



Study and Investigation of Bioprinters in Bone Tissue Engineering

Golnaz khoshbakht

Department of Biomedical Engineering, Tehran medical Branch ,Islamic Azad University, Tehran, Iran

Minoosh Lalinia

Department of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

Department of Biomedical Engineering, Tehran medical Branch, Islamic Azad University, Tehran, Iran

Reyahaneh Reshadi

Department of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract

Tissue engineering has transformed significantly due to 3D bioprinting innovations, which utilize bioinks to construct complex three-dimensional structures for cell culture. Bioinks, defined by their material characteristics, biological interactions, gelation behavior, and viscosity, play a pivotal role in the success of bioprinting processes. Techniques such as autonomous self-assembly, micro-tissue building blocks, and biomimicry enable 3D bioprinting to create tissues or organ-like structures by meticulously layering biological materials and living cells. Despite the substantial promise of 3D bioprinting in tissue engineering, replicating physiologically relevant 3D structures effectively remains a significant challenge. Progress in 3D bioprinting for therapeutic applications hinges on enhancing bioink materials and understanding their physiological properties. Researchers are focusing on developing sophisticated bioink compositions and advanced printing procedures to create bioprinted tissues and organs that closely mimic native tissues. This advancement paves the way for personalized regenerative medicine and organ transplantation, offering hope for more effective and customized medical treatments in the future. Furthermore, the ongoing improvements in bioink formulations and bioprinting technologies are expected to overcome current limitations, potentially revolutionizing the field of tissue engineering and regenerative medicine.

Keywords: Bone-Bioink-Bioprinter-Tissue-Calsium phosphate

1. Introduction

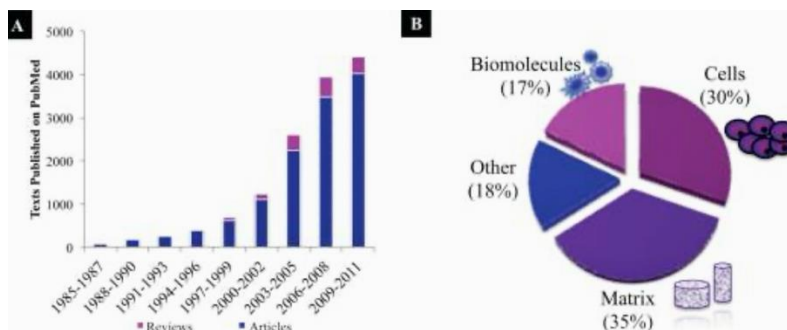
A novel biomedical method called 3D bioprinting uses biological molecules, living cells, and biomaterials to create complex structures. This novel approach is revolutionizing cell culture methods by making it easier to create intricate and medically accurate three-dimensional habitats. A key component of 3D bioprinting's success is bioink, a relatively new development in tissue engineering and regenerative medicine. The bioink selection has a significant effect on the biocompatibility and functionality of the 3D cultures that simulate biological tissues. Bioinks can be derived from synthetic polymers like polyethylene glycol or natural polymers like collagen and gelatin. They are classified according to their scaffold approach, which includes



both scaffold-free and scaffold-based methods, and their role, which includes support, sacrificial, curing, and matrix. For bioactivity, cytocompatibility, and biocompatibility to be guaranteed post-bioprinting, bioinks' biological and material characteristics are essential. Biodegradation rates, mechanical characteristics, and post-bioprinting maturation processes are important considerations when choosing a bioink. Notwithstanding the intricacy of bioinks, choosing the right bioink for a given application requires careful consideration of material characteristics, biological interactions, gelation processes, and viscosity. The development of scalable and repeatable micro-scale tissues and maybe completely functional organs is made possible by this rigorous selection procedure. 3D printing technologies are essential for the creation of biomimetic scaffolds with tailored mechanical and biological properties in the context of bone tissue engineering. The scaffold geometry, pore size, porosity, and physiological properties can all be precisely controlled with these techniques. Biomaterials containing calcium phosphate (CaP), like hydroxyapatite and β -tricalcium phosphate, are commonly employed in these applications because of their capacity to promote bone regeneration and similarity to the composition of bone mineral. Synthetic polymers do not have the bioactive qualities required for efficient hard tissue regeneration, despite having appropriate mechanical and bioinertness qualities. 3D bioprinting is rapidly advancing to create scaffolds with macrostructure, microstructure, submicrostructure, and nanostructures that closely resemble the hierarchical structure of genuine bone. In order to inhibit stress shielding and promote bone cell proliferation and tissue regeneration, these scaffolds must be customized. Although it complicates the bioprinting procedure and biomaterial selection, adding living cells to 3D-printed scaffolds can expedite bone formation and tissue regeneration. With the help of cutting-edge 3D printing technology and the thoughtful application of bioinks, 3D bioprinting has great potential for producing intricate tissues and organs. The goal of this field's ongoing research and development is to create biomedical structures that are functional, scalable, and repeatable so they can transform tissue engineering and regenerative medicine.

2. Bone tissue engineering (BTE)

Since its inception almost thirty years ago, the field of bone tissue engineering (BTE) has seen significant growth and interest as seen by the increasing number of research and reviews available on PubMed since the mid-1980s. In order to address the drawbacks of current therapeutic approaches, such as immunological rejection, pathogen transfer, donor site morbidity, and restricted availability, BTE aims to provide substitute therapies.. The joint efforts of scientists, engineers, and surgeons are required to realize the goal of developing bone grafts that improve bone repair and regeneration. Several essential components compose the core BTE framework: The components of the bone tissue matrix are as follows: (1) a biocompatible scaffold that closely resembles the extracellular matrix of natural bone; (2) osteogenic cells; (3) morphogenic signals that guide the cells to the appropriate phenotype; and (4) enough vascularization to eliminate waste and supply nutrients. The construct can affect the host after implantation by releasing growth factors that are osteogenic and/or vasculogenic, or by incorporating cells that naturally release these factors through genetic engineering. At the site of the defect, this causes faster cell homing, vascularization, and bone regeneration. Even with the great advancements, there are still numerous important obstacles to overcome before BTE is used in clinical settings. This paper examines the developments and challenges encountered in obtaining effective BTE.



Fig(1)A. Published articles on BTE since mid-1980s on PubMed. B. Breakdown of the articles published in 2011 according to BTE focus (i.e., bio molecules, cells, matrices and other, including vascularization approaches and bioreactors). [7]

3. Phases of 3D Bioprinting

The entire bioprinting workflow can be condensed into three primary stages: Pre-bioprinting, Bioprinting, and Post-bioprinting.

3.1. Pre-bioprinting

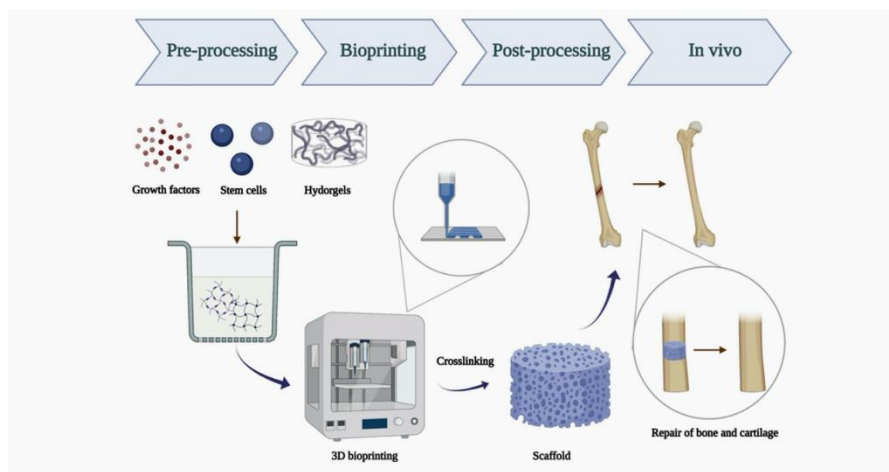
The preliminary phase entails creating a digital model using specialized software, which will subsequently be bioprinted into a three-dimensional structure. A biological template is acquired through a biopsy of the target tissue employing imaging techniques like MRI or CT scans. These scans are transformed into a 2D format to serve as the design template. Following this, cells are chosen and cultured in an appropriate medium to generate the bioink.

3.2. Bioprinting

During this phase, the bioink is loaded into the bioprinter and deposited onto a scaffold to construct a three-dimensional structure based on the 2D design from the software. This is a sophisticated stage as various cell types are printed in accordance with the specific requirements of the target tissues and organs.

3.3. Post-bioprinting

The concluding phase involves stabilizing the bioprinted structure to ensure it maintains its form and biological function. Physical and chemical stimuli are employed to stabilize the mechanical properties, facilitating cell reorganization and encouraging tissue development.



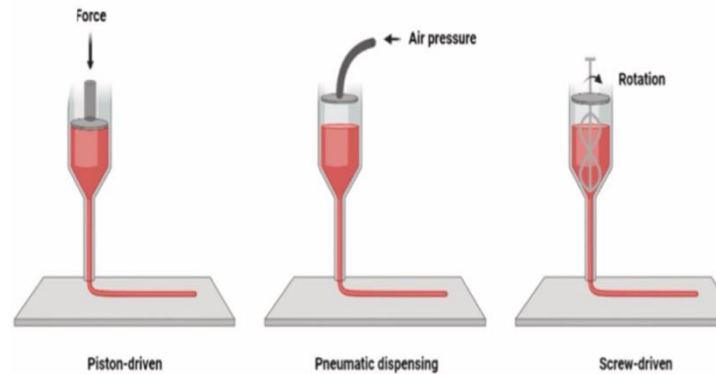
Fig(2) phases of 3D bioprinting [3]

4. Overview of 3D bioprinting techniques

4.1. Extrusion-Based Bioprinting (EBB)

EBB is used by many research facilities for tissue and organ studies. Using a piston to apply mechanical or pneumatic pressure, bioink is inserted into a cylindrical container and extruded onto a substrate either continuously or in pulses. For accurate material manipulation and deposition, the bioprinters used by EBB are outfitted with a stage and a temperature-regulated system that can move in any direction. Because it can bioprint different materials at different sites on a single construct utilizing several print heads, EBB is a popular tissue engineering technology. This makes it easier to create structures with tailored variations in signaling chemicals, cell kinds, densities, and biomaterials. Furthermore, in comparison to other bioprinting methods, EBB allows for larger cell densities. However, one major drawback of EBB is the limited supply of materials suitable for usage as bioinks. Furthermore, during extrusion, cells undergo shear stress and pressure inside the nozzle, both of which may reduce the survival and functionality of the

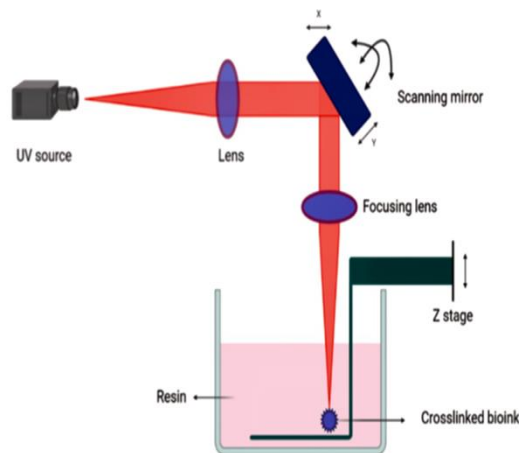
cells. Even though EBB has access to a larger variety of biomaterials than other bioprinting techniques, a significant obstacle is still the lack of appropriate bioinks.



Fig(3) Extrusion-Based Bioprinter schematic [1]

4.2. Stereolithography (STL)

Stereolithography (STL), often called vat photopolymerization, is a process that creates three-dimensional structures by layering and curing a liquid photopolymer resin using a light-emitting device (typically a UV laser). With this method, a thermosetting plastic is used. With the use of UV light or a UV laser, STL is the first additive manufacturing technique to selectively harden a liquid photosensitive resin. Stunning details may be produced with minimal stair-stepping effects and a smooth surface finish thanks to STL. Complicated chemical formulas are utilized in the liquid-based ingredients of every STL bioprinter. Powder-based and solid thermoplastic polymers such as PLA, ABS, and PA are utilized in STL. A thin coating of liquid resin is bound to the biofabrication bed during the procedure by exposure to UV light. The fake item is then left on the bed until it is taken off and cleaned using solvents containing alcohol. The part may require additional chemical post-treatment or further curing under intense UV light, depending on the material used. Post-processing may be required for the STL process, which frequently calls for additional support structures. The greatest precision in biofabrication is provided by STL, which also permits the use of a wide variety of materials.

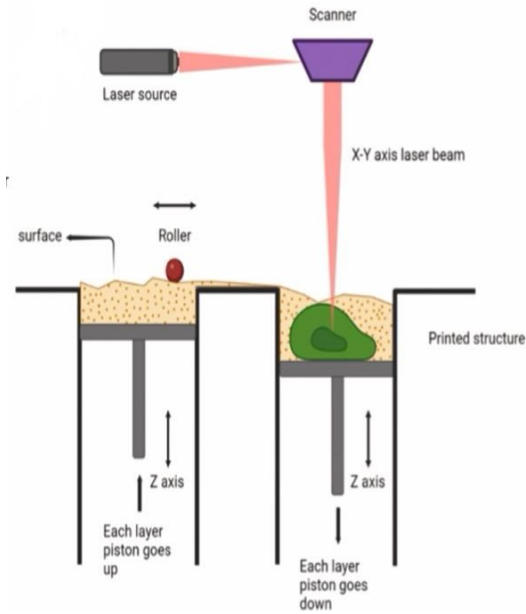


Fig(4) STL bioprinter schematic [1]

4.3. Selective Laser Sintering (SLS)

The 3D bioprinting technique known as selective laser sintering (SLS) makes use of a computer-directed, pre-programmed laser beam that is controlled by CAM/CAD software. With this method, particles are fused together to create three-dimensional objects using powerful CO₂ lasers. Scaffolds are made by laser sintering powders made of

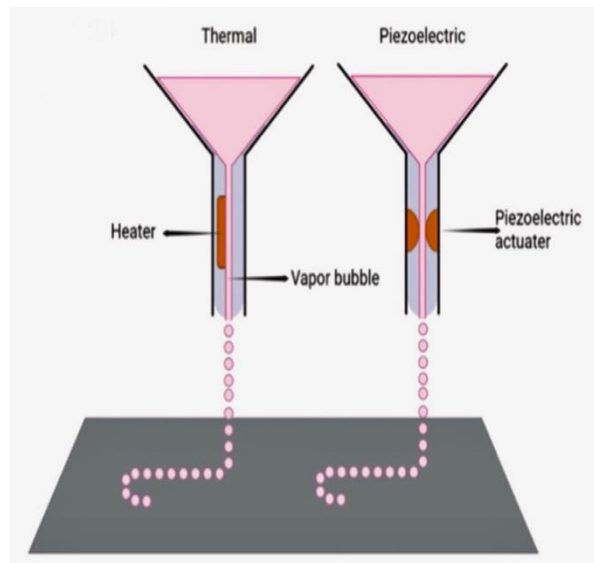
metal, ceramic, or polymers like white nylon. The process entails designing the part on top of a powder bed using 3D modeling software. The building platform or bed is lowered after every laser scan. This technique is unique in that it keeps doing this step by step until the item reaches the desired height. Since unsintered powder envelops and holds the model in place, no additional support materials are needed during construction. The unsintered particles are manually removed following bioprinting. SLS is perfect for prototypes and fully functional end-use parts since it can work with a variety of materials and produce strong, accurate pieces. SLS and stereolithography are comparable in terms of quality and speed.



Fig(5) SLS bioprinter schematic [1]

4.4. Inkjet-Based Bioprinting (IBB)

The most economical bioprinting method is inkjet-based bioprinting (IBB). Thermal or acoustic forces are employed to dispense the bioink onto a substrate from a cartridge. The print head in thermal inkjet bioprinting is electrically heated to generate pressure, which causes droplets to emerge from the nozzle. By creating an acoustic wave inside the print head, a piezoelectric crystal in acoustic inkjet bioprinting breaks the liquid into droplets. The piezoelectric material rapidly changes shape when electricity is supplied, creating the pressure required to release droplets. Because each has unique benefits and drawbacks, the choice between thermal and acoustic approaches depends on the particular application. Because inkjet bioprinting prints quickly and allows cells and biomaterials to be directly deposited into flaws, it is frequently used to regenerate healthy skin and cartilage tissues. Furthermore, IBB maintains the functionality and survival of the cells while enabling the homogeneous deposition of primary or stem cells upon wounds. The successful fusion of inkjet bioprinting and electrospinning has produced layered cartilage constructs.



Fig(6) IBB bioprinter schematic [1]

5. Bioink

In order to maximize tissue regeneration and guarantee the effectiveness of bioprinted regenerative implants, the choice of ink material is essential. Much work has been done to develop 3D bioprinting and recreate functional tissue architectures using a range of materials, including naturally generated biomaterials and synthetic polymers. Materials used in 3D bioprinting are categorized as either biomaterial inks or bioinks based on whether or not cells are printed into the constructed objects. In order to create cell-friendly habitats with the right physical and biochemical characteristics, hydrogel biomaterials, bioactive chemicals, and/or individual or clustered cells are commonly mixed with bioink, a printed material. Tissue printing requires careful consideration of the fundamental qualities of bioink, including rheological and mechanical properties, printability, biomimicry, and biodegradability. The common biomaterials utilized in orthopedic tissue engineering applications and performance for 3D bioprinting are examined in this section.

5.1. Hydrogels

Hydrogels are thought to be the best bioinks for 3D bioprinting since they are made from both natural and synthetic polymers, including chitosan, collagen, gelatin, fibrin, elastin, agarose, and alginate. These polymers, which come from both non-mammalian and mammalian sources, successfully mimic the natural porosity of bone, which encourages the growth of new cells. Excellent cell adhesion, consistent biodegradability, non-toxicity, good rheological properties (ideal viscosity and shear thinning for filament extrusion), quick gelation, and sufficient mechanical strength and stiffness are only a few of the essential qualities they meet.

5.1.1. Advanced Hydrogel-Based Bioinks

Hydrogel biomaterials, known for their high water content, are frequently used to encapsulate cells and additives, fostering cellular behaviors such as adhesion, proliferation, and differentiation within 3D environments. These bioinks possess viscoelastic properties that minimize shear-induced cell damage during printing and provide a supportive matrix that mimics native complex microenvironments. Naturally sourced hydrogels like alginate, gelatin, collagen, silk fibroin, and decellularized extracellular matrix (dECM) are particularly noteworthy for their biocompatibility, inherent bioactivity, and structural similarity to natural ECM.

Alginate: Because of its affordability, biocompatibility, readily changeable rheological and mechanical qualities, and quick crosslinking with divalent cations like calcium, alginate, a naturally occurring polysaccharide derived from brown algae, is frequently employed as a bioink. But in order to increase bioactivity for tissue engineering, its poor biological activity makes it necessary to combine it with other materials or change it with RGD functionalization.

Collagen The main protein found in musculoskeletal tissue, collagen, has good biological qualities and offers a large number of binding sites for cell attachment. Because collagen type I hydrogel thermally gels at 37°C, is biocompatible, and causes no immunological reaction, it is widely employed. Nevertheless, chemical alterations or blending with high-viscosity biomaterials can enhance the mechanical and structural stability of pure collagen bioink.



Gelatin is a non-toxic, water-soluble, biocompatible, biodegradable substance derived from denatured collagen that has a low immunogenicity. Because of their RGD patterns and thermoresponsive nature, gelatin-based bioinks are preferred. Through quick UV-induced crosslinking, methacrylated gelatin (GelMA), a cutting-edge bioink, improves printability and mechanical stability.

Produced by silkworms, **silk fibroin (SF)** is a highly sought-after bioink because of its mechanical strength, biocompatibility, tunable degradation, and ease of processing. SF bioink, however, has drawbacks such poor viscosity, sluggish gelation, and frequent nozzle clogging. These problems are addressed by blending with high-viscosity biomaterials or by enzymatic, chemical, and physical changes.

Extracellular matrix (dECM) has become a material of interest for 3D bioprinting applications. Decellularization is the process of eliminating cells from tissues or organs while leaving the extracellular matrix (ECM) intact, retaining its distinct makeup and structure. dECM-based bioinks have the ability to mimic the intricate, tissue-specific natural ECM characteristics that regulate cellular activity and tissue function. Even with all of its potential, dECM bioinks still need to be improved technically because of issues including batch-to-batch variance, uneven compositions, limited mechanical stability, and potential immunological reactions. To sum up, there are advantages and disadvantages to each of the main bioink materials utilized in 3D bioprinting for orthopedic applications. The performance and efficacy of these bioinks are significantly influenced by the crosslinking techniques used.

5.2. Synthetic Polymers as Biomedical Inks

The mechanical properties of biocompatible synthetic polymers, like polycaprolactone (PCL), polylactic acid (PLA), and polyurethane (PU), can be tailored to specific needs, and their exceptional printability makes them ideal for 3D bioprinting. For example, FDA-approved PCL is well known for its slow rate of decomposition and compatibility with biological systems, which makes it perfect for a wide range of biomedical applications. On pure PCL structures, however, its hydrophobic properties lead to negligible cellular activity. Because of its suitable biodegradability, processability, biocompatibility, and high flexibility and hardness, PLA is a widely used biodegradable polymer in the biomedical industry. Despite this, PLA's brittleness and the acidic byproducts it releases during breakdown limit its use in tissue engineering. Since PU materials are highly elastic, biocompatible, and have tunable chemical and physical properties, they are favored in biomedical settings. PUs are used in many different types of medical equipment, such as cardiovascular apparatus, catheters, tubing, and surgical drains. Most synthetic polymers are not bioactive, but they are used largely as structural materials to provide important mechanical and physical support for the tissue build in 3D bioprinting for tissue scaffolds. In orthopedic tissue engineering, combinatorial approaches using thermoplastic polymers in combination with biological components (such as cells and growth factors) are preferred to generate bioactive and mechanically strong cellular scaffolds for bone, cartilage, and tendon/ligament regeneration.

6. Calcium phosphates

Calcium phosphates are widely used in bioprinting for bone tissue engineering as well as three-dimensional printing. Because of their beneficial qualities, certain calcium phosphates, like hydroxyapatite (Hap) and tricalcium phosphate (TCP), are frequently used as biomaterials for bone regeneration.

6.1. Hydroxyapatite

With a calcium to phosphorus molar ratio of 1.67, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) typically forms a hexagonal crystal structure, yet occasionally, depending on the orientation of the hydroxyl groups, it can display a monoclinic structure. Because of its exceptional biocompatibility and bioactivity, it is an essential bioceramic that is frequently used to simulate bone tissue and treat bone abnormalities. In a physiological setting, HAp interacts with surrounding tissues and fluids to cling to natural bone. At first, their mechanical characteristics are similar to those of cancellous bone: they are strong when compressed, but brittle and weak when under stress or shear. It is enhanced by various materials, structural modifications, sophisticated production methods, growth factor addition, and co-cultivation of bone regrowth cells, and when implanted in vivo, it promotes new bone formation and revascularization. HAp's ability to resorb is, however, limited by its high crystallinity and calcium to phosphorus ratio. HAp is widely applied as a coating on metallic implants to enhance tissue engineering and regenerative medicine because of its stability in vivo.

6.1.1. Hydroxyapatite (HAp)-Based 3D-Printed Scaffolds

3D-Printed Scaffolds Made of Hydroxyapatite (HAp) Because it allows for fine control over scaffold design and can replicate the internal structure and shape of defects, three-dimensional bioprinting technology is being researched extensively for the manufacture of scaffolds. This accuracy makes it possible to place tissue-engineering scaffolds steadily and precisely, which facilitates efficient bone conduction and tissue regeneration. HAp was one among the



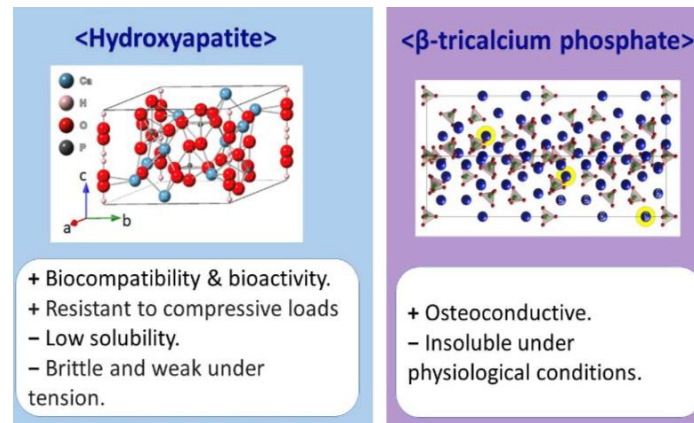
first calcium phosphates used in 3D printing because it contains chemicals that are similar to those found in natural bone. Nevertheless, pristine HAp's low tensile strength and low fracture toughness restrict its therapeutic application. Several improvements have been made to lessen this. Polymer integration into HAp-based scaffolds enhances mechanical characteristics and permits 3D printing of multilayer compositions. Graphene oxide has been used in some studies to improve the mechanical properties of HAp/polymeric biomaterials. In order to preserve minimal cytotoxicity while enhancing structural integrity, homogeneity, cell survival, and proliferation, cerium ions can be substituted for calcium ions in HAp. Biological investigations have demonstrated the positive effects of magnesium and zinc on osteogenic development. Strontium substitutions control the swelling behavior and ion release of scaffolds, while stoichiometric and strontium-containing HAp crystals both enhance the quality of scaffold printing. Drug-loaded HAp/polymer 3D scaffolds promote the growth of new bone, and the shape, size, and orientation of the pores in the scaffold control how the pharmaceuticals release.

6.2. Crystalline Tricalcium Phosphates

Tricalcium phosphate crystals Tricalcium phosphate (TCP) has a stoichiometric formula of $\text{Ca}_3(\text{PO}_4)_2$ and can exist in at least two crystalline forms: α -TCP and β -TCP. At temperatures higher than 1125°C , β -TCP transforms into α -TCP. α -TCP is metastable at room temperature and does not change much in dry conditions. The solubility of both forms is intermediate when compared to orthophosphates; however, α -TCP is more reactive in water and can easily hydrolyze into calcium phosphates that are more stable. Because α -TCP cannot be created by precipitating it from aqueous solutions and must be synthesized at high temperatures, its production presents problems that make it less suitable for tissue engineering applications. On the other hand, β -TCP is typically characterized in the trigonal setting and crystallizes in the rhombohedral space group. β -TCP has been more and more popular as an alternative to bone grafting. β -TCP is deemed osteoconductive despite being insoluble at physiological pH 7.4, as it is reabsorbed by a cell-mediated mechanism that includes macrophages and multinucleated giant cells (MNGCs). In an acidic environment, the rate at which β -TCP dissolves rises because MNGCs release an acid that dissolves β -TCP locally before it is absorbed and phagocytosed.

6.2.1. Beta-Tricalcium Phosphate (β -TCP)-Based 3D-Printed Scaffolds

Insufficient tissue regeneration, bone abnormalities, and a higher long-term fracture risk around HAp-based implants are among the issues that might arise from the lack of HAp degradation and may require permanent osteosynthesis fixation. On the other hand, β -TCP is resorbable and easily substituted by new bone tissue. Just like HAp, tricalcium phosphate is combined with printable polymers to improve the mechanical and bioactive properties of scaffolds that are 3D printed. We go over some recent research on β -TCP-infused 3D-printed scaffolds below. By combining β -TCP and poly(hydroxyl alkanoate), Ye et al. created scaffolds that had compressive strengths that were similar to those of real bone. The combination of thermoplastic polyurethane, poly(lactic-co-glycolic acid) (PLGA), and β -TCP produced 3D-printed scaffolds that were superior to commercially available calcium phosphate ink (OsteoInk®) in terms of mechanical characteristics, cytocompatibility, and osteoconductivity. The ability of polycaprolactone (PCL)/ β -TCP scaffolds to promote osteogenic cell development and a mineralized matrix has garnered interest. Poly(tri-methyl carbonate) (PTMC)/poly(ϵ -caprolactone)/ β -TCP scaffolds demonstrated little cytotoxicity, outstanding biocompatibility, and stimulated osteoblast growth. PCL frequently envelops the bioceramic during 3D printing, reducing the material's ability to interact with cells. Liu, K. et al. tackled this by incorporating PEG into scaffolds made of 3D-printed PCL/ β -TCP. Because of this, β -TCP release could be regulated, which enhanced wettability and in vitro mineralization. The combination of PEG and β -TCP resulted in considerable cell proliferation in the scaffolds after five days. In a clinical assessment, eight patients with complex zygomatic-maxillary abnormalities were treated using 3D-printed, patient-specific PCL/ β -TCP scaffolds. The assessment revealed acceptable volume conformity between preoperative simulations and actual implants. Jeong, W.S. et al. It is possible to add carbon nanotubes to improve mechanical qualities. Increased stiffness and enhanced early osteogenic responses were observed in 3D-printed scaffolds made from PCL combined with CNTs and calcium phosphates (HAp and β -TCP). β -TCP has the potential to decrease mechanical characteristics, as demonstrated by 3D-printed scaffolds made of polylactic acid that have better early osteogenic responses and more rigidity. On the other hand, β -TCP can occasionally lessen mechanical qualities. This is demonstrated by 3D-printed scaffolds based on polylactic acid, where β -TCP exhibits reduced mechanical properties in comparison to scaffolds made entirely of polylactic acid. Moreover, lignin addition has enhanced the mechanical robustness and cellular adherence of sodium acetate/ β -TCP 3D-printed scaffolds. Adding drugs to 3D-printed scaffolds is an additional way to promote bone growth. Dipyrindamole was added by Xu and colleagues via 3D printing to a poly(vinyl alcohol)/ β -TCP composite to improve the osteogenic differentiation, cell proliferation, and hydrophilicity of stem cells.



Fig(7) Characteristics of presented calcium phosphates.[2]

7. Perspective

Looking ahead, to fully realize the potential of 3D bioprinting in orthopedic tissue engineering, several crucial areas need to be advanced. Enhancing the biomimicry of bioprinted tissues remains a major challenge, with decellularized extracellular matrices (dECMs) holding promise as biomimetic ink materials due to their tissue-specific characteristics. However, these often originate from animals, leading to compatibility issues, inadequate printability, and poor mechanical qualities that hinder precise tissue fabrication. Innovations are needed to improve these characteristics and create bioinks that closely mimic the original tissue environment. Optimizing signaling tactics, such as co-culturing procedures, growth factor inclusion, and bioreactor systems, is imperative to enhance the biofunctionality of bioprinted constructs, ensuring full tissue repair and maturation. Additionally, more advanced techniques with higher resolutions are required to create structures that closely resemble biological tissues. Establishing a viable vascular network within bioprinted constructs is essential for regenerating highly vascularized tissues like bone and muscle, ensuring adequate nutrient and oxygen supply and waste removal. Although recent research has explored various bioprinting techniques to produce prevascularized tissue constructs, challenges remain in manufacturing three-dimensional thick tissues due to limitations in oxygen and nutrient diffusion. Further developments are necessary to mimic native-like hierarchical vascular organization and function. Bioprinted tissue structures must exhibit both biomechanics and biodegradability for successful implantation in living organisms. Scaffolds should provide initial mechanical stability and structural integrity during regeneration, with degradation rates matching the rate of new tissue formation. For load-bearing applications like bone and cartilage, scaffolds need to be mechanically strong with tunable biodegradability. More attention must be given to scaffold design and material qualities for optimal results. Reliable animal models are crucial for confirming the therapeutic effectiveness of bioprinted tissues. While smaller animal models can cause hesitancy in initiating human clinical trials, larger animal disease models can enhance the translational capabilities and predictive validity of bioprinted tissues for clinical practice. Addressing regulatory and ethical considerations is also imperative to ensure the quality, efficacy, and safety of bioprinted products for clinical use. In summary, despite significant advances in 3D bioprinting techniques for musculoskeletal tissue engineering, further research and development are needed to overcome current obstacles and implement these innovations in clinical settings. If 3D bioprinting techniques continue to evolve and expand, they hold the potential to revolutionize orthopedic tissue engineering and regenerative medicine.

8. Conclusion

In today's communities, orthopaedic disorders are common and can cause severe chronic pain, limited mobility, and a reduced quality of life. Global healthcare systems and patients alike bear significant socioeconomic costs as a result of these problems. The field of orthopaedic tissue engineering holds tremendous potential for creating functional tissue constructions for



regenerative treatments thanks to the significant advancements in 3D bioprinting technology in recent decades. These structures have shown great therapeutic promise in improving tissue repair and healing. However, despite these developments, the field of 3D bioprinting for orthopaedic purposes is still in its infancy and has a long way to go before it can be used in real-world applications and implemented in clinical settings. To fully harness the potential of 3D bioprinting, ongoing research must address several critical challenges, including the development of bioinks that closely mimic native tissue environments, the creation of functional vascular networks within bioprinted constructs, and the optimization of biomechanical properties and biodegradability of scaffolds. Furthermore, reliable animal models and comprehensive regulatory frameworks are essential to ensure the safety and efficacy of bioprinter tissues before transitioning to human clinical trials. Interdisciplinary collaboration between engineers, biologists, clinicians, and regulatory bodies will be crucial in overcoming these obstacles. The future of orthopaedic tissue engineering lies in the continuous improvement and refinement of bioprinting technologies and materials. With sustained effort and innovation, 3D bioprinting has the potential to revolutionize regenerative medicine, providing customized and effective treatments for patients suffering from orthopedic disorders. The journey from laboratory research to clinical application is complex and challenging however, the potential improvements in patient care and quality of life make this a valuable pursuit.

References

- [1] Sania Raees, Faheem Ullah, Fatima Javed, Hazizan Md. Akil, Muhammad Jadoon Khan, Muhammad Safdar, Israf Ud Din, Mshari A. Alotaibi, Abdulrahman I. Alharthi, M. Afroz Bakht, Akil Ahmad, Amal A. Nassar, Classification, processing, and applications of bioink and 3D bioprinting: A detailed review, International Journal of Biological Macromolecules, Volume 232, 2023, 123476. <https://doi.org/10.1016/j.ijbiomac.2023.123476>. (<https://www.sciencedirect.com/science/article/pii/S0141813023003689>)
- [2] Tolmacheva, N.; Bhattacharyya, A.; Noh, I. Calcium Phosphate Biomaterials for 3D Bioprinting in Bone Tissue Engineering. Biomimetics 2024, 9, 95. <https://doi.org/10.3390/biomimetics9020095>.
- [3] Yang Z, Yi P, Liu Z, Zhang W, Mei L, Feng C, Tu C and Li Z (2022) Stem Cell-Laden Hydrogel-Based 3D Bioprinting for Bone and Cartilage Tissue Engineering. Front. Bioeng. Biotechnol. 10:865770. doi: 10.3389/fbioe.2022.865770.
- [4] K.C.R. Kolan, J.A. Semon, A.T. Bindbeutel, D.E. Day, M.C. Leu, Bioprinting with bioactive glass loaded polylactic acid composite and human adipose stemcells, Bioprinting 18 (2020) e00075.
- [5] H. Cui, W. Zhu, M. Nowicki, X. Zhou, A. Khademhosseini, L.G. Zhang, Hierarchical fabrication of engineered vascularized bone biphasic constructs via dual 3D bioprinting: integrating regional bioactive factors into architectural design, Adv. Healthc. Mater. 5 (17) (2016) 2174–2181.
- [6] L.K. Narayanan, P. Huebner, M.B. Fisher, J.T. Spang, B. Starly, R.A. Shirwaiker, 3D-bioprinting of Polylactic Acid (PLA) nanofiber–alginate hydrogel bioink containing human adipose-derived stem cells, ACS Biomater. Sci. Eng. 2 (10)(2016) 1732–1742.
- [7] Ami R. Amini and Cato T. Laurencin and Syam P. Nukavarapu, Bone Tissue Engineering: Recent Advances and Challenges ,Critical Reviews™ in Biomedical Engineering,0278-940,2011,volume (40)(5)(363-408).
- [8] S. Vanaei, M.S. Parizi, S. Vanaei, F. Saleemizadehparizi, H.R. Vanaei, An overview on materials and techniques in 3D bioprinting toward biomedical application, Eng. Regen. 2 (2021) 1–18.
- [9] C.H. Cheng, M.Y. Shie, Y.H. Lai, N.P. Foo, M.J. Lee, C.H. Yao, Fabrication of 3Dprinted poly(lactic acid)/polycaprolactone scaffolds using TGF-β1 for promoting bone regeneration, Polymers 13 (21) (2021) 3731.
- [10] A. Asti, L. Gioglio, Natural and synthetic biodegradable polymers: different scaffolds for cell expansion and tissue formation, Int. J. Artif. Organs 37 (3)(2014) 187–205.
- [11] A.O. Inyang, C.L. Vaughan, Functional characteristics and mechanical performance of PCU composites for knee meniscus replacement, Materials 13 (8)(2020) 1886.
- [12] M. Griffin, N. Castro, O. Bas, S. Saifzadeh, P. Butler, D.W. Hutmacher, The current versatility of polyurethane three-dimensional printing for biomedical applications, Tissue Eng. Part B Rev. 26 (3) (2020) 272–283.
- [13] H.W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity, Nat.Biotechnol. 34 (3) (2016) 312–319.
- [14] J.Y. Park, J.H. Shim, S.A. Choi, J. Jang, M. Kim, S.H. Lee, D.W. Cho, 3D printing technology to control BMP-2 and VEGF delivery spatially and temporally to promote large-volume bone regeneration, J. Mater. Chem. B 3 (27) (2015)5415–5425.
- [15] J.H. Shim, K.M. Jang, S.K. Hahn, J.Y. Park, H. Jung, K. Oh, K.M. Park, J. Yeom, S.H. Park, S.W. Kim, J.H. Wang, K. Kim, D.W. Cho, Three-dimensional bioprinting of multilayered constructs containing human mesenchymal stromal cellsfor osteochondral tissue regeneration in the rabbit knee joint, Biofabrication8 (1) (2016) 014102.
- [16] Y. Sun, Y. You, W. Jiang, B. Wang, Q. Wu, K. Dai, 3D bioprinting dual-factor releasing and gradient-structured constructs ready to implant for anisotropic cartilage regeneration, Sci. Adv. 6 (37) (2020) eaay1422.
- [17] M. Chen, Z. Feng, W. Guo, D. Yang, S. Gao, Y. Li, S. Shen, Z. Yuan, B. Huang, Y. Zhang, M. Wang, X. Li, L. Hao, J. Peng, S. Liu, Y. Zhou, Q. Guo, PCL-MECM-based hydrogel hybrid scaffolds and meniscal fibrochondrocytes promote whole meniscus regeneration in a rabbit meniscectomy model, ACS Appl. Mater. Interfaces 11 (44) (2019) 41626–41639.
- [18] S. Chae, S.S. Lee, Y.J. Choi, D.H. Hong, G. Gao, J.H. Wang, D.W. Cho, 3D cell-printing of biocompatible and functional meniscus constructs using menis-cus-derived bioink, Biomaterials 267 (2021) 120466.
- [19] X. Jiang, S. Wu, M. Kuss, Y. Kong, W. Shi, P.N. Streubel, T. Li, B. Duan, 3D printing of multilayered scaffolds for rotator cuff tendon regeneration, Bioact.Mater. 5 (3) (2020) 636–643.
- [20] Y. Cao, S. Yang, D. Zhao, Y. Li, S.S. Cheong, D. Han, Q. Li, Three-dimensional printed multiphasic scaffolds with stratified cell-laden gelatin methacrylate hydrogels for biomimetic tendon-to-bone interface engineering, J. Orthop. Transl. 23 (2020) 89–100.
- [21] S. Chae, Y. Sun, Y.J. Choi, D.H. Ha, I. Jeon, D.W. Cho, 3D cell-printing of tendon-bone interface using tissue-derived extracellular matrix bioinks for chronic rotator cuff repair, Biofabrication 13 (3) (2021) 035005.
- [22] S. Chae, U. Yong, W. Park, Y.M. Choi, I.H. Jeon, H. Kang, J. Jang, H.S. Choi, D.W. Cho, 3D cell-printing of gradient multi-tissue interfaces for rotator cuff regeneration, Bioact. Mater. 19 (2023) 611–625.

- [23] W. Sun, B. Starly, A.C. Daly, J.A. Burdick, J. Groll, G. Skeldon, W. Shu, Y. Sakai, M. Shinohara, M. Nishikawa, J. Jang, D.W. Cho, M. Nie, S. Takeuchi, S. Ostrovidov, A. Khademhosseini, R.D. Kamm, V. Mironov, L. Moroni, I.T. Ozbolat, The bioprinting roadmap, *Biofabrication* 12 (2) (2020) 022002.
- [24] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, *Prog. Polym. Sci.* 37 (1) (2012) 106–126.
- [25] E. Axpe, M.L. Oyen, Applications of alginate-based bioinks in 3D bioprinting, *Int. J. Mol. Sci.* 17 (12) (2016) 1976.
- [26] J. Jia, D.J. Richards, S. Pollard, Y. Tan, J. Rodriguez, R.P. Visconti, T.C. Trusk, M.J. Yost, H. Yao, R.R. Markwald, Y. Mei, Engineering alginate as bioink for bioprinting, *Acta Biomater.* 10 (10) (2014) 4323–4331.
- [27] Q. Gao, B.S. Kim, G. Gao, Advanced strategies for 3D bioprinting of tissue and organ analogs using alginate hydrogel bioinks, *Marine Drugs* 19 (12) (2021) 708.
- [28] S. Nemati, A. Reza bakhsh, A.B. Khoshfetrat, A. Nourazarian, Ç. Biray Avci, B. Goker Bagca, H. Alizadeh Sardroud, M. Khaksar, M. Ahmadi, A. Delkhosh, E. Sokullu, R. Rahbarghazi, Alginate-gelatin encapsulation of human endothelial cells promoted angiogenesis in in vivo and in vitro milieu, *Biotechnol. Bio-eng.* 114 (12) (2017) 2920–2930.
- [29] J.A. Semba, A.A. Mieloch, J.D. Rybka, Introduction to the state-of-the-art 3D bioprinting methods, design, and applications in orthopedics, *Bioprinting* 18 (2020) e00070.
- [30] L. Cen, W. Liu, L. Cui, W. Zhang, Y. Cao, Collagen tissue engineering: development of novel biomaterials and applications, *Pediatr. Res.* 63 (5) (2008) 492–496.
- [31] R. Parenteau-Bareil, R. Gauvin, F. Berthod, Collagen-based biomaterials for tissue engineering applications, *Materials* 3 (3) (2010) 1863–1887.
- [32] S. Rhee, J.L. Puetzer, B.N. Mason, C.A. Reinhart-King, L.J. Bonassar, 3D bioprinting of spatially heterogeneous collagen constructs for cartilage tissue engineering, *ACS Biomater. Sci. Eng.* 2 (10) (2016) 1800–1805.
- [33] Y.B. Kim, H. Lee, G.H. Kim, Strategy to achieve highly porous/biocompatible macroscale cell blocks, using a collagen/genipin-bioink and an optimal 3D printing process, *ACS Appl. Mater. Interfaces* 8 (47) (2016) 32230–32240.
- [34] M. Yeo, J.S. Lee, W. Chun, G.H. Kim, An innovative collagen-based cell-printing method for obtaining human adipose stem cell-laden structures consisting of core-sheath structures for tissue engineering, *Biomacromolecules* 17 (4) (2016) 1365–1375.
- [35] S. Vanaei, M.S. Parizi, S. Vanaei, F. Saleemizadehparizi, H.R. Vanaei, An overview on materials and techniques in 3D bioprinting toward biomedical application, *Eng. Regen.* 2 (2021) 1–18, <https://doi.org/10.1016/j.engreg.2020.12.001>.
- [36] R.F. Pereira, P.J. B´artolo, 3D bioprinting of photocrosslinkable hydrogel constructs, *J. Appl. Polym. Sci.* 132 (48) (2015), <https://doi.org/10.1002/app.42458>.
- [37] P. Rider, “Z.P. Ka” carevi’c, S. Alkildani, S. Retnasingh, M. Barbeck, Bioprinting of tissue engineering scaffolds, p. 2041731418802090, *J. Tissue Eng.* 9 (Oct. 2018), <https://doi.org/10.1177/2041731418802090>.
- [38] H. Gudapati, M. Dey, I. Ozbolat, A comprehensive review on droplet-based bioprinting: past, present and future, *Biomaterials* 102 (Sep. 2016) 20–42, <https://doi.org/10.1016/j.biomaterials.2016.06.012>.
- [39] M. Kotlarz, A.M. Ferreira, P. Gentile, S.J. Russell, K. Dalgarno, Droplet-based bioprinting enables the fabrication of cell-hydrogel-microfibre composite tissue precursors, *Bio-Des. Manuf.* 5 (3) (Jul. 2022) 512–528, <https://doi.org/10.1007/s42242-022-00192-5>.
- [40] Z. Wang, R. Abdulla, B. Parker, R. Samanipour, S. Ghosh, K. Kim, A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks, *Biofabrication* 7 (4) (Dec. 2015), 045009, <https://doi.org/10.1088/1758-5090/7/4/045009>.
- [41] S.S. Mahdavi, M.J. Abdekhodaie, H. Kumar, S. Mashayekhan, A. Baradaran-Rafii, K. Kim, Stereolithography 3D bioprinting method for fabrication of human corneal stroma equivalent, *Ann. Biomed. Eng.* 48 (7) (Jul. 2020) 1955–1970, <https://doi.org/10.1007/s10439-020-02537-6>.
- [42] L. Elomaa, C.-C. Pan, Y. Shanjani, A. Malkovskiy, J.V. Sepp” al” a, Y. Yang, Three-dimensional fabrication of cell-laden biodegradable poly(ethylene glycol-co-depsipeptide) hydrogels by visible light stereolithography, *J. Mater. Chem. B* 3 (42) (Oct. 2015) 8348–8358, <https://doi.org/10.1039/C5TB01468A>.
- [43] S. Wasti, S. Adhikari, Use of biomaterials for 3D printing by fused deposition modeling technique: a review, *Front. Chem.* 8 (2020). Accessed: Nov. 26, 2022. [Online]. Available: <https://www.frontiersin.org/articles/10.3389/fchem.2020.00315>.

- [44] Filip, D.G.; Surdu, V.A.; Paduraru, A.V.; Andronesu, E. Current Development in Biomaterials-Hydroxyapatite and Bioglass for Applications in Biomedical Field: A Review. *J. Funct. Biomater.* 2022, 13, 248. [CrossRef]
- [45]. Feng, C.; Zhang, K.; He, R.; Ding, G.; Xia, M.; Jin, X.; Xie, C. Additive manufacturing of hydroxyapatite bioceramic scaffolds: Dispersion, digital light processing, sintering, mechanical properties, and biocompatibility. *J. Adv. Ceram.* 2020, 9, 360–373. [CrossRef]
- [46] Niu, Y.; Chen, L.; Wu, T. Recent Advances in Bioengineering Bone Revascularization Based on Composite Materials Comprising Hydroxyapatite. *Int. J. Mol. Sci.* 2023, 24, 12492. [CrossRef]
- [47]. Trzaskowska, M.; Vivcharenko, V.; Przekora, A. The Impact of Hydroxyapatite Sintering Temperature on Its Microstructural, Mechanical, and Biological Properties. *Int. J. Mol. Sci.* 2023, 24, 5083. [CrossRef]
- [48] Anita Lett, J.; Sagadevan, S.; Fatimah, I.; Hoque, M.E.; Lokanathan, Y.; Léonard, E.; Alshahateet, S.F.; Schirhagl, R.; Oh, W.C. Recent advances in natural polymer-based hydroxyapatite scaffolds: Properties and applications. *Eur. Polym. J.* 2021, 148, 110360. [CrossRef]
- [49]. Bohner, M.; Santoni, B.L.G.; Dobelin, N. beta-tricalcium phosphate for bone substitution: Synthesis and properties. *Acta Biomater.* 2020, 113, 23–41. [CrossRef] [PubMed]
- [50] C.R. Alcala-Orozco, X. Cui, G.J. Hooper, K.S. Lim, T.B.F. Woodfield, Converging functionality: strategies for 3D hybrid-construct biofabrication and the role of composite biomaterials for skeletal regeneration, *Acta Biomater.* 132 (2021)188–216.