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Advances in 3D bioprinting to enhance translational applications in bone tissue engineering and regenerative medicine

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Summary. Bone defects are due to trauma, infections, tumors, or aging, including bone fractures, bone metastases, osteoporosis, or osteoarthritis. The global burden of these demands research into innovative strategies that overcome the limitations of conventional autografts. In this sense, the development of three-dimensional (3D) bioprinting has emerged as a promising approach in the field of tissue engineering and regenerative medicine (TERM) for the on-demand generation and transplantation of tissues and organs, including bone. It combines biological materials and living cells, which are precisely positioned layer by layer. Despite obtaining some promising results, 3D bioprinting of bone tissue still faces several challenges, such as generating an effective vascular network to increase tissue viability. In this review, we aim to collect the main knowledge on methods and techniques of 3D bioprinting. Then, we will review the main biomaterials, their composition, and the rationale for their application in 3D bioprinting for the TERM of bone.

Key words: 3D bioprinting, Bioink, TERM, Bone, Biomaterials, Hydrogel

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Introduction

Three-dimensional (3D) bioprinting, an additive bio-fabrication process, employs living cells and biomaterials to construct functional tissues and organs layer by layer, guided by 3D digital models (Skeldon et al., 2018; Theus et al., 2020). In the late 1990s, the healthcare sector saw the advent of 3D printing, initially utilized by surgeons for printing dental implants, customized prostheses, and kidney bladders. Subsequently, the concept of 3D bioprinting emerged, featuring bioink as the material, comprising living cells, biomaterials, or active biomolecules (Mendoza-Cerezo et al., 2023). The term bio-fabrication describes a process yielding a defined product with biological function, utilizing cells as building blocks and other materials as cement to craft 3D constructions (Salaris and Rosa, 2019). It is crucial to distinguish 3D bioprinting from 3D printing, as the former uses cell-loaded bioinks and other biological products to construct living tissues, while the latter produces a porous polymeric scaffold for cell seeding (Vijayavenkataraman et al., 2018). Aligned with the principles of additive manufacturing, 3D bioprinting involves the gradual deposition of bioink layer by layer to fabricate 3D structures, such as tissues or organs. In this context, the evolving domain of tissue engineering and regenerative medicine (TERM) has contributed significantly to the advancement of 3D bioprinting (Leon-Oliva et al.,

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2023).

The classification of 3D bioprinting broadly encompasses extrusion, laser bioprinting, and droplet techniques. Extrusion-based bioprinting utilizes mechanical, solenoid, or pneumatic dispensing systems to deposit bioinks in the form of continuous filaments (Ozbolat and Hospodiuk, 2016). Conversely, laser-based bioprinting employs laser power and the photo-polymerization principle to print 3D structures, offering precise cell positioning through techniques like laser direct writing and laser-induced direct transfer (LIFT). Droplet-based bioprinting relies on the generation of bioink droplets via thermal, electrical, or acoustic stimulation (Gudapati et al., 2016).

The choice of bioinks varies for each bioprinting modality, depending on factors such as rheology, viscosity, crosslinking chemistry, and biocompatibility. Extrusion-based bioprinting requires shear-thinning bioinks, while droplet or inkjet bioprinting demands low-viscosity materials. To overcome challenges, bioinks can be extruded onto a granular support bed incorporating yield-strength hydrogels, preventing collapse by solidifying around the extruded structure, additionally 3D bioprinting is instrumental in creating *in vitro* tissue models for drug screening, disease modeling, and other *in vitro* applications (Heo et al., 2020). The technique of 3D bioprinting finds extensive applications in tissue engineering (Gao and Cui, 2016), which involves combining cells, biomaterials, biochemical factors, and engineering technologies to generate biomimetic organ and tissue substitutes, addressing damage caused by injury or disease (Heinrich et al., 2019). Research in tissue engineering, particularly in regenerative medicine, aims to design functional tissue or organs to replace dysfunctional or damaged ones (Cui et al., 2017).

Presently, a significant challenge in healthcare is the scarcity of organs for transplantation and the potential risk of immunological rejection of donated tissues. This underscores the need for novel methodologies to address these problems. Bioprinting emerges as a promising solution to the organ shortage crisis, by combining biomaterials and various cell types, replicating the native microenvironment and biological behavior of the tissue (Zhu et al., 2016). Over the last three decades, 3D bioprinting has undergone substantial development and is extensively used to fabricate 3D cell scaffolds and medical implants with notable applications in regenerative medicine (Park et al., 2017). In this sense, 3D bioprinting is a potential solution to alleviate the problems posed by arthritis and other causes of bone defects in the field of orthopedics. Bioprinting the construction of intricate bottom-up tissue constructs. It can enhance the potential for creating intrinsic vascular structures by allowing the printing of internal channels containing vascular cells within the constructs facilitating *in vivo* blood vessel growth (Murphy and Atala, 2014). In contrast, the conventional tissue engineering method of seeding cells into a prefabricated scaffold lacks the precision required to place cells or biological content in 3D, limiting the ability to build complex hierarchical tissue formations (Shafiee and Atala, 2016). Bioprinters often have multiple printing nozzles, allowing the incorporation of various combinations of cells and biomaterials into a printed construct. This allows a high level of spatial control over the architecture and content of the construct. After printing, the construct can be implanted directly into a patient or undergo maturation *in vitro*. Bioreactors, which serve as biologically active culture environments, are available to guide and support cell growth toward specific tissue types (Li et al., 2016).

On the other hand, 3D printing can be advantageous for plastic and reconstructive surgeons to create patient-specific tissue substitutes with tissue-like functions and mechanical properties. 3D bioprinting offers an alternative approach to fabricating patient nose/ear structures suitable for implantation (Fulco et al., 2014). Unlike traditional scaffold fabrication methods, 3D bioprinting can produce patient scaffolds/constructs with controlled architectures without the need for molding. Also, cells can be precisely printed, which offers greater special precision compared with conventional cell seeding in porous scaffolds (Kang et al., 2016).

In this review, we aim to collect the main knowledge on methods and techniques of 3D bioprinting. Then, we will review the main biomaterials, their composition, and the rationale for their application in 3D bioprinting for the TERM of bone and cartilage.

3D Bioprinting methods and techniques

The success of 3D bioprinting depends on several biomechanical parameters, such as the rheological properties of the bioinks, the surface tension, the printing flow rate, and crosslinking/solidification of the bioinks, and the maintenance of cell viability, as cells undergo post-bioprinting stress caused by environmental factors, e.g. pH and temperature, as well as the duration and intensity of these factors (Ning et al., 2020). Over the last decade, a wide variety of bioprinting strategies have been developed to obtain viable functional constructs for application in TERM. The main bioprinting techniques can be classified into four modalities: extrusion-based, inkjet-based, laser-assisted, and stereolithographic. The characteristics of these technologies must be considered for the different biomedical applications.

Methods

The technique of 3D bioprinting finds extensive applications in tissue engineering (Gao and Cui, 2016), which involves combining cells, biomaterials, biochemical factors, and engineering technologies to generate biomimetic organ and tissue substitutes, addressing damage caused by injury or disease (Heinrich et al., 2019). Research in tissue engineering, particularly in regenerative medicine, aims to design functional

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3D bioprinting incorporates fundamental elements of conventional two-dimensional (2D) printing, such as a desktop printer (3D printer), print file (3D model file), ink (bioink), and printing platform (paper) (Shapira and Dvir, 2021). However, in contrast to 2D printing, 3D bioprinting constitutes a process that involves various design considerations, including the use of imaging, modeling, printer selection, choice of bioinks, culture conditions, and the development of 3D constructs (Masaeli et al., 2019). In general terms, the bioprinting process can be broken down into three distinct phases: pre-bioprinting, bioprinting, and post-bioprinting (Fig. 1) (Zhu et al., 2016). The pre-bioprinting or modeling phase involves obtaining 3D images of the anatomical structure of the tissues, 3D digital design, and the choice of biomaterials/bioinks depending on the type of 3D bioprinting model. An essential requirement for replicating complex and heterogeneous tissues or organs is a thorough understanding of their components (Gao et al., 2023). To obtain information on 3D structure and function at the cellular, tissue, and organ level, various imaging technologies, such as computed tomography (CT) or magnetic resonance imaging (MRI), are applied. From the images obtained, 3D models are constructed using computer-aided design (CAD) programs, which are stored as stereolithography (STL) files, a format commonly used in bioprinters (Di Somma et al., 2019).

As for the choice of biomaterial or bioink, this depends on the type of bioprinter and the required properties of the final product. Suitable bioinks with properties that mimic the tissue to be printed are selected and primary cells are collected from the patient. These cells are suspended in the bioinks, generating cell-loaded bioinks that are used as ink for the bioprinters (Xia et al., 2021).

A 3D structure with a patient-specific design is printed in the layer-by-layer deposition process during the bioprinting phase. In this phase, the printer interprets the STL file and deposits successive layers of liquid, powder, or other materials to build the 3D model from a series of 2D cross-sections (Jakus et al., 2016).

The post-bioprinting phase encompasses the maturation of the cell-loaded printed constructs using bioreactor technologies that provide an environment conducive to tissue cell development (Guyette et al.,

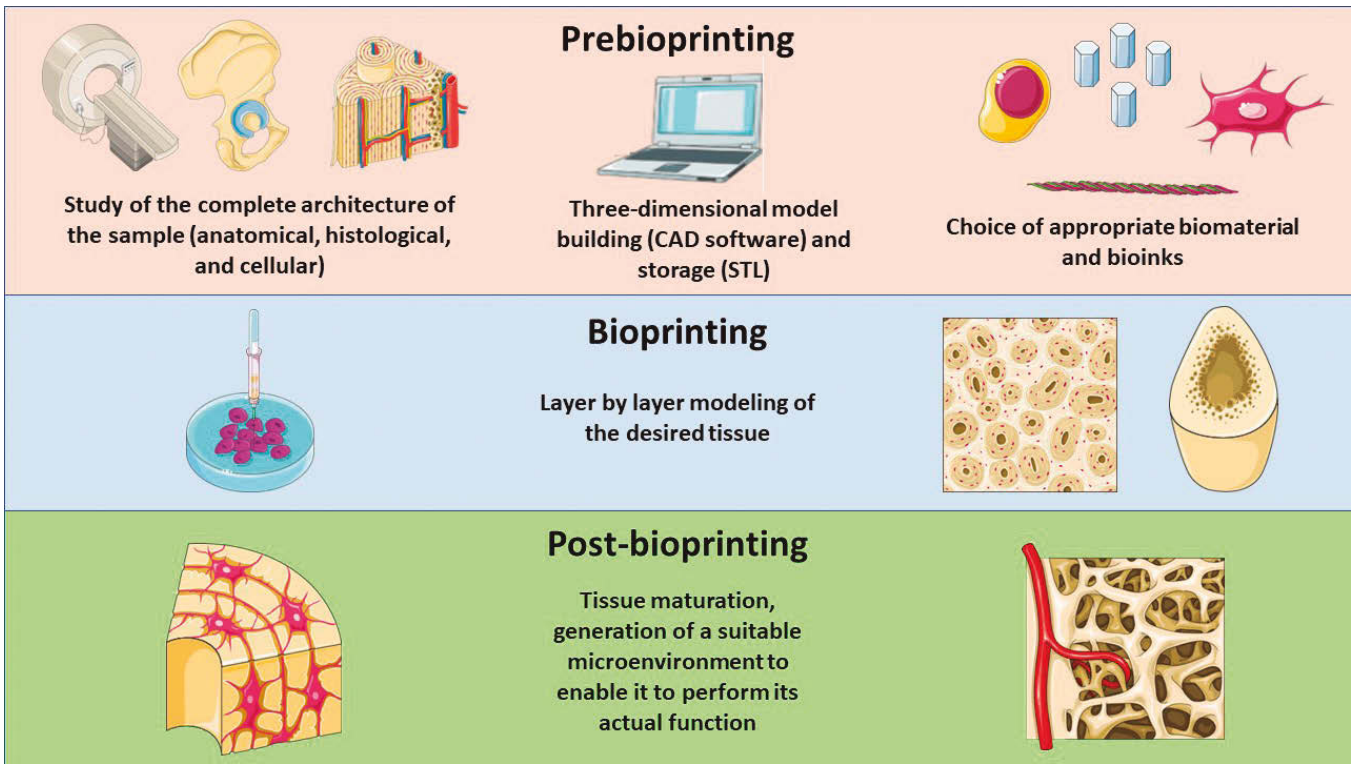

Fig. 1. Schematic representation of the end-to-end bioprinting workflow, including pre-bioprinting preparatory phases and post-printing procedures.

2016). This stage, crucial for the development of biomimetic structures, mechanical supports, and biological functionality is essential for establishing a suitable microenvironment for the growth of mature tissues/organs (Patrocinio et al., 2023). Cells must proliferate to establish cell-cell connections and communicate with each other, as well as secrete extracellular matrix components and perform specific biological functions to integrate into the host tissue effectively.

Tissue 3D bioprinting strategies

In the last decade, a wide variety of bioprinting strategies have been developed to obtain viable functional constructs for application in TERM. The main bioprinting techniques can be classified into four modalities: extrusion-based, inkjet-based, laser-assisted, and stereolithography-based (Murphy and Atala, 2014; Bejoy et al., 2021). Each of them has different characteristics, such as biological materials, resolution, printing speed, and cell viability. The advantages and limitations of the different 3D bioprinting methods must be considered for the construction of functional tissue in the recipient.

Extrusion-based 3D bioprinting consists of the deposition of biomaterials through nozzles to create 3D structures. It can be driven by either a pneumatic pressure-, piston-, or screw-based system to dispense cell-laden bioink (Ozbolat and Hospodiuk, 2016). It is one of the most employed methods because it is versatile and affordable. It presents the advantages of printing various biologics, including cells, tissues, tissue constructs, organ modules, and microfluidic devices (Ramesh et al., 2021). It can print materials with a wide range of viscosities (30 mPa/s to $>6\times10^7$ mPa/s) and elevated cell density (including cell spheroids) (Gillispie et al., 2020). However, the cells are subjected to mechanical forces, especially shear stress, and, along with the high viscosity of some bioinks, this induces cell stress and damage, reducing the cellular viability of this technique (Boularaoui et al., 2020). Pneumatic extrusion-based bioprinting uses a pressurized air pump to disperse the bioink through the nozzle (Wenger et al., 2022). In piston-driven systems, the extrusion of material from the nozzle occurs through the application of a force generated by a piston, usually attached to a motor via a screw. In contrast, screw-driven systems use the rotary movement of the screw directly to propel the material (Gu et al., 2020; Liu et al., 2023). An additional advantage of this methodology is its scalability and the ability to print tissues on a human scale. This is facilitated by the uninterrupted flow of bioink and the high deposition and printing speeds. However, the resolution of the constructs produced remains relatively low, from 30 to 100 μm, mainly due to the dimensions of the printing nozzle (Fakhruddin et al., 2018; Fu et al., 2021).

Another type of nozzle-based bioprinting technique is an inkjet-based bioprinting system. Inkjet bioprinting, the first bioprinting technology released in 2003 by Wilson and Boland, originates from the modification of traditional 2D inkjet printing (Wilson and Boland, 2003). Inkjet bioprinters deliver a regulated volume of bioink onto the printing surface, inducing a continuous flow of the substance (in continuous inkjet printing) or drop out from the nozzle as needed (in droplet on demand (DOD) inkjet printing) (Takagi et al., 2019). The latter is preferred due to the regulation of the droplets. The DOD printer head exerts thermal or piezoelectric forces to deposit droplets onto a substrate, which can be included in the final product (Park et al., 2023). Inkjet bioprinting makes it possible to control the size and deposition of the bioink, presenting high resolution and printing speed and low cost. Some of the drawbacks of this technique are the limitation of the high viscosities of the bioink and the biomaterial has to be in a liquid form because of the low mechanical strength and size of the nozzle (Li et al., 2020a; Yumoto et al., 2020).

Laser-assisted bioprinting (LAB) was developed by the Naval Research Laboratory. LAB substitutes the nozzle for a laser source and a focusing system layer of liquid bioink solution, which employs the laser for the accurate deposition of biomaterials in a receiving substrate, usually placed on a 3D movable platform (Chang and Sun, 2023). When the laser hits the ribbon, the biomaterial starts evaporating and forms droplets that are deposited on the substrate. The absence of a nozzle and mechanical forces allows high resolution, high cell viability (>95%), and the ability to deposit high viscous bioink (Dou et al., 2021; Ventura, 2021). However, LAB printing modules make this method highly expensive and, therefore, unsuitable for generating extensive tissue structures. In addition, the choice of materials is limited to photosensitive polymers, which restricts the range of biomaterials compatible with this method.

Lastly, stereolithography-based bioprinting employs ultraviolet (UV) light or visible light to induce polymerization of a photocrosslinkable biomaterial in a layer-by-layer process to create 3D structures (Li et al., 2023). A computer-controlled laser beam cures a liquid photocrosslinkable bioink accumulated in a vat, and a build platform lifts up or down when a layer has been completely printed via point-by-point curing (Kumar and Kim, 2020). This technique presents high resolution (~ 6 μm), rapid printing, and the ability to print highly viscous materials (Wang et al., 2015). In addition, it allows the creation of implantable scaffolds with precise anatomical geometry, controlled surface characteristics, and adjustable physical and chemical properties (Grigoryan et al., 2021).

Bioinks

"Bioink" is the printed biomaterial defined as an ink formulation that facilitates the printing of living cells and macromolecules, such as GFs. The success of bioprinting projects depends on the careful selection of a suitable bioink. The ideal bioinks should be compatible

with biological materials and with the printing process, offering the biological, mechanical, and functional properties necessary for the development of tissue constructs (Gungor-Ozkerim et al., 2018). Some of the ideal properties include good biocompatibility and biodegradability, degradability, easy crosslinking mechanism, and robust mechanical properties. The bioinks are cross-linked or stabilized during or immediately after the bioprinting to generate the programmed tissular construction (Hospodiuk et al., 2017). The printability of the bioink depends on its rheological properties and crosslinking mechanisms. During the printing process, several biomechanical parameters that affect the bioink must be considered for achieving the generation of a suitable tissue construct with high shape fidelity. This includes flow pattern, viscosity, viscoelasticity, surface tension, flow rate, and mechanical forces such as hydrostatic pressure, shear stress, and extensional stress, which will define the printability of a biomaterial (Ning et al., 2020; Schwab et al., 2020; Kim, 2023).

The biomaterials serve as the basis for the construction of scaffolds for the delivery of cells and growth factors (GFs) to support cell growth, as well as for the creation of a vasculature network that ensures the long-term survival of the construct. A wide variety of biomaterials have been employed in 3D bioprinting, highlighting the use of hard biomaterials (ceramics), soft biomaterials (hydrogels), and nanoparticles, and their application will be reviewed in the next section (Hospodiuk et al., 2017; Mao et al., 2020; Vanaei et al., 2021). Three different types of cells are loaded in the biomaterial: primary cells, cell lines, and stem cells (SCs). SCs are capable of self-renew and differentiate into different phenotypes under guidance. Mesenchymal stromal cells (MSCs) require an osteogenic medium, which includes β-glycerophosphate, ascorbic acid, and dexamethasone, for their differentiation into osteoblasts and osteocyte lineages (Levato et al., 2014). Lastly, GFs are sometimes added to help regeneration and angiogenesis or stimulate SC differentiation, including transforming growth factor-β (TGF-β), insulin-like growth factors (IGF), bone morphogenetic proteins (BMP), vascular endothelial growth factor (VEGF), and parathyroid hormone (PTH) (Yazdanpanah et al., 2022).

Biomaterials used in 3D bioprinting in TERM of bone

Hard biomaterials

Bioinks for hard tissues originate from natural or synthesized materials incorporating metallic, ceramic, or polymeric (thermoplastic) components; these make it possible to create structures with robust mechanical properties, which makes them suitable for bone tissue bioprinting (Chia and Wu, 2015). Due to the inherent self-healing and regenerative capabilities of bone, most scaffolds produced focus on sending regenerative signals to osteogenic cells to promote further regeneration and repair. A key challenge in designing foundations for load-bearing applications is the simultaneous customization of competing biomaterial requirements (Chocholata et al., 2019). Hard tissue scaffolds must not only be biocompatible, integrate seamlessly with native tissue, and be easy to fabricate, but must also exhibit an optimal replacement rate and a highly porous structure without significantly compromising mechanical properties. Commonly used alloplastic synthetic scaffolds for bone grafting, which emulate native bone tissue and provide structural and mechanical support, include metals, biomimetic ceramics, and composites (Nikolova and Chavali, 2019). Bone is a hard tissue that presents a mineralized matrix and different cell types that act through the cycle of deposition and resorption of the matrix (Fig. 2).

Much research has been conducted in the field of dentistry on the application of the selective laser melting (SLM) method, which uses a laser to build 3D dental prostheses using metal powder (Hong et al., 2017). Therefore, the use of Co-Cr as a hard material in the use of 3D bioprinting has been studied and the results suggest that the biocompatibility of 3D bioprinted Co-Cr alloy is promising and may find wide applications in dental prosthodontics (Ganbold et al., 2019).

Due to its inherent degradability within the body, magnesium (Mg) and its alloys are gaining increasing interest for applications in orthopedics and cardiovascular procedures (Arif et al., 2022). The inclusion of micro-nano-scale Mg particles in biodegradable polymers significantly improves the strength, biocompatibility, and degradability of scaffolds and implants. The use of biodegradable polymer implants also improves overall quality of life, especially in an aging society, by eliminating the need for secondary surgeries often necessary to remove permanent implants, thus substantially reducing healthcare costs (Tsakiris et al., 2021).

Titanium alloy (Ti6Al4V) is one of the most widely used synthetic bone substitutes due to its biocompatibility and adequate mechanical properties, using a material extrusion technique, 3D PLA-Ti6Al4V (Ti64) scaffolds with open pores and interconnected channels were successfully fabricated (Zhao et al., 2021). The strategy of incorporating cells into 3D bioprinted porous Ti6Al4V scaffolds filled with bone marrow stromal cells (BMSCs) and EPC-loaded hydrogel as a composite implant aims to induce angiogenesis and osteogenesis, thus promoting osseointegration (Omorphos et al., 2021).

Zinc (Zn) is an essential trace element for several physiological functions of the human body, such as enzyme production, gene expression, signal transmission, nucleic acid metabolism, apoptosis control, growth promotion, and tissue regeneration (Sorahinobar et al., 2023). Recent research has identified potent anti-atherogenic properties associated with Zn. In recent years, zinc (Zn) has emerged as a promising substitute for iron and magnesium alloys in the production of

biodegradable medical implants, due to its near-ideal degradation rate (Venezuela et al., 2019). Consequently, it has attracted attention as a potential candidate for dental implants, in its application in degradable cardiovascular stents. However, the creation of zinc implants by selective laser melting (SLM) is complicated by their low melting and boiling points, and high susceptibility to oxidation, resulting in increased porosity in the fabricated components (Guillory et al., 2019).

Zirconium dioxide (ZrO_2), commonly known as zirconium, is a white crystalline oxide of zirconium (Kozakiewicz et al., 2021). Its outstanding mechanical properties, characterized by high compressive strength, flexural strength, tensile strength, and flexibility, make it ideal for applications requiring load-bearing in bone. However, its bio-inertness and high stiffness make zirconia unsuitable for situations where flexibility and osseointegration are crucial (Aboushelib and Shawky, 2017).

Soft biomaterials

Hydrogels are cross-linked 3D polymeric networks capable of swelling without dissolving in an aqueous medium (Gao et al., 2019). These hydrogels effectively mimic various properties of the cellular environment, known as the extracellular matrix (ECM), allowing homogeneous and efficient cell seeding within a mechanically stable network (Bedell et al., 2020). These characteristics make hydrogels desirable materials for biomedical applications (Jungst et al., 2016). In the field of tissue engineering, hydrogels have attracted a great deal of attention due to their flexible synthesis, methods, diverse structures, biocompatibility, desirable physical properties, and adjustable pore size (Dutta et al., 2019).

Hydrogels can maintain their distinctive 3D structures, offering mechanical support to cells in engineered tissues and replicating the ECM (Luo et al., 2019). Hydrogels with a high water content and a construction that mimics native tissues can create an ideal environment for cell survival in tissue engineering applications (Chinga-Carrasco, 2018). The main criteria for the use of hydrogels in 3D printing include maintaining a high viscosity so that they remain fluid during the printing process and solidifying and retaining their original shape after printing without the need for additional post-processing (Chinta et al., 2021). In addition, printed layers must exhibit strong adhesion to adjacent layers and retain their shape without the need for supplementary support (Podstawczyk et al., 2020).

Gelatin is the result of the partial hydrolysis of

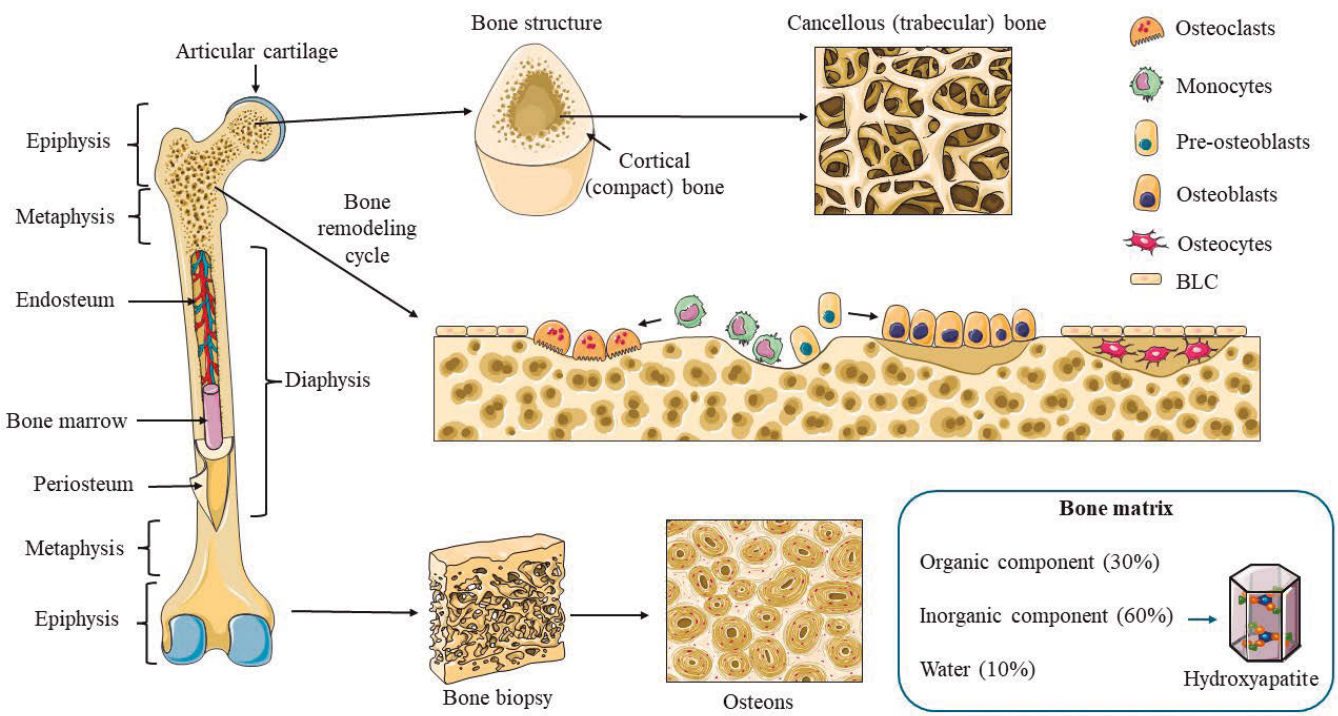

Fig. 2. Bone anatomy and histology. The figure shows both the anatomical and histological structure of bone, including the bone remodeling cycle. Bone tissue or bone is composed of cells and matrix. The latter is composed of the inorganic element, calcium salts mainly represented by hydroxyapatite (60%), the organic component (30%), type I collagen and other proteins, and water (10%). At a macroscopic level, it can be distinguished as both cancellous and compact bone. The epiphysis is formed by a thin layer of compact bone, and within it is cancellous bone containing red bone marrow. The bone remodeling cycle takes place due to the opposing actions of osteoclasts, which mediate bone resorption, and osteoblasts, which deposit bone matrix. The osteoblasts become embedded in the mineralized bone matrix and transform into osteocytes. BLC: bone-lining cells.

collagen, obtained by acid or basic hydrolysis of animal bones and tendons. It has remarkable biocompatibility characteristics and low immunogenicity (Gungor-Ozkerim et al., 2018). In addition, it has RGD sequences in its structures that promote cell adhesion and matrix metalloproteinase (MMP) target sequences that facilitate cell remodeling. Methacryloyl-modified gelatin hydrogels (GelMA), like gelatin, are biodegradable, biocompatible, and non-immunogenic, retaining the cell adhesive properties of gelatin. In this sense, they meet the requirements of integrity and mechanical resistance of printed constructions (Zhang et al., 2016). As for their applications, they are mainly used as sacrificial materials for channel formation, facilitating the transmission of oxygen and nutrients that promote cell proliferation and differentiation (Zhang and Wang, 2019).

Hyaluronic acid (HA) is a glycosaminoglycan present in the natural ECM. Similar to gelatin, it can be modified with methacrylate (HAMA) to create a hydrogel with mechanical properties ideal for bioprinting cellular constructs (Ding et al., 2023). Due to its structural and biological properties, hyaluronic acid can facilitate cell signaling, wound repair, and extracellular matrix organization. In conclusion, it promotes cell viability and stem cell differentiation when combined with gelatin to form hydrogels (Poldervaart et al., 2017).

Fibrinogen, which is found in high levels in PRP (platelet-rich plasma), can create a contact-polymerized fibrin network (Li et al., 2020b). Some studies formulated a silk fibroin bioink containing PRP for cartilage regeneration; similarly, other studies detailed the creation of a PRP-based bioink for cartilage tissue engineering, incorporating Gel-MA to enhance PRP with calcium and thrombin (Fernandes and Yang, 2016). However, an extrusion-based printing method is not suitable for a cross-linked fibrin network, as the resulting structure would lack robustness (Ng et al., 2016).

Chitosan proves to be a favorable natural polysaccharide for bioink applications due to its attractive advantages such as biodegradability, biocompatibility, cost-effectiveness, and non-immunogenicity (Lazaridou et al., 2022). Various improvements, such as crosslinking by chemical agents or irradiation, the inclusion of thickening molecules or nanoparticles, and functionalization of the chitosan molecule, present potential solutions to improve the integrity and function of articular cartilage and bone regeneration (Foyt et al., 2018; Marques et al., 2019; Li et al., 2021).

Oligo (poly(ethylene glycol) fumarate) (OPF) is a biocompatible polymer composed of hydrophilic poly (ethylene glycol) (PEG) chains with bonded double bonds (Liu et al., 2017b). When exposed to UV radiation, these double bonds open and crosslink with each other, creating a soft hydrogel (Liu et al., 2017a). The cross-linked OPF hydrogel undergoes *in situ* biodegradation by hydrolysis of the ester bond, demonstrating remarkable biocompatibility in various *in vitro* and vivo studies focused on bone and nerve tissue regeneration (Olthof et al., 2018).

Nanoparticles

Nanohydroxyapatite (nHA), a calcium phosphate mineral very similar to the inorganic components of bone, is being used in bone tissue engineering for its supportive functions for bone growth and osseo-integration. Other nanomaterials have been used to increase the strength of 3D bioprinted bone constructs. Controlling the phosphate ion concentration allows the thickness of the nHA to be easily regulated, resulting in higher modulus in alginate/gelatin scaffolds compared with scaffolds without the nHA coating (Wasti and Adhikari, 2020).

Cellulose nanocrystals (CNC) obtained from the abaca plant were used to improve the durability of 3D bioprinted biomaterial constructs by taking advantage of their mechanical strength and inherent stiffness (Yang et al., 2013). Wood-derived nanocellulose exhibits excellent aqueous dispersion, enabling its direct use in additive manufacturing to accurately 3D bioprint precise structures. Systematic evaluation of the repeatability of extrusion 3D bioprinted constructions was carried out, along with a thorough examination of volumetric and anisotropic deformation (Gillispie et al., 2020).

Conclusions

In summary, advances in 3D bioprinting for bone tissue engineering present promising prospects for regenerative medicine. The ongoing search for new biomaterials to optimize tissue regeneration, along with strategies to create vascular networks within constructs, are a key focus for future research. In addition, 3D bioprinting models and techniques need to be refined to extend the feasibility and integration of bioprinted tissues into living organisms. These limitations are critical to achieving the translational potential of 3D bioprinting in bone regenerative applications. As research progresses, the impact of 3D bioprinting on the field of bone TERM is becoming increasingly evident.

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