effectively support bone regeneration.

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3.2. Bamboo and wood as multi-scale structural models

Bamboo and wood are example of fiber-reinforced cellulose materials that exhibit highly interconnected porous structures and mechanical characteristics similar to bone [125]. These materials are consisted of fiber bundles surrounded by a thin-walled cell matrix. They contain various vessels, sieve tubes, or pores of different sizes, facilitating nutrient transport and reducing overall weight. Furthermore, both bamboo and wood are composed of co-axial layered arrangements, where cellulose molecules are embedded within the lignin matrix to form microfibers. These microfibers are densely packed through interchain/intramolecular hydrogen bonding and the numerous hydroxyl groups on the cellulose surface. This unique multi-scale structure provides bamboo and wood with a high resistance to bending. Bamboo, in particular, exhibits a refined distribution of fiber density gradients to further enhance its mechanical efficiency in resisting bending [126]. Due to their exceptional mass transfer capabilities and remarkable mechanical properties, both bamboo and wood are valuable models for imitation in the design and fabrication of BTE scaffolds. Therefore, Wu's group utilized natural bamboo as a biological template to maintain the inherent hierarchical porous structure. After removing lignin and residual organelles, they obtained a bamboo-based mineralized biological scaffold through biomimetic mineralization [127]. This scaffold exhibits high strength, excellent transport properties, and high biological activity, making it a promising candidate for BTE application. Similarly, natural wood represents a multi-scale hierarchical and anisotropic structure selected through natural evolution. Ren's group impregnated a biocompatible hydrogel into the micro-channels of wood and subsequently mineralized HA in situ, resulting in the fabrication of highly anisotropic, super-strong, and rigid composite bone scaffold. The scaffold holds great potential for bone repair [128]. These biological template methods provide an effective biomimetic strategy for producing hierarchical multi-scale structural material [129]. Such biomimetic structure offers significant advantages in the development of BTE scaffolds with strong mechanical characteristics, excellent substance transport capabilities, and high biological activity [130]. Therefore, biological structural materials can serve as valuable guidelines for researching, designing, and manufacturing future artificial BTE scaffolds.

In conclusion, the mechanical strength and mass transfer capabilities displayed by bamboo or wood, with their co-axial layered structure, have significant potential in BTE scaffolds. However, utilizing bamboo or wood as templates for fabricating BTE scaffolds poses certain challenges. These challenges include the presence of residual lignin, organelles, and demolding agents, as well as the immunogenicity and limited degradability of cellulose. These issues indicate the necessity of exploring alternative and more environmentally-friendly fabrication techniques to construct BTE scaffolds that accurately replicate the properties of bamboo or wood. This approach avoids solely relying on naturally occurring cellulose-based biomaterials that have been stripped of their lignin content.

3.3. Nacre as multi-scale structural models

Through millions of years of evolution, nacre has achieved a perfect combination of microstructural organization and functional adaptation to the environment [131]. Comprising an assemblage of brittle aragonite platelets interspersed with biopolymer layers, nacre exhibits extraordinary levels of strength and toughness, surpassing the mechanical properties of both biopolymers and minerals by several orders of magnitude. The outstanding mechanical properties can be attributed to its distinctive hierarchical micro/nano-scale structure, which encompasses the classical "brick and mortar" multilayered arrangement and the interface interactions between organic layers and aragonite platelets [132]. The construction of layered structures in biomaterials holds great promise for attaining optimal mechanical performance. Taking inspiration from the layered structure of nacre and the multilayer concentric structure of cortical bone, Wu's group has developed an innovative BTE scaffold with similar compressive strength, bending strength, and toughness by orderly assembling graphene oxide (GO), chitosan (CS), and hydroxyapatite (HA) (Figure 8). The scaffold was fabricated using membrane filtration and tube rolling techniques, enabling its potential use in vascularized bone regeneration. This innovative material demonstrates promise for application

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in this field [133]. Based on the structure of nacre, they also successfully prepared large-sized layered silicate-based bio-ceramic with ordered layered microstructure using a bidirectional freezing technique in another study. To further strengthen the layered bio-ceramic, resin was infiltrated into the layered ceramic blocks to achieve a "brick-and-mortar" structure [134]. Through this method, the production of centimeter-scale layered bio-ceramic composites was made possible, allowing for mass production. Furthermore, these layered composite materials demonstrated outstanding strength and modulus, comparable to cortical bone tissue. This surpasses the limitations of conventional bone implant materials. Importantly, the layered structure played a pivotal role in promoting new bone integration, highlighting the exceptional bioactivity of the fabricated materials.

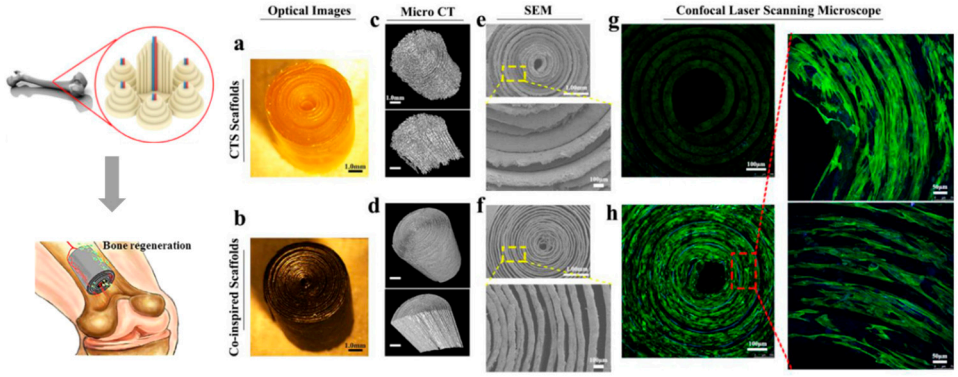

Figure 9. Morphology and adhering cells fluorescence images of cortical bone macrostructure-inspired scaffolds [133].

The biomaterial composed of the composite BTE scaffold, featuring a "brick and mortar" structure that mimics the properties of nacre, is an exceptional material for load-bearing bone regeneration. This scaffold exhibits remarkable mechanical properties that closely resemble those of cortical bone, while also demonstrating outstanding bioactivity. The combination of these factors makes it a highly promising biomaterial for potential applications in load-bearing bone regeneration.

3.3. Other multi-scale structural models

One of the essential objective of a porous scaffold is to facilitate the infiltration of nutrients and cells into its interior, promoting vascularization and inward growth of bone tissue for effective repair of bone defects [135]. Nevertheless, it is crucial to note that improving the transport capacity of substances within the scaffold may potentially compromise its mechanical performance. Therefore, designing aligned and straight pore structures with low fluidic resistance is an effective solution. Inspired by the lotus root, researchers have developed a biomimetic scaffold with high porosity (80%) using improved 3D printing. The scaffold exhibits mechanical strength exceeding 40 MPa, meeting the requirements for bone defect repair. This approach is not only applicable to various materials such as bio-ceramic and polymer, but also allows for the fabrication of lotus root-inspired scaffolds with heterogeneous shapes, numbers of channel, and channel diameter. Furthermore, the porosity and mechanical strength of this biomimetic lotus root scaffold can be controlled by regulating the elemental stacking and number of channels in the 3D scaffold [136]. Interlocking materials inspired by barbs utilize mechanical interlocking mechanisms to withstand tensile or shear forces. An example of a reversible interlocking device inspired by this concept is Velcro, which employs a basic hook and loop design with repetitive interlocking properties. The aforementioned concept has served as inspiration for researchers to develop an innovative biomaterial that combines electrostatic flocking-based interlocking with enhanced extracellular matrix (ECM) properties. This novel biomaterial aims to withstand shear and compressive loads in biological conditions, while simultaneously promoting cell viability even when subjected to mechanical deformation. In addition, several significant findings have been established: i) Interlocking scaffolds have been shown to provide support and enable cell proliferation within the pores and between the interlocking fibers. ii) The anisotropic nature of these scaffolds can offer protection to cells against compression-induced cell death pathways. iii) The

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mechanical strength of the interlocking scaffold is further enhanced by ECM secreted by the cells that are seeded within the scaffold. [137].

Similar to all biomaterials, these structural design elements are composed of biopolymers and biological hard substances, which are assembled and layered from the nano-scale to the macro-scale. Nevertheless, the remarkable mechanical properties and substance transport capabilities observed in these natural materials can be attributed to the intricate structural organization present at various spatial scales within these structural design elements. The synthesis of natural structural materials is achieved through a bottom-up self-assembly process, utilizing materials from the surrounding environment and under gentle conditions. Therefore, the prospect of emulating this natural process to create novel materials with comparable advantageous properties as natural structural materials represents a promising approach for BTE.

4. The manufacturing technology of multi-scale hierarchical scaffolds

In addition to the challenges in the structural design of BTE scaffolds, their fabrication also presents greater difficulties [138]. The overall strategy for developing biomimetic multi-scale BTE scaffolds involves selecting specific natural structural materials with unique functionalities or structures, studying and refining them, and integrating them into BTE scaffolds to achieve the desired mechanical and biological performance. Achieving designs inspired by natural structural materials requires new material fabrication methods for multi-scale assembly. Hierarchical multi-scale composite materials, which are manufactured using various techniques, exhibit a combination of mechanical and biological properties. Therefore, it is necessary to summarize the strengths and limitations of current manufacturing technologies and the latest design strategies for different biomimetic structures to provide valuable references for biomimetic fabrication. Based on the knowledge acquired, appropriate material systems and fabrication techniques are selected to replicate the structure of natural structural materials and obtain the desired functionalities.

BTE scaffolds are crucial materials used in bone regeneration and can be fabricated using either natural polymers or synthetic polymers, or ceramics in certain cases [139-144]. It is essential to design these scaffolds to simulate the multi-scale hierarchical structure in natural bone, promote integration with host bone tissue, and facilitate new bone formation. Biomimetic BTE scaffolds need to offer structural support for cell biological behaviors, metabolite transport, and ECM generation [80]. As a result, parameters, including porosity, pore diameter and pore structure of the scaffolds should be carefully considered [74]. While most research in the development of BTE scaffolds has focused on single-scale structures that do not fully mimic the biological environment, recent studies have recognized the importance of multi-scale structural materials for bone regeneration [74]. Inspired by the multi-scale hierarchical structure in natural tissues like bone, osteocartilage, skin, nerve, vascular tissue, and bladder, it is increasingly evident that more complex multi-scale structure scaffolds are necessary for optimal tissue regeneration. Porous multi-scale 3D scaffolds with interconnected pore networks have gained significant attention as they can better simulate bone tissue and provide an appropriate environment for cell growth and function, ensuring the physical and biochemical stimulation required for optimal regeneration of the target tissue. The formation of biomaterials involves multi-scale processes from the nano-scale to the macro-scale. Various manufacturing techniques have been developed for creating such biomimetic scaffolds, falling into two categories: conventional and advanced methods. These techniques have differing degrees of success and can be employed to fabricate scaffolds using one or more components through similar or different approaches [8,145-148]. Many excellent review articles have summarized the manufacturing methods of porous BTE scaffolds [149]. To provide a brief overview, Table 2 presents a simple introduction to these techniques.

Table 2. The describes of the advantages and disadvantages of different manufacture techniques for BTE scaffolds.

Technique	Advantages	Disadvantages

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Solvent casting/particle leaching	low-cost; effective control and tunable of appropriate structures	organic solvents residues; time consuming; limited bulk volume
Freeze-drying	high porosity and interconnection; low-cost	poor control of the location of pore space
Phase separation	high degree of adjustability; easy to combine with other manufacturing techniques	utilization of organic solvents; Inadequate regulation of pore interconnection
additive manufacturing	creating scaffolds with precise design	high-cost; time-consuming
electrospinning	produce nano-scale fibers to mimic nano features	high-cost; time-consuming

4.1. Traditional manufacturing techniques

Solvent casting/particle leaching is a commonly used method for preparing scaffolds with interconnected porous networks [150-152]. This method allows for effective control and tunability of porosity location and pore size, enabling the construction of structure with desired mechanical and biological properties [153]. However, it is limited to fabricating scaffolds up to 3mm thickness and is time-consuming due to the casting and solvent evaporation steps. Additionally, residual organic solvents can be challenging to completely remove from the scaffold, and incorporating pores with precise spatial control simultaneously is not possible using this method.

Freeze-drying is a widely used technique to obtain high-porosity 3D porous materials without the addition of pore-inducing agents [154-156]. It involves the complete sublimation of ice crystals formed during freezing to eliminate the solvent and acquire a porous structure scaffold with the aid of a lyophilizing machine [157]. By utilizing this technique, it becomes possible to fabricate scaffolds with desirable characteristics such as high porosity and interconnectivity. Adjustable by modifying parameters such as polymer properties, concentration, and freezing temperature. Nevertheless, the control over pore space localization is constrained when using this method.

Phase separation is a straightforward technique that relies on thermal energy variations to induce the separation of homogeneous polymer/solvent solutions [158,159]. The solidification or precipitation of the polymer-rich phase leads to the formation of a nanofiber structure within the polymer matrix, while the solvent-rich phase is eliminated through processes such as evaporation, sublimation, or extraction, thereby creating pores within the polymer matrix [160]. Phase separation technology provides a broad spectrum of pore sizes in 3D scaffolds and can be easily combined with other manufacturing techniques, such as particle leaching or 3D printing, to customize scaffolds for bone tissue engineering (BTE) applications [161,162]. However, the utilization of organic solvents in this method can result in inadequate pore interconnectivity, and it is only applicable to a restricted range of polymers possessing low melting points

While traditional manufacturing processes may lack precise control over pore structure, multi-scale hierarchical porous scaffolds can still be produced by combining different manufacturing processes, as outlined in Table 3.

Table 3. The describes of the multi-scale structure characteristics obtained based on the multi-assembly process.

Assembly process	Manufacture of multi-scale structure	Characteristics of multi-scale structure	Ref
Gas foaming + porogen leaching	The size and concentration of the pore-foaming agent were utilized to control the formation and size of macro-	The multi-scale porosity was beneficial to cell adhesion,	[163]

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	pores. The micro-pores were controlled by adjusting the gas foaming parameters.	proliferation and differentiation.	
Dynamic freeze casting (DFC) + micro-arc oxidation (MAO)	DFC was used to produce a porous structure with relatively large pores and high porosity, MAO was used to form microporous surface.	The compressive strength and elastic modulus were controlled by manipulating the porosity. The multi-scale porous scaffolds showed better biological response.	[164]
Freeze casting + porogen leaching	Freeze casting was used to produce relatively large dendritic pores with several tens of microns size, porogen leaching was used to form micron-sized small spherical pores.	The two methods are combined to readily tailored the porosity and compressive strength.	[165]
Thermally induced phase separation (TIPS) + porogen leaching	The size of the nanofibers could be regulated by adjusting the concentration of the polymer and phase separation temperature. The large pore size can also be customized according to the template sphere size.	The hierarchical structure of the scaffolds provided a large surface area, enhancing both the bioactivity and the potential as a drug delivery depot.	[166]
Photolithography + chemical modification	The multi-scale features of the hole/column/groove/ridge based on 3D were prepared on the PDMS carrier by photolithography, and the nano surface were obtained by chemical modification.	The multi-scale structures synergically affected the cell behaviors.	[167]
3D printing + MAO	The macroporous titanium implants prepared by 3D printing were treated with MAO to obtain nano-morphology.	Such scaffolds with micro-nano morphology were beneficial to enhance apatite induction ability in vitro and bone integration ability in vivo.	[168]
3D printing + electrospinning	The macro- /micro-scale porous scaffolds with multi-scale (bimodal) pore diamerter distribution of 300μm and 20μm were prepared by combining 3D printing and electrospinning techniques.	The scaffolds could promote the expression of osteogenic markers in MC3T3 cells.	[169]
3D printing + electrospinning	Polyester warp knitted scaffolds were utilized to	The multi-scale scaffold can provide	[170]

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	mimic the collagen fiber layer of the natural ECM with a pore size range of 1-5 μm. Electrospinning were used to prepare nano-scale morphology and 3D printing were employed to replicate micro-scale morphology.	structural and mechanical support.	
3D printing + near-field electro-spinning (NFES)	3D printing were used to prepare the macro-scaffold, the NFES were used to build tissue micro-morphology .	The macro- and micro-structures have high controllability, which meet the needs of mechanical properties in tissue engineering.	[171]
Melt deposition modeling (FDM)+ electro-hydrodynamic (EHD)printing	Through the manipulation of modules to regulate the electric field and extrusion, it is possible to convert between FMD and EHD modes during the printing process. This enables the creation of a multi-scale direct writing system wherein both coarse and fine fibers can be effortlessly printed using a single device.	Within this multi-scale composite scaffold, the coarse fibers offer adequate strength, while the fine fibers create a microenvironment that is conducive to cell adhesion	[172]
Melt deposition modeling (FDM)+ Melt electrospinning (MEW)+ solution electrospinning (SE)	The meso-, micro-, and nano-fibers fabricated via FDM, MEW, and SE can provide structural support, promote cell alignment, and create a biomimetic microenvironment to facilitate cell function.	Multi-scale hierarchical scaffolds can improve cell adhesion and proliferation, as well as facilitate cell alignment.	[173]

4.2. Advanced manufacturing techniques

In contrast to traditional manufacturing techniques, additive manufacturing and electrospinning have emerged as prominent techniques in the fabrication of medical implants with highly repeatable and customizable complex structures [38,174-176]. Electrospinning involves charging a polymer melt or solution under high pressure to produces fine fibers, and the arrangement of these fibers determines the surface structure of the porous scaffold [38,177]. Electrospinning technique can produce nano-scale fibers that mimic nano features found in natural bone ECM, providing appropriate surfaces for cellular adhesion because of their elevated surface area-to-volume ratio [178-182]. However, controlling the placement of fibers to form an ordered structure is a challenge in electrospinning due to the instability of the electrostatically stretched polymer. Additive manufacturing, on the other hand, can surpass these challenges by enabling the fabrication of scaffolds with precise designs [183]. Nevertheless, the achievable resolution of commonly employed additive manufacturing techniques, particularly extrusion-based methods, is generally confined to the micro-scale. To accurately replicate the nano-scale structure of the ECM, it is essential to consider alternative technologies such as electrohydrodynamics (EHD) processes, which can produce submicron fibers through techniques like electrospinning, electrostatic spraying, and melt electrospinning, are needed. However, it is important to note that the incorporation of charge

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dissipation introduce limitations on the thickness of the scaffold, and the process can be time-consuming to achieve desired outcomes like high-density and well-ordered structures.

Additive manufacturing techniques, along with electrospinning, have demonstrated significant potential in the design and fabrication of multi-scale hierarchical scaffolds for BTE. These approaches enable the replication of the intricate structure and complexity found in natural bone [184]. A variety of investigations have leveraged the integration of additive manufacturing and electro-hydraulic processes to fabricate hybrid scaffolds. This approach provides a versatile and uncomplicated platform for generating structures with diverse materials and a broad range of scales (Table 3). These scaffolds are particularly appealing as they can feature multi-scale pore diameter distributions. Macro-pores are deliberately designed to facilitate cell penetration, while micro-pores play a crucial role in facilitating protein adsorption and transfer. Furthermore, even nano-pores have the potential to significantly influence cell behavior. For example, Chen et al. created a hierarchical 3D gelatin and sodium alginate composite scaffold composed of macro-pores, honeycomb micro-pores, and nanofibers by combining biological 3D printing, electrospinning and vacuum freeze-drying technologies [100]. The macro- and micro-scale features of the scaffold facilitated nutrients infiltration, new vessels formation, cell migration, and cell adhesion on the rough surface formed by honeycomb micro-pores and nanofibers. Li et al. employed a biomimetic multi-scale scaffold with high porosity, a large specific surface area, mechanical support and cell-scale oriented structure created through high-precision near-field direct writing printing to produce. They loaded Schwann progenitor cells onto the scaffold, resulting in excellent repair effect [105]. PCL materials suitable for clinical treatment were employed to print multi-scale scaffold using the near-field direct writing process. Precise structural regulation without additional material modification achieved excellent biological activity. Therefore, the combination of multi-scale structures and clinical implants through 3D printing holds great potential for the development of BTE scaffold with enhanced performance. Inspirations from natural structure, such as climbing tree trunks, Gao et al. believed that cells around 10μm could easily grasp and adhere to microfibers with a diameter of 2-3 microns, rather than scaffolds with a size of 100 microns. Therefore, they proposed the development of multi-scale scaffold that combine macro-scale fibers for mechanical strength and micro-scale fibers for creating a favorable microenvironment for cell adhesion, proliferation, and differentiation [185]. An integrated 3D printing system has been proposed to fabricate multi-scale hierarchical scaffold, based on existing technology including melting deposition, near-field direct writing. The main feature of the platform is the ability to manufacture macro- and micro-scale scaffolds on the same platform with the same nozzle through the coordinated response of the control system. This approach allows for the fabrication of scaffolds with desirable mechanical properties, biomimetic microenvironment by simply adjusting the structure without changing the material composition.

Cell arrangement plays a critical role in cytoskeletal reorganization, ECM remodeling, and biomechanical regulation. A wide range of biomimetic scaffolds have been developed, taking inspiration from the natural tissue orientation of cells. These scaffolds incorporate well-aligned microfibers to induce cell alignment and promote tissue regeneration. Nevertheless, there are still challenges in manufacturing scaffolds that possess suitable mechanical characteristics, biomimetic microenvironments, and the ability to effectively support cell organization and arrangement. Therefore, Wang et al. proposed a unified 3D printing system for fabricating multi-scale hierarchical scaffold by bonding macro-, micro- and nanofiber filaments. Macro-, micro- and nanofibers were prepared by melt deposition modeling, melt electrospinning and solution electrospinning, respectively [107]. This strategy provides for the fabrication of multi-scale hierarchical scaffolds that possess desirable mechanical characteristics, biomimetic microenvironments, and the capacity to facilitate cell arrangement.

4.3. Combination of advanced and traditional manufacturing techniques

Organisms have undergone a lengthy process of evolution, utilizing a bottom-up approach to create highly precise and refined multi-scale hierarchical structures that meet the specific needs of life activities. This natural method of structural formation produces structures that span multiple

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scales and far surpass materials of the same category developed in laboratories. Although researchers have made significant progress in using various bottom-up and top-down manufacturing techniques to fabricate BTE scaffolds, each technique has its own advantages and disadvantages. Therefore, the effective combination of multi-assembly techniques and biomimetic fabrication principles may be a promising strategy for developing novel and efficient multi-scale hierarchical BTE scaffolds (Table 3). For instance, previous research group prepared BTE scaffolds with macro-pores and micro-pores using 3D printing and non-solvent-induced phase separation. The resulting porous scaffolds exhibit mechanical properties similar to cancellous bone, and the micro-pores in the scaffolds can promote cell attachment, proliferation, and differentiation [110]. Previous study utilized indirect 3D printing to manufacture porous scaffolds, aiming to simplify the design of the desired scaffold shape and control its macro- and micro-structure. By pouring a composite suspension into a soluble 3D-printed negative mold, a combination of macro- and micro-pores in the scaffold material can be achieved through freeze-drying/particle leaching methods. Optimization of the composite material formulation allows for control over the morphology, porosity, mechanical properties, and degradation of the final scaffold made of polylactic acid/poly(ε-caprolactone)/hydroxyapatite (PLA/PCL/HA) composites. The composite scaffolds exhibit improved performance in terms of biocompatibility, bioactivity, and osteoinductive capabilities [186].

Constructing the best biomimetic multi-scale hierarchical scaffold that match bone repair remains a challenge due to the complexity of multi-scale structure that simulate natural tissues, the complexity of interactions between cells and porous scaffolds, and the limitations of current manufacturing processes. Addressing this challenge will require future efforts on multiple fronts, such as exploring the relationship between multi-scale hierarchical structure and biological response, as well as developing efficient and economical manufacturing processes to produce multi-scale hierarchical BTE scaffolds [187]. Based on current research, it is challenging to achieve multi-scale hierarchical BTE scaffolds through a single manufacturing technology [90,188]. In the future, the advantages of various manufacturing processes will be combined to realize multi-scale hierarchical BTE scaffolds with superior composite properties [6,189-192]. For instance, additive manufacturing techniques have been recognized for their ability to assemble structural materials inspired by natural materials. However, the ability to use these technologies to create biomimetic composites will depend on solving difficult problems, such as expanding the range of materials that additive manufacturing can process [193,194]. Currently, only a limited number of metals, polymers, and ceramics are available for processing. For instance, the high thermal stability of ceramics has somewhat hindered the use of melting or in situ sintering techniques, which require the addition of “inks,” usually colloidal suspensions in water or other solvents. Furthermore, biomimetic materials are often a combination of materials, such as ceramics and polymers, that are challenging to create with a single technique [76]. Printing materials with nano-scale accuracy presents some challenges, with a final resolution of only tens or hundreds of microns. However, some studies have shown that electrospinning has advantages in constructing nano-scale materials and can compensate for some of these deficiencies. Therefore, it is significant in the field of BTE to explore the advantages and disadvantages of various manufacturing processes and integrate them to construct multi-scale hierarchical BTE scaffolds.

5. Conclusion and outlook

Through billions of years of evolution, living organisms have highly organized multi-scale structures that range from the nano-scale to the micro-scale and to the macro-scale. This bottom-up self-assembly of multi-scale structures serves as the foundation for constructing their macroscopic 3D structures and reflects the optimization of structure and performance at each scale. In nature, these multi-scale, multifunctional, and hierarchical organizational structures result in extraordinary properties of hybrid materials, even though they are achieved only by ordinary composition combinations. This far exceeds the achievements of many engineering fields. An important question, however, is achieving materials with the same form and function remains a monumental challenge using existing technologies [195]. In the field of BTE, biomaterials must interact actively with the

ECM while providing support stability to injured regions. Understanding the relationship between bone healing responses and chemical and physical cues is crucial for designing optimal BTE scaffolds [99]. As bone regeneration and healing pathways are current subjects of extensive research, the development of improved scaffolds for BTE that can create an optimal environment for bone healing poses a significant challenge. Currently, a large number of researchers are focusing on constructing multi-scale BTE scaffolds by simulating the multi-scale hierarchical structure of organisms [196]. Traditional manufacturing techniques have the capability to produce hierarchical pores that replicate the natural structure of bone. However, these techniques have limitations when it comes to simultaneously incorporating multiple materials. Furthermore, the type of porous structure also influences the deposition of ECM within the scaffold, consequently impacting its ability to facilitate bone healing. Despite advancements in manufacturing techniques, there are still limitations in creating multi-scale hierarchical 3D structures that accurately simulate complex natural systems. Regrettably, it is challenging to envision a single strategy that can encompass all the required solutions in the field of BTE [6]. Therefore, combining multiple manufacturing technologies is obtaining attention as the most effective approach in mimicking multi-scale hierarchies [75]. By gaining a comprehensive understanding of tissue composition and properties, it is reasonable to anticipate that future manufacturing methods will be able to synergistically combine various technologies to overcome existing limitations and enhance the functionality of biomedical stents. Current manufacturing processes can be complex and time-consuming, hindering the generation of large-scale 3D architectures. This greatly restricts the scale and commercialization of biomimetic technology. Researchers in the field of BTE scaffolds face two challenges: the first is to devise general methods for designing structures that mimic natural materials, and the second is to create economical and practical methods that can be mass-produced [76,197]. To surmount these limitations from a manufacturing standpoint, persistent endeavors need to be made to cultivate inventive multi-scale models and manufacturing techniques that enable adaptable control of multi-scale characteristics within substantial structures. One solution is to combine two or more manufacturing technologies to develop more reliable, efficient, and versatile biomimetic BTE scaffold systems by compensating for the limitations of each technology. Scaffold manufacturing necessitates finding a delicate equilibrium between simplicity and complexity in order to replicate the intricate structure of natural organization. Scaling up and advancing manufacturing can be a protracted and intricate undertaking when multiple components and/or methods are employed. Hence, it is imperative for scaffolds to exhibit a high simplicity-to-clinical-efficacy ratio while simultaneously meeting the following criteria: ease of scaling up, low production costs, optimization of biomolecules and additives used as ingredients, and a straightforward conditioning phase that provides substantial benefits to patients. Nevertheless, there is a hopeful anticipation that in the future, a one-step approach can be employed to attain BTE scaffold materials possessing intricate hierarchical multi-scale structures. This possibility serves as the future focus of collective endeavors.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, Zuolin Wang; investigation, Yun Wang, Yufeng Meng; resources, Zuolin Wang; writing—original draft preparation, Yun Wang, Yufeng Meng; writing—review and editing, Yun Wang, Yufeng Meng.; supervision, Yanhuizhi Feng, Yuning Wang, Shuai Yuan, Yiyuan Li; project administration, Zuolin Wang; funding acquisition, Zuolin Wang and Yufeng Meng. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Acknowledgments: This work was supported by the National Key Research and Development Program of China (2018YFE0202201, 2018YFE0202200), the Clinical Research Plan of SHDC (Grant SHDC2020CR1030B), the National Natural Science Foundation of China (22305240).

Conflicts of Interest: The authors declare no conflict of interest.

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