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Review

Prioritizing Biomaterial Driven Clinical Bioactivity Over Designing Intricacy during Bioprinting of Trabecular Microarchitecture: A Clinician's Perspective

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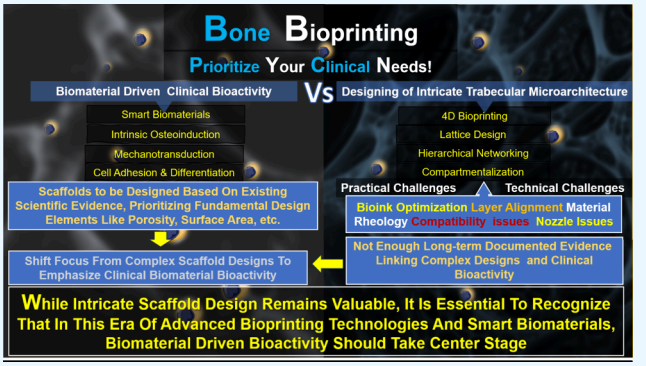
ABSTRACT: Bone tissue engineering has witnessed a historical shift from three perspectives. From a biomaterial perspective, materials have now become smarter and dynamic; from a bioengineering perspective the bioprinting techniques have now advanced to 4D bioprinting; and from a clinical perspective scaffold bioactivity has progressed toward enhanced osteoinductive scaffolds driven by intricate biomechanical, biophysical, biochemical, and biological cues. Though all of these advancements are indicative of improvised scaffold engineering, a pivotal question regarding the critical role and need of designing and replicating the intricacies of trabecular microarchitecture for enhanced, clinically appreciable osteoangiogenicity needs to be answered. This review hence critically evaluates the rationale and the need of investing substantial effort into designing complex microarchitectures amidst the era of "smart biomaterials" and dynamic 4D bioprinting aimed toward enhancing clinically appreciable bioactivity. The article explores the concept of integrating intricate designs into a scaffold microarchitecture to bolster bioactivity and the practical challenges encountered in 3D bioprinting of complex designs and meticulously examines the pivotal role of biomaterials in scaffold bioactivity, proposing a comprehensive approach to bioprinting geared toward achieving clinical bioactivity and striking a judicious balance between design intricacy and functional outcomes in bone bioprinting.



INTRODUCTION

The growing clinical demand for effective and durable reconstructive solutions for bone defects secondary to trauma or post cancer resections should be predictable with long-term success and biomechanical stability. This clinical need has driven the exploration of alternatives to current reconstructive solutions such as autografts and allografts, which suffer from limitations such as donor site morbidity, immune rejection, and availability constraints. Bioprinting bone is a viable alternative in such situations and has revolutionized bone tissue engineering, providing high hopes for patients in need of such reconstructions.[1,2]

The ultimate requirement of any scaffold in bone tissue engineering is to demonstrate its efficacy in forming a predictable quantity of good quality bone.[3] The unique arrangement of trabecular structures influences tissue properties at various levels from cellular behavior to biomechanical performance. Cells within these architectures experience diverse microenvironments, affecting their proliferation, differentiation, and overall function.[4] Moreover, the high surface area-to-volume ratio of trabeculae supports efficient nutrient diffusion and waste removal, critical for sustaining cell viability in engineered tissues.[5] Replicating such complexity is a technical challenge as it not only demands precise control over characterization and optimization of bioink for efficient osteoangiogenic activity but also poses a challenge to bioengineers to design and standardize bioprinting parameters to achieve high printing efficacy for a stable scaffold construct from physical, chemical, and biological perspectives.[6]

The initial years of tissue engineering technology focused on osteoconductive biomaterials as scaffolds where bone formation was called creeping substitution from the periphery to the center of the scaffold. The following years, the scaffolds were constructed to become osteoinductive. This osteoinduc-

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tion is a simple culmination of complex cellular signaling triggered by biomechanical, biophysical, and biochemical and biological cues.[7] Though bioprinting is establishing itself as the best alternative to autografts for bone tissue engineering, a critical point that needs to be focused on is the requirement of mimicking trabecular architecture for clinical osteoangiogenicity. The scientific literature is divided on this issue. A section of researchers claim that the design of a scaffold intricate microarchitecture plays a major role in its bioactivity,[8−11] and the other section claims that only basic microarchitecture requirements like optimal porosity and specific surface area need to be focused on during scaffold designing. The bioactivity is inherently related to biomaterial used for bioprinting[12−14] the scaffold construct.

This review hence engages in a thought-provoking debate, critically analyzing the current rationale behind investing substantial effort into designing an intricate microarchitecture in the era of "smart biomaterials" and dynamic 4D bioprinting for improving clinically important bioactivity. The review has been organized into sections which deal not only with the concepts analyzing the idea of incorporating intricate designs into scaffold microarchitectures aimed toward bioactivity, and practical challenges in 3D bioprinting of complex designs, but also on the concept of biomaterial driven scaffold bioactivity and a holistic approach toward bioprinting aimed toward clinical bioactivity.

SCAFFOLD MICROARCHITECTURE DRIVEN BIOACTIVITY

The sophisticated network of trabecular microarchitecture is an engineering marvel (Figure 1). These intricate patterns provide bone with strength, stiffness and the ability to heal. By replicating these patterns in bioprinted scaffolds, the clinical outcome becomes more biomimetic with predictable functional properties. One of the paramount advantages of this mimicry lies in its profound impact on cellular interactions. The intricate topography of trabecular microarchitecture facilitates the creation of multifaceted niches and microdomains, fostering an environment conducive to cellular crosstalk, signaling cascades, and intricate choreography.[15] Cells receive various signals that help them to communicate, differentiate, and specialize. These intricate interactions at the microscopic level show how complex structures can influence cell behavior and maintain tissue balance.

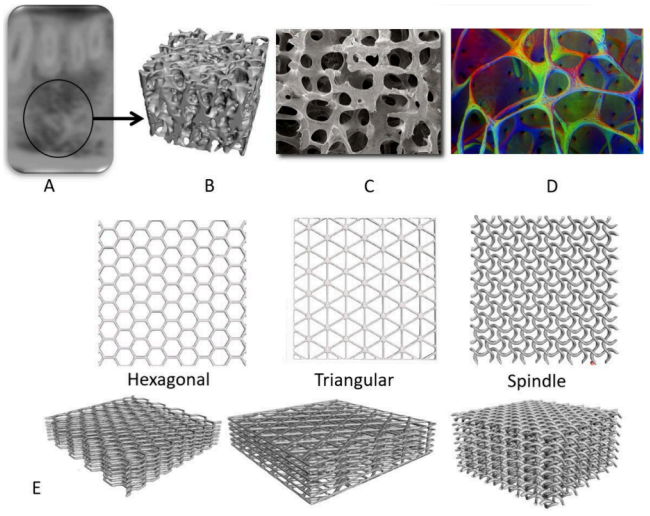

Figure 1. (A) Section of mandibular CBCT. (B) 3D model generated from CBCT representing the trabecular microarchitecture in the mandibular bone of a 30-year-old woman. (C) Low-power scanning electron microscope image of the normal trabecular bone microarchitecture in the third lumbar vertebra of a 30-year-old woman, marrow, and other cells removed to reveal thick, interconnected plates of bone. (D) Backscatter SEM of trabecular bone. (E) Various sophisticated scaffold designs and their corresponding scaffold bioprint constructs which mimic the trabecular microarchitecture (Figure 1C and 1D). Adapted with permission from https://boneresearchsociety.org/resources/images/public/. (C) Creator of the image - Tim Arnett. (D) Creator of the image - Duncan Bassett, Alan Boyde, and Graham Williams.

ROLE OF TRABECULAR MICROARCHITECTURE IN SCAFFOLD BIOACTIVITY

A1. Porosity in Bone Scaffold Design. Optimizing porosity is critical during the microarchitectural design of bone scaffolds.[8,9,16] It allows cellular infiltration, adhesion, and differentiation. Both macro- and microporosities within a biomaterial play crucial roles in promoting osteogenesis and angiogenesis (Figure 2).[17−19] Larger macropores, with an average size exceeding 100 μm, facilitate osteogenesis, and macropores exceeding 500 μm facilitate angiogenesis.[10,11]

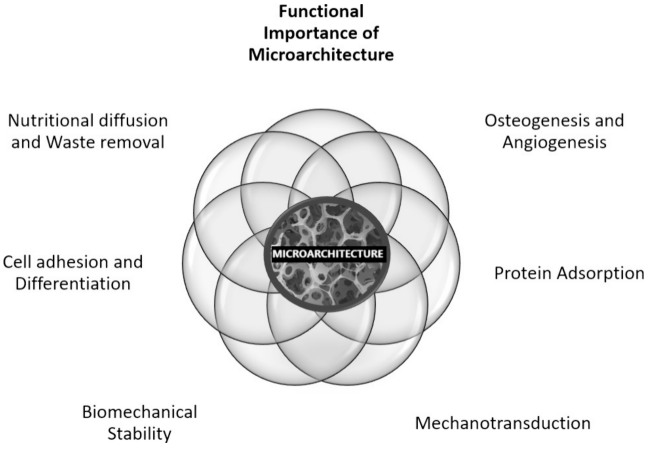

Figure 2. Functional importance of scaffold microporosity.

Microporous hydroxyapatite (HA) scaffolds with 0.4 μm pore sizes exhibit osteogenic potential, as hydroxyapatite nanocrystals display superior adsorption of albumin and fibronectin.[19−21] Further, it is also observed that structural design influences bioactivity; concave scaffolds induce bone formation within pores and its concave surfaces,[22] while osteoblasts preferentially attach to grooved scaffold surfaces.[23] Microporosities not only enhance the surface area for protein adsorption but also induce capillarity, anchoring, and accommodating cells within micropores, even if their dimension is slightly smaller than the cell.[24]

Calcium and phosphate ions from body fluids are absorbed into microporosities,[25,26] aiding apatite deposition, often accompanied by coprecipitation of osteogenic proteins that encourage cell differentiation. Scaffold architecture needs to be carefully controlled to regulate vascular ingrowth and hence calcium and phosphate concentration. Mechanotransduction, driven by scaffold−bone interface micromotions, governs bone cell proliferation and differentiation.[27]

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B1. Impact of Microarchitecture on Bone Regeneration. Recently, the influence of scaffold microarchitecture on the regulation of angiogenesis and osteogenesis for regeneration of bone in rat femoral defects has been investigated. The investigators explored the impact of scaffold microarchitecture on healing large bone defects using emerging 3D printing technologies. Specifically, fused deposition modeling (FDM) created scaffolds with decreased porosity and increased fiber diameter, and melt electrowriting (MEW) created scaffolds with increased porosity and decreased diameter. After 12 weeks, both scaffold types showed increased defect vascularization compared with controls, but MEW scaffolds demonstrated higher new bone formation. Interestingly, this superior healing with MEW scaffolds was not linked to increased angiogenesis, implying that vascular ingrowth into the scaffold is necessary but not mandatory for osteoactivity, and that the unique microarchitecture of MEW scaffolds, with small fiber diameter, high porosity, and surface area, positively influenced bone regeneration. This is suggestive of the fact that scaffold porosity can significantly impact angiogenesis and tissue regeneration, obviating the need for complex growth factors.[11]

Furthermore, the replication of nature's intricate complexity offers tangible physiological advantages that have far-reaching impacts on the construct. The interconnected network of trabeculae enhances nutrient diffusion and waste removal, overcoming the constraints of simpler geometries.[28] This improved accessibility to essential resources fosters a conducive microenvironment for sustained cell growth and vitality, promoting tissue maturation and organized structural development.

From a biomechanical perspective, integrating intricate microstructures into bioprinted constructs introduces a paradigm shift in load distribution and mechanical robustness. These microstructures act as well-designed reinforcements that mirror the natural tissue's ability to effectively distribute forces and endure stresses. The strategic arrangement of interwoven struts and lattice formations ensures that bioprinted constructs replicate the biomechanical integrity of native tissues, establishing a foundation for enhanced durability, resilience, and overall stability.[29] This biomechanical resemblance bridges the elegance of natural architecture with the practical demands of functional tissue engineering.

From a physiological perspective, embracing an intricate microarchitecture strategically addresses the physiological challenges inherent in tissue engineering. These designs improve oxygen and nutrient diffusion, departing from conventional limitations. In thicker constructs, where diffusion distances pose constraints, an intricate microarchitecture establishes a network of pathways that expedite vital resource exchange. This physiological optimization enhances cellular viability, metabolic activity, and overall construct functionality, augmenting the potential for engineered tissues to closely emulate their natural counterparts.[30]

The paradigm-shifting potential of intricate designs further extends to tailored microenvironments. The compartmentalization enabled by these designs empowers researchers to fabricate constructs that harbor microdomains that cater to distinct cell types or functions. This orchestration of tailored environments transforms tissue engineering into a realm of limitless possibilities, allowing researchers to create spatially segregated zones that nurture specific cellular behaviors, facilitate optimal differentiation, or simulate the complexities of native tissue interfaces.

Kang et al. leveraged advanced bioprinting techniques to engineer bone grafts with precisely tailored microstructures. These grafts replicated the natural hierarchical organization of bone tissue, harnessing a combination of mineralized and nonmineralized regions. The outcome was 2-fold: enhanced mechanical properties attributed to biomimetic microarchitecture and augmented cellular responses, culminating in improved osteogenic differentiation and bone formation. This study thus illustrated that intricate bone microarchitecture can orchestrate a harmonious interplay between material mechanics and cellular behavior, elevating the regenerative potential of engineered constructs.[31]

A compelling demonstration of intricate bone microarchitecture's potential in overcoming clinical challenges emerged in a study by Murphy et al. This study focused on mandibular reconstruction utilizing 3D bioprinted bone constructs to regenerate critical-size mandibular defects. The intricate scaffold design, mirroring the trabecular network, not only facilitated osseointegration but also supported neovascularization. The result was not only functional bone regeneration but also the establishment of a vascular network that is crucial for nutrient supply and overall graft survival. This pioneering application showcased the significance of intricate microarchitecture in addressing multifaceted clinical demands, emphasizing its potential to bridge the gap between biomaterial constructs and physiological complexities.[32]

PRACTICAL CHALLENGES IN BIOPRINTING COMPLEX DESIGNS

Navigating the intricate landscape of bioprinting complex designs requires a holistic approach that acknowledges and addresses the multifaceted challenges at play (Figure 3). As researchers strive to bridge the gap between conceptual design and tangible bioprinted constructs, a dynamic synergy of scientific ingenuity and technological advancement becomes paramount.

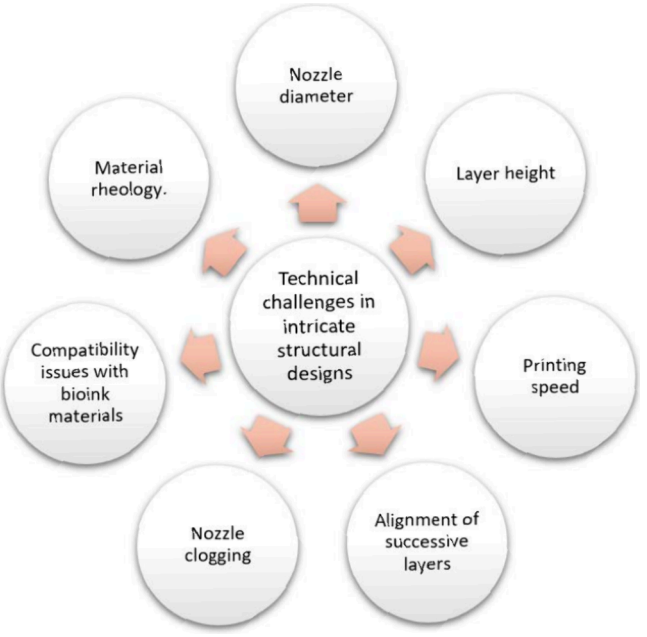

Figure 3. Technical challenges for incorporating intricate microstructural designs in bone tissue engineering.

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A2. Overview of Technical Challenges in Printing Complex Structures. In the realm of bioprinting, the pursuit of intricately structured constructs necessitates a profound understanding of the underlying technical challenges, akin to precision required in fine craftsmanship. To materialize intricate trabecular designs with accuracy, the orchestration of printer parameters, including nozzle diameter, extrusion pressure, layer height, and printing speed,[33−36] becomes pivotal. Achieving such precision, however, is a delicate balance, where the alignment of successive layers, avoidance of nozzle clogging, and compatibility of bioink materials assume roles of paramount importance.[37,38] Each parameter influences the outcome, and deviations can lead to distortions, inaccuracies, or even failure to reproduce the intended intricate structure.

As the complexity of the design increases, introducing multiple material printing nozzles into the performance amplifies the intricacy of the choreography.[39] The synchronization of multiple nozzles to realize multimaterial or multicellular designs though demands rigorous calibration and harmonization.[40] This multifaceted challenge arises due to the interplay of various factors, including nozzle dynamics, deposition rates, and material rheology. Precision in nozzle synchronization becomes a prerequisite to achieving seamless transitions between different materials or cell types, ensuring a cohesive and integrated final construct.

B2. Challenges in Optimizing the Bioink Parameters toward Intricate Design. The realm of bioprinting intricate trabecular architectures is a captivating endeavor, fraught with intricate challenges that extend to the very core of bioink formulation. Among these, the optimization of parameters such as viscosity, shear stress, cross-linking, and biocompatibility emerges as a complex battleground of scientific considerations and practical hurdles.[41−44] While these challenges may at times seem like insurmountable barriers, they also represent opportunities for innovation and growth, making the debate surrounding their optimization both compelling and essential (Figure 4).

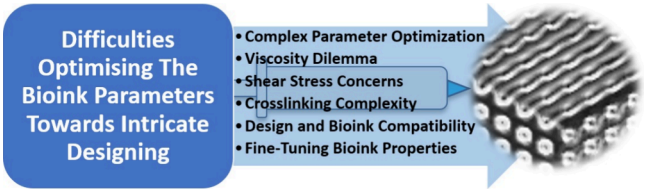

Figure 4. Difficulties in bioink optimization for intricate microarchitecture bioprinting.

Viscosity, a pivotal parameter, must strike a delicate balance between fluidity and stability. A higher viscosity ensures structural integrity during printing but risks clogging the nozzles and impeding cell encapsulation. Studies have shown that with a rising cell concentration at a consistent shear rate the viscosity experiences an upward trend. This phenomenon arises because as suspended cells interact within the bioink flow energy dissipation increases. This heightened energy dissipation occurs due to the combined effects of flow field distortion caused by the cells and friction generated by the bioink flow at the cell's surface.[45,46] Conversely, lower viscosity enhances cell viability and distribution but may compromise intricate structural fidelity.[47] The debate lies in determining the optimal viscosity that facilitates precise deposition while supporting cellular needs. Striking this balance demands rigorous experimentation, with the scientific rationale rooted in understanding the rheological behavior of bioinks and their impact on both structural and cellular outcomes.

Shear stress is a formidable adversary in bioprinting, as it can disrupt delicate cell structures and compromise their viability.[48] The majority of bioink hydrogels are non-Newtonian fluids, as their viscosity is altered in response to an applied force. Considering this viscous characteristic, they are primarily categorized as either exhibiting shear thickening or shear thinning.[49] The challenge lies in optimizing the printing speed and nozzle dimensions to minimize shear stress while maintaining accurate deposition. The debate hinges on whether to prioritize speed for efficiency or reduce it to safeguard cells, prompting a scientific exploration of how shear stress thresholds influence cellular responses and architectural precision.[50]

Cross-linking, a critical step, plays a pivotal role in determining scaffold stability and cellular interaction. Hydrogel bioinks used in 3D bioprinting can be cross-linked through various methods, including chemical, physical, enzymatic, or a combination. Chemical cross-linking involves irreversible covalent bonding between polymeric chains, achieved through chemical cross-linkers or reactions like Schiff base chemistry, azide−alkyne cycloaddition, and more. Physical cross-linking relies on noncovalent bonds like H-bonds and electrostatic attraction, yielding mechanically weaker but cell-friendly hydrogels. To enhance stability, nanofillers or chemical functionalities can be added.[51] Balancing cross-linking density is crucial; lower levels enable smoother bioink flow, while higher levels yield stiffer structures that can hinder printability. Careful control of cross-linking kinetics is essential to prevent nozzle blockage during printing. The physicochemical characteristics of bioinks, both prior to and following cross-linking, play a vital role in the bioprinting of intricate tissue structures.[52]

Yet, the challenge is multifaceted−organic cross-linking may hinder accurate deposition, while slow cross-linking may lead to bioink spreading. The debate centers on identifying dynamic cross-linking strategies that balance structural integrity with cellular functionality.[53] Scientific rationale dictates a comprehensive evaluation of the cross-linking kinetics' influence on structural stability, cell behavior, and long-term construct viability.

In the crucible of these bioink-specific challenges, a debate rages—is the pursuit of intricate trabecular architectures an impractical task or a gateway to transformative breakthroughs? The rationale behind this debate rests on the recognition that the path to success is paved with scientific rigor and innovation, which needs to be explored at a greater length in the future years.

The success of the intricate design of every scaffold layer to mimic the microarchitecture hinges on the fine-tuning (optimizing) of bioink properties in accordance with the technical and clinical demand. Bioink's viscosity, shear-thinning behavior, and cross-linking kinetics, for instance, must be meticulously modulated to ensure optimal extrusion and precise deposition, preserving structural integrity during layer-by-layer assembly. A well-aligned design and bioink compatibility enable the fabrication of intricate features and delicate structures, allowing for the realization of biomimetic

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tissue models that faithfully mirror the complexities of native tissues.

C2. Current Level of Accuracy Achievable in 3D Bioprinting. Achieving high accuracy while maintaining cell viability is a persistent challenge. Some studies have demonstrated successful bioprinting with cellular resolutions; however, optimization is very challenging, and many more studies need to be carried out. Next is the advancement in the technology where the emerging technologies such as extrusion-based, laser-based, and inkjet-based bioprinting have shown improved accuracy in replicating complex biological structures. Advancements in these technologies aim to improve the precision and resolution of printed structures.[54−58]

Although over the past few years' researchers have given their opinion on assessment methods of precision of bioprint,[59] there is limited standardized data to validate high level bioprinting accuracy. This is because of the fact that the precision of 3D bioprinting technology varies based on multiple factors, including the printing technique, materials, and intended application. This being the case, recent development of newer bioprinters for high precision bioprinting has started to provide standardized settings for reproducible complex bioprinted constructs.

Tashman et al.[60] have converted a commercially available 3D printer and converted it into a bioprinter. They achieved extrusion bioprinting accuracy (resolution) near 20 μm. They validated the accuracy by initially designing a square scaffold lattice consisting of 1000 and 500 μm filament spacing, and then the 3D volumetric image of grid space in the bioprint construct was captured using Optical Coherence Tomography (OCT). Further, a more complex bioprint of an adult human ear was validated using the same method. The results revealed a deviation of -29 ± 107 μm (mean $\pm$ STD) between the bioprint and the original computer-generated mode.

Recently, Li et al.[61] developed a multichannel 3D high precision bioprinter with high positional accuracy. They have also developed a control program with motion, pneumatic, and temperature subsystem controls for a multichannel bioprinting platform. The motion accuracy of the printer was controlled at the submicrometer level with the displacement error range of 0.6 to 0 μm after error compensation using the Laser Doppler Frequency Method.

The printing accuracy was verified by changing the printing speed, nozzle diameter, and extrusion pressure (Table 1) at a constant room temperature of 25°. The filaments extruded were steady, uniform, and accurate to produce a stable and accurate construct. The whole process was reproducible.

Table 1. Bioprinting Parameters[61]

Needle Guage	Inner Diameter	Outer Diameter	Extrusion Pressure
34G	0.04 mm	0.23 mm	0.18 MPa
32G	0.08 mm	0.23 mm	0.08 MPa
30G	0.15 mm	0.29 mm	0.04 MPa

SCAFFOLD BIOMATERIAL IS CRITICAL FOR BIOACTIVITY

The role of the scaffold biomaterial in scaffold bioactivity cannot be overstated. It forms the very foundation upon which the complex process of tissue regeneration hinges. Osteoinduction, an intrinsic process vital for scaffold functionality, can sometimes take a considerable amount of time, spanning months or even years following scaffold implantation. In this context, a novel mechanism of intrinsic osteoinduction within scaffolds has emerged as a groundbreaking concept.

This innovative theory proposes that the initial formation of a biologic apatite layer on the scaffold's surface is of paramount importance, surpassing the traditional focus on the local supply of calcium and phosphate ions through the bioprinted scaffolds.[62] While the scaffold's architectural design certainly plays a role, it primarily serves as a network that augments microporosities, just enough to facilitate microfluidic imbibition for cellular entry. Therefore, it is the formation of this apatite layer that emerges as the linchpin for the actual "bioactivity" of the scaffold, a concept articulated by Kukubo.[63]

This apatite layer plays a multifaceted role in scaffold bioactivity, involving crucial processes (Figure 5):

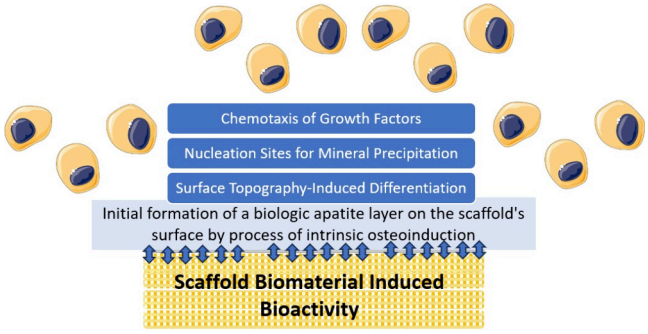

Figure 5. Intrinsic osteoinduction.

Chemotaxis of the Growth Factors: The surface of the scaffold attracts and encourages the migration of the growth factors. This chemotactic effect is instrumental in fostering the cellular processes necessary for tissue regeneration.

Surface Topography-Induced Differentiation: The topographical features of the scaffold's surface trigger the differentiation of mesenchymal stem cells into osteoprogenitor cell lineages. This differentiation process is pivotal for the formation of new bone tissue.

Nucleation Sites for Mineral Precipitation: The concavities within the scaffold's structure accumulate calcium and phosphate ions, creating nucleation sites for heterogeneous precipitation. This mineralization process is a critical step in bone formation.

The concept of scaffold bioactivity as a mechanism for material-driven heterotopic ossification has been comprehensively explored in an opinion paper by Bohner and Miron.[62] The authors opine that the scaffold's architecture should be viewed only to be a basic framework for a tissue-engineered construct and that the true scaffold bioactivity arises from the intrinsic osteoinductivity of the biomaterial used. This biomaterial provides the necessary porosity for vascular ingrowth and facilitates the transport of cells into the scaffold material.

In the context of bioprinting, this perspective leads to the intriguing idea of formulating bioinks composed of osteoinductive biomaterials specifically designed for scaffold bioprinting. This approach would significantly enhance the bioactivity of the bioprinted scaffold, aligning with the mechanism described above. By carefully selecting and engineering

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biomaterials that possess inherent osteoinductive properties, bioprinting can become a powerful tool in accelerating the complex process of tissue regeneration, ultimately improving patient outcomes and revolutionizing regenerative medicine.

"SMART" BIOMATERIAL DRIVEN 4D BIOPRINTING TOWARD DYNAMIC SCAFFOLD BIOACTIVITY

Traditional 3D printing is evolving now into 4D printing within the realm of tissue engineering, where materials are designed to respond dynamically to stimuli, creating an adaptive environment for tissue regeneration.[64] From the perspective of 4D bioprinting, the primary focus now lies in harnessing the potential of biomaterials to drive bioactivity. In this context, the ongoing debate among researchers who are currently dedicated to developing scaffolds with intricate architectural designs requires a critical revaluation. This advancement in 4D bioprinting places a strong emphasis on bioactivity being primarily governed by the unique properties of biomaterials. These biomaterials are referred to as "smart" due to their ability to mimic and enhance biological processes. Smart biomaterials represent the pinnacle of biomaterial research, as they not only faithfully replicate biological and biomechanical cues but also possess the remarkable capability to generate biomimetic bioelectric fields within the vicinity of tissue regeneration sites.[65]

The concept of 4D bioactivity revolves around understanding the changes that unfold within the implanted scaffold over time. This dynamic transformation can be achieved through various means, such as the utilization of injectable stimuli-responsive hybrid hydrogels, the deployment of shape memory scaffolds, or the innovative bioprinting of 3D scaffolds using piezoelectric materials.[66]

These smart biomaterials are particularly well-suited for bioprinting owing to their favorable rheological properties. Furthermore, they possess remarkable in vivo self-setting ability, making them excellent carriers for osteoblasts—the cells responsible for bone formation. For example, thermoresponsive polysaccharide hydrogels exhibit a critical solution temperature that conveniently falls between physiological and room temperature, allowing them to transition into a gel state at body temperature.[67,68] This inherent property ensures seamless integration with the body's natural processes. Piezoelectric materials, on the other hand, rely on a "functional transformation mechanism" to stimulate a physiological electrical microenvironment in response to applied stress. This unique behavior promotes osteoactivity, providing biomimetic bioelectrical cues without the need for external stimulation devices.[69] Consequently, scaffold bioactivity can be carefully modulated to influence cellular behavior and contribute significantly to osteogenesis.[70]

Perhaps one of the most remarkable features of shape memory scaffolds[71,72] and smart hybrid hydrogels[73,74] is their exceptional ability to recover their original shape. They serve as self-adapting implants that conform precisely to the dimensions and shape of bone defects, ensuring a snug fit and optimal support for tissue regeneration. This adaptability effectively eliminates any void spaces postimplantation. In addition to their "smart bioactivity", these biomaterials boast optimal porosity and specific surface area, which play a crucial role in facilitating cellular attachment and proliferation.[75] Thus, the fusion of 4D bioprinting and smart biomaterials promises groundbreaking advancements in tissue engineering and regenerative medicine, with the biomaterials taking center stage in driving bioactivity to new heights.

PRACTICAL APPROACH TOWARD SCAFFOLD LATTICE STRUCTURE DESIGN FOR CLINICAL OSTEOACTIVITY

From a biomechanical point of view, it is important to analyze the efficiency of biomimicking the natural trabecular architecture. It becomes imperative to understand that in cancellous bones the microarchitecture consists of transversely oriented platelike struts and longitudinally oriented rodlike struts. In this regard, Torres et al. have explored the effects of microarchitectures on failure of a scaffold construct.[76] They studied the microscopy of natural trabeculae using the morphological decomposition approach and isolated individual structures within the architecture. They measured the microscopic damage in the trabecular architecture for specific amounts of cyclic loading. They found that microscopic damage correlated with the strain and was not related to density and amount of platelike struts (primary load bearing element). They also found that the pattern of strut failure (biomechanical failure) was directly proportional to the orientation of the struts, and for every unit increase in the diameter of rodlike structures, the fatigue life increased by two times the magnitude of load. Conversely, every unit increase in the size of platelike structures increased the failure of the construct by five times the magnitude of load. The transversely oriented platelike struts are sacrificial elements that are capable of accumulating a large amount of stress before overt failure. Hence, biomimicking the exact trabecular architecture incorporating platelike struts would not always be efficient to avoid biomechanical failure of the scaffold construct.

Structural design of a bioprinted construct should thus be guided by basic geometric optimized parameters consisting of 3D Struss like lattice structures composed of interconnected struts and nodes.[77] To optimize such a design of a bone scaffold, two prominent mathematical modeling methods are utilized: Voronoi Tessellation and a Triply Periodic Minimal Surface (TPMS). These methods play crucial roles in creating scaffolds with specific structural and mechanical properties to facilitate bone tissue regeneration.

Voronoi Tessellation. This approach closely emulates the natural bone's porous structure. It is particularly useful when designing scaffolds with irregular pore shapes while maintaining a uniform porosity. Such a structure is beneficial for cell migration and proliferation within the scaffold. Compared to traditional rod-based scaffolds, Voronoi-based structures exhibit reduced stress−concentration in typical lattice network structures and offer favorable mechanical and biological properties. Such a lattice structure is thus a porous 3D spatial structure formed and tessellated by unit cells with different topological geometries. This unit cell strut design is optimized for specific performance. The Voronoi method of scaffold design optimizes the bioprinted construct design where first a structural template of the scaffold is designed and then cells progressively adhere and proliferate. This approach to scaffold design is based on hierarchical structures (lattice) created by the repetition of the unit cell of known geometry (based on Adam and Zimmer criteria) and known properties so that the bioactivity of a scaffold construct could be predicted based on the geometry of the cell.

Triply Periodic Minimal Surface (TPMS). Currently, TPMS is a highly suitable method for constructing porous

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scaffolds with regular pore structures through repetitive unit cells, encompassing various customizable TPMS types like primitive, diamond, gyroid, and I-WP surfaces to cater to specific design needs.[78]

Zhu et al. focused on designing porous meniscal implants using TPMS for knee joint reconstruction and assessing their biomechanical properties. Finite element simulations included healthy knees, knees with solid implants, and knees with TPMS-based porous implants. Their results showed that porous implants reduce stresses on cartilage compared with solid ones, resembling natural menisci. Pore properties impact implant effectiveness, with primitive surface implants distributing stress more effectively.[79]

TPMS-based scaffolds offer even stress distribution, enhancing the mechanical and biological properties. They can lower the elastic modulus of materials such as titanium alloys while maintaining high yield strength, with the modulus inversely tied to porosity. Adjusting the unit type or porosity allows precise control of the elastic modulus to mimic cortical and trabecular bone. In a recent study, titanium alloy porous scaffolds with TPMS design were explored for bone tissue engineering. These scaffolds reduce stress-shielding, endure complex stress environments, and facilitate nutrient transport. Fused Gyroid and Diamond TPMS scaffolds exhibited stable mechanical performance in compression tests with strengths of 367.741 to 419.354 MPa and moduli of 10.617 to 11.252 GPa in different loadings.[80]

Further, the porous architecture of TPMS scaffolds has also been studied for its impact on the scaffold's overall permeability behavior. This study investigated the porous structure of triply periodic minimal surface (TPMS) scaffolds (Schwartz D, Schwartz P, and Gyroid, each with 70% porosity) and its impact on scaffold permeability. The scaffolds were 3D printed, and permeability was calculated using Darcy's Law. Finite element simulations in ABAQUS assessed the fluid flow and concentration areas under compression. Unit cell design significantly influenced the permeability and fluid flow velocity, regardless of consistent porosity. Gyroid had a higher permeability, while Schwartz P showed less fluid trapping. Schwartz D was less favorable in both the experimental and numerical assessments. Gyroid and Schwartz P appear promising for specific bone tissue engineering applications.[81]

A recent study addresses the challenge of tailoring pore architecture in porous scaffolds to enhance osteogenesis. Using a digital light processing technique, the study fabricates Mg-doped wollastonite scaffolds with interconnected pore networks and curved pore structures (TPMS), akin to cancellous bone. The sheet-TPMS geometries (s-Diamond and s-Gyroid) exhibit initial compressive strength four times higher and release Mg ions 20%−40% faster than other TPMS variants (Diamond, Gyroid, and I-graph-Wrapped Package). Notably, Gyroid and Diamond pore scaffolds significantly induce osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). In vivo rabbit experiments reveal delayed bone tissue regeneration in sheet-TPMS structures, while Diamond and Gyroid scaffolds foster rapid neobone tissue formation, ultimately filling the porous network. These findings offer valuable insights into optimizing bioceramic scaffold pore architecture design to accelerate osteogenesis, facilitating their clinical use in bone defect repair.[82]

Thus, a general perspective for designing a scaffold is to have a scaffold with its external form conforming to the customized shape of a bone defect and the internal architecture with optimized degrees of interconnectivity between the struts (degrees of interconnectivity directly proportional to scaffold bioactivity), favoring cell adhesion, differentiation, and proliferation. Thus, unit cell approaches for scaffold design do not aim to mimic the exact intricate trabecular microstructure but generate a constructive solid geometry optimized for specific scaffold bioactivity (osteoinduction).

The future of bioprinting is moving toward dynamic scaffold bioactivity post implantation. Hence, considering the evidence-based structural design of the scaffold with an osteoangiogenic biomaterial satisfying the biomechanical and clinical needs, the researchers need to focus more on the bioink formulations and optimization of bioprinting parameters aimed toward clinical bioactivity.

SUMMARY

In the realm of bone bioprinting, the advent of 4D printing and smart biomaterials prompts a re-evaluation of priorities. While intricate scaffold design and the emulation of trabecular architecture have long been central in tissue engineering, it is imperative to reconsider our focus. Complex designs in bioprinting present formidable practical and technical challenges, necessitating a balanced approach that places paramount importance on improving clinical outcomes. The shift toward emphasizing biomaterial bioactivity allows us to harness the potential of smart biomaterials to create bioprinted constructs that actively promote tissue regeneration, aligning with the clinical efficiency demanded in bone bioprinting.

Furthermore, this review underscores the significance of anchoring our design decisions in existing empirical evidence, especially concerning fundamental design elements such as porosity and surface area. This ensures that bone bioprinting continues to progress with a clear orientation toward enhancing patient well-being and addressing clinical requirements. While intricate scaffold design remains valuable, it is essential to recognize that in this era of advanced bioprinting technologies and smart biomaterials biomaterial driven bioactivity should take center stage. This shift in focus empowers us to make substantial advancements in bone bioprinting, ultimately enhancing clinical outcomes.

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