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Review

Application of 3D- printed hydrogels in wound healing and regenerative medicine

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ABSTRACT

Hydrogels are three-dimensional polymer networks with hydrophilic properties. The modifiable properties of hydrogels and the structure resembling living tissue allow their versatile application. Therefore, increasing attention is focused on the use of hydrogels as bioinks for three-dimensional (3D) printing in tissue engineering. Bioprinting involves the fabrication of complex structures from several types of materials, cells, and bioactive compounds. Stem cells (SC), such as mesenchymal stromal cells (MSCs) are frequently employed in 3D constructs. SCs have desirable biological properties such as the ability to differentiate into various types of tissue and high proliferative capacity. Encapsulating SCs in 3D hydrogel constructs enhances their reparative abilities and improves the likelihood of reaching target tissues. In addition, created constructs can simulate the tissue environment and mimic biological signals. Importantly, the immunogenicity of scaffolds is minimized through the use of patient-specific cells and the biocompatibility and biodegradability of the employed biopolymers. Regenerative medicine is taking advantage of the aforementioned capabilities in regenerating various tissues-muscle, bones, nerves, heart, skin, and cartilage.

1. Introduction

3D bioprinting is a revolutionary technique that involves layering different materials. Similar to 2D printing, it requires "ink" which will be a biological compound, and paper replaced by biodegradable material [1]. Thus, it is well-known that 3D bioprinting focuses on cells or tissues, and it is necessary to ensure the biocompatibility of the material, the appropriate printing method, and parameters to provide cell viability or delivery of growth factors. Optimal process conditions are conducive to creating constructs with properties similar to those observed in vivo, such as the specific organization of the extracellular matrix (ECM) [2]. 3D bioprinting methods include inkjet bioprinting, fused deposition modeling (FDM), extrusion-based bioprinting, laser-assisted bioprinting, stereolithography, and VAT polymerization. FDM, which involves printing thermoplastic polymers is the most commonly used one. Stereolithography then utilizes a UV laser to polymerize the polymers. Inkjet bioprinting is based on 2D inkjet printers, while extrusion bioprinting employs mechanical and pneumatic force. Laser-assisted bioprinting deposits cells on a metal film using a laser. VAT polymerization, meanwhile, exploits a laser solidification mechanism [3].

An essential element of 3D bioprinting is the bioink used. Biomaterials utilized for printing can be divided into natural and synthesized. The advantages of natural biomaterials are biocompatibility, biodegradability, ability to self-organize or mimic the structure of ECM. Synthetic polymers are still used because they can be designed to mimic mechanical properties and degradation specifics of natural tissues and organs, enable controlled drug release, offer versatile design options (e.g. ability to control mechanical stability or pH and temperature responses), and contribute to research and development efforts. However, synthetic polymers have limited applications due to toxic solvents used during their preparation, melting points higher than the temperature of the human body, and lack of features enabling their biological properties e.g. sites for cellular recognition, promotion of cell growth or differentiation. It is also more difficult to encapsulate cells in synthetic polymers

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than in the natural ones. Synthetic compounds most commonly used in 3D structures include polyethylene glycol (PEG), poly(lactic-co-glycol), polycaprolactone (PCL), polyvinylpyrrolidone (PVP), poly(L-lactic acid) (PLA). In turn, natural compounds include agarose, alginate, collagen, or hyaluronic acid applied individually or as mixtures [4,5]. Currently, hydrogels, which have a three-dimensional structure of polymer chains, are also increasingly used in 3D bioprinting. Adapting hydrogels to the affected area's shape and size allows tissue mapping and replacement while retaining appropriate biological and mechanical properties. A limitation in the application of hydrogels is the remaining compounds after polymerization such as catalysts, and activators, which can cause side effects. Radical polymerization techniques like separating molecules are used in the process to reduce the risk of complications. Another important element of the bioink is the cells, such as SCs, primary cells isolated from tissues or cell lines derived from primary cell cultures. SCs are cells with a high capacity for division and differentiation. The first and most well-known stem cells were hematopoietic stem cells (HSCs). Key types in 3D bioprinting include bone marrow mesenchymal stromal cells (BM-MSCs), AD-MSCs, and induced pluripotent stem cells (iPSCs) [6]. Proliferation of cells within the 3D constructs is vital to ensure tissue viability. In addition, cells should exhibit immunologic neutrality and relative resistance to printing conditions. Functionality and vitality of cells are widely studied and explored [7,8].

3D bioprinting techniques are widely used in medicine. A lot of research is focused on repairing skin defects, cartilage, or bone restoration using 3D constructs. As well as applications include the liver, pancreas, nerves, heart, or vascular system regeneration. 3D scaffolds also provide a valuable model for studying metabolic diseases or producing drugs and their delivery systems [9,10].

2. Hydrogels classification

Hydrogels have physical properties similar to living tissue due to their high water content, softness, and plasticity. They can be classified in several ways depending on the parameter that will be taken as a differentiating factor (Fig. 1). Hydrogels can vary due to the source of origin, ionic charge, size, crosslinking methods, chain composition, biodegradability, and response to different factors. In the next chapters, characterizations of each class of hydrogels are detailed. However, each type of hydrogel has different physical, chemical, and biological properties due to which they can be used for a wide variety of applications [11–13].

2.1. Source

Due to the source of origin hydrogels are divided into natural and

Fig. 1. Classification of hydrogels.

synthetic polymers. Natural polymers are biomolecules found in nature that are made up of repeating subunits. They are formed through biological processes and can be found in many living organisms, such as plants, animals, and microorganisms. The group of hydrogels with natural origin mainly includes collagen, gelatin, fibrin, hyaluronic acid, Matrigel®, alginate, and others. On the other hand, synthetic polymers are man-made polymers that are produced through chemical reactions in a laboratory. While both natural and synthetic polymers have their unique properties and applications, they share some common characteristics, such as high molecular weight, flexibility, and the ability to be formed into various shapes and sizes. [11–13].

2.1.1. Natural polymers

The natural hydrogels most used as biomaterials are described below.

2.1.1.1. Collagen. **Collagen** is one of the most important proteins forming the ECM in the body. It is built with a motif of repeating amino acid blocks Gly-X-Y, where proline and hydroxyproline are the most common at the X and Y positions [14]. As a biomaterial, collagen has numerous functions that are vital for maintaining structural integrity and providing mechanical strength to tissues such as skin, bone, cartilage, and others. It binds water in these tissues which helps to regulate their hydration, and participates in blood clotting processes, wound healing, scar formation, and bone fusion after fractures [15,16]. Additionally, collagen has potential applications in tissue engineering and regenerative medicine where it can be used to replace malfunctioning or diseased tissues as well as combined with other materials such as synthetic polymers to improve its functionality [17].

2.1.1.2. Gelatin. **Gelatin** is made of the same amino acids as collagen. Gelatin is derived from collagen through a process of hydrolysis, which breaks down the collagen molecules into smaller peptides. It is obtained by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of collagen from the animal bones, skin, and muscles. This process involves heating collagen in water, which causes the collagen fibers to unravel and release the smaller peptides. These peptides can then be further processed to form gelatin. Besides being widely used in the food industry, gelatin also has several beneficial biological properties that make it ideal for use in engineering and regenerative medicine applications. Its biocompatibility, low antigenicity, biodegradability, and ability to stimulate cell growth and attachment make it an invaluable resource for these fields [18–20]. Gelatin is widely used as a biomaterial in various medical applications. It serves as a tissue engineering scaffold, providing support for cell growth and tissue regeneration. Gelatin-based dressings promote wound healing by creating a favorable environment. It is also utilized for controlled drug delivery and as a hemostatic agent during surgical procedures [21, 22].

2.1.1.3. Fibrin. **Fibrin** belongs to the group of fibrillar proteins, formed from fibrinogen through the activity of thrombin [23]. Fibrin has natural biostatic and bioactive properties. It induces processes such as angiogenesis, synthesis of cytokines and ECM, and enhanced cell migration and proliferation. Fibrin plays a very important role in the aspect of regeneration, i.e. filling tissue defects. The mechanical strength of fibrin gel can be regulated by increasing or decreasing the presence of divalent ions such as Ca^{2+} [24,25]. Fibrin hydrogels have several advantages as a biomaterial, including their biocompatibility, biodegradability, and ability to support cell growth and differentiation [26]. Fibrin, as a biomaterial, has many applications in medicine. It serves as a scaffold for tissue engineering, facilitating cell growth and regeneration. Fibrin-based hemostatic agents effectively control bleeding during surgeries. It is utilized as a drug delivery system, enabling controlled release of therapeutic agents [25,27–29].

2.1.1.4. Hyaluronic acid (HA). **Hyaluronic acid (HA)** is a polysaccharide belonging to the group of glycosaminoglycans. Its structure consists of alternating mers of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine linked by β(1→4) and β(1→3) glycosidic bonds. HA is well known for its strong hydrophilicity due to the numerous carboxyl groups that can form hydrogen bonds with water molecules. This property enables 1 g of HA to bind up to 6 liters of water, making it an ideal component for numerous cosmetic products. Such products are favored due to the biocompatibility, biodegradability, low toxicity, viscosity, and plasticity of HA [30]. HA is commonly used in tissue engineering as a scaffold for promoting cell adhesion, proliferation, and tissue regeneration. HA-based dermal fillers are used for cosmetic purposes to reduce the appearance of wrinkles and restore volume to the skin. It is utilized in ophthalmic surgeries as a viscoelastic agent to protect and lubricate the eye. Additionally, HA plays a crucial role in drug delivery systems, facilitating controlled release of pharmaceuticals [31,32].

2.1.1.5. Matrigel. **Matrigel®** is a commercial, well-known, and widely used cell culture matrix composed of natural polymers secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. This matrix is enriched in laminin, collagen type IV, and various other molecules such as perlecan, entactin, growth factors, and cytokines which are crucial for normal cellular growth and development [33,34]. Matrigel is commonly used as a 3D matrix for cell culture and tissue engineering, providing a supportive environment for cell growth and differentiation. MatriGel is also utilized as a drug delivery system, enabling controlled release of therapeutic agents. In regenerative medicine, it serves as a scaffold for promoting tissue regeneration and wound healing [35].

2.1.1.6. Alginate. **Alginate** is a well-known polysaccharide extracted from marine algae, especially from brown algae (*Phaeophyceae*). It's a native co-polymer consisting of β -D-mannuronic acid (M-blocks) and α -L-guluronic acid (G-blocks) residues linked by glycosidic bonds. The M and G blocks can occur in different proportions and different arrangements along the polymer chain. Alginate possesses strong sorption properties, with low total pore volume and internal surface area. Its high water-binding capacity is achieved through hydrogen bonding between the -OH groups on alginic acid fibers, as well as to unsubstituted groups in the case of divalent metal alginates. The process of alginate hydrogel formation is triggered by divalent ions, especially Ca2 + [36,37]. Alginate hydrogels are used in drug delivery systems, allowing controlled release of pharmaceuticals. Alginate-based dressings are used for wound healing, providing a moist environment to promote tissue regeneration [38].

2.1.2. Synthetic polymers

The second group of hydrogels consists of synthetic-derived hydrogels. Synthetic-derived hydrogels are produced through a controlled process in which monomers are polymerized to form macromolecules with distinct properties. This process can be used to create synthetic polymers with specific physical and chemical characteristics that enable them to be used for a wide variety of applications. The synthetic polymers most used as biomaterials are described below.

2.1.2.1. Polyacrylamide. **Polyacrylamide (PA),** a water-soluble linear polymer, is composed of acrylamide monomers or a combination of acrylamide and acrylic acid monomers. This polymer is widely used in agriculture, food processing, and other industries. Its hydrogel form consists of a covalent polymeric network combined with varying amounts of water. The water content in polyacrylamide hydrogel can range from 70% to 90%, making it extremely useful for applications such as absorption, filtration, sedimentation, and flocculation [39]. PA hydrogels have several advantageous properties that make them suitable for a range of biomedical applications. Due to their composition and extensive crosslinking, these materials are highly biocompatible,

enabling safe and non-toxic interactions with living cells. Furthermore, the physical and chemical properties of PA hydrogels can be tailored by adjusting their composition or adding functional groups or bioactive molecules. This allows for tuning the mechanical properties to match those of healthy tissues and organs in the human body. Moreover, such hydrogel scaffolds are capable of creating 3D structures that closely resemble the ECM, further increasing their potential use in TE strategies [40].

2.1.2.2. Polyethylene glycol. **Polyethylene glycol (PEG)** is a polyether compound derived from petroleum, consisting of long chains of ethylene oxide molecules. It exhibits excellent water solubility and has been used extensively in biomedical applications due to its biocompatibility and ability to be modified covalently with functional groups and bioactive molecules, allowing for tuning of mechanical properties of hydrogels to match those found in tissues within the body. PEG-based hydrogels are popular in medical applications as they possess many advantageous characteristics. For example, these hydrogels can be formed into a variety of shapes and sizes, enabling them to fit into specific anatomic locations. Furthermore, PEG-based hydrogels are capable of controlling drug release kinetics by altering the mesh size or incorporating chemical moieties that can respond to external stimuli such as temperature or pH levels [41,42].

2.1.2.3. Peptide hydrogels. **Peptide hydrogels** are hydrogels composed of short peptide chains that self-assemble in water to form a 3D network structure. Peptides are short chains of amino acids and can be designed to have specific chemical and physical properties. By controlling the sequence and concentration of the peptides, it is possible to create hydrogels with a wide range of properties, such as stiffness, porosity, and biocompatibility. Peptide hydrogels are attractive materials for tissue engineering and regenerative medicine applications because they can mimic the ECM of natural tissues. The ECM is a complex network of proteins and other molecules that provides structural support and biochemical signals to cells [43–45].

2.1.2.4. Hybrid hydrogels. **Hybrid** hydrogels are a rapidly expanding class of materials that show promise for biomedical applications due to their versatility, functionality, and improved performance over pure polymer networks. To create hybrid hydrogels, two or more different types of polymers must be combined, such as natural polymers (e.g., gelatin, chitosan) and synthetic polymers (e.g., polyethylene glycol). These combinations of polymers can provide increased mechanical strength and biocompatibility compared to purely synthetic hydrogels. Furthermore, these hybrid hydrogels can have their properties tailored by different concentrations and types of polymers, as well as by controlling the degree of crosslinking between them. This ability to tailor properties enables hybrid hydrogels to be used in a wide range of applications, including tissue engineering, drug delivery, and diagnostic biosensors [46].

2.2. Size of particles

Hydrogels can be classified based on various characteristics, including their chemical composition, crosslinking density, and mechanical properties. Another way to classify hydrogels is based on the size of their particles. Based on particle size, three types of hydrogels can be identified: nanogels, microgels, and bulk hydrogels. The size of the particles in a hydrogel can have a significant impact on its properties and potential applications. By selecting the appropriate size and characteristics of the particles, hydrogels can be tailored to meet specific requirements for a wide range of biomedical and biotechnological applications. Particles smaller than 100 nm form nanogels, subsequently microgels are formed by particles reaching micrometric size. Hydrogels with molecules larger than 100 μm are called **bulk hydrogels** [47]. Microgels, due to the high presence of solvent in their structure, usually form soft structures. According to that, they are prone to exchange solvents with the fluid that is in the external environment of the hydrogel. **Microgels**, due to their hydrophilic nature, have a high ability to absorb water, and their swelling properties are reversible depending on external stimuli, which can be a change in temperature, pH, ionic strength, and solvent [48,49]. **Nanogels** have high water absorption and swelling. This is caused by the presence of hydrophilic functional groups such as -OH, -CONH-, -$CONH_2$-, -COOH, and -SO_3H. Due to the hydrophilic nature of the nanogels, they have a wide range of properties such as biocompatibility and high transferability of hydrophilic biotherapeutics, high stability, and biodegradability [50,51].

2.3. Crosslinking

Crosslinking is a critical step in the formation of hydrogels, as it imparts structural stability, mechanical strength, and biocompatibility to the material. Chemical, physical, and enzymatic crosslinking can be distinguished. Hydrogels need to be cross-linked to maintain their shape and integrity, as well as to control their properties and functionality. Crosslinking refers to the process of linking polymer chains together to form a 3D network structure, which gives hydrogels their unique properties, such as high water content, softness, and flexibility. Crosslinking is an essential step in the formation of hydrogels, as it provides the structural stability, mechanical properties, and biocompatibility needed for a wide range of biomedical and biotechnological applications The enzymatic method is one of the most gentle ways of crosslinking. Enzymes mostly exhibit a high degree of substrate specificity. This allows for the avoidance of potential side reactions during the process and makes it possible to control and predict kinetics, and the overall crosslinking rate [52,53]. An example of enzymatic crosslinking of hydrogels is the crosslinking of gelatin and chitosan in the presence of transglutaminase and tyrosinase. Chemical crosslinking of hydrogels involves the use of covalent bonding between polymer chains to produce a stable hydrogel. Most commonly, small crosslinking molecules, polymer-polymer conjugation, or photosensitizers are used for this purpose. Covalent bonds are formed primarily between functional groups of polymers (-OH, -COOH, -NH_2), which provide solubility to water-soluble polymers. Covalent bonds are formed between two groups that have complementary reactivity. Among the most commonly used reactions are Schiff base formation, Michael addition, peptide bonds formation, and "click chemistry'' reactions [54–57]. Physical crosslinking of hydrogels refers to the formation of a 3D network structure through physical interactions between polymer chains, rather than through chemical reactions. This process typically involves the use of non-covalent interactions, such as hydrogen bonding, hydrophobic interactions, or electrostatic interactions, to create a stable network. There are several ways to physically crosslink hydrogels, including temperature-induced gelation, ionic gelation, and hydrogen bonding. Physical crosslinking has several advantages over chemical crosslinking, including better biocompatibility, easier fabrication, and the ability to reversibly change the properties of the hydrogel. However, physical crosslinking can also be weaker than chemical crosslinking, and the properties of the hydrogel may be more sensitive to changes in the environment, such as temperature or pH [58].

2.4. Biodegradability

The biodegradability of hydrogels is a highly desirable trait for numerous biomedical applications, particularly those that require the use of these materials as temporary scaffolds for tissue regeneration or drug delivery. Depending on the intended purpose, hydrogels can be designed to be biodegradable or non-biodegradable, depending on the intended use and application. Biodegradable hydrogels can break down into smaller molecules over time, which allows for their safe elimination from the body or environment. There are several methods for achieving

biodegradability in hydrogels, including using natural or synthetic polymers that are inherently biodegradable, incorporating cleavable chemical bonds into the hydrogel structure, or designing the hydrogel to respond to specific environmental cues that trigger degradation [59]. In the context of biomedical applications, biodegradable hydrogels are particularly attractive for drug delivery, tissue engineering, and wound healing. They can be designed to release therapeutic agents over time while gradually breaking down and being eliminated from the body. It is very important to be able to control the mechanism and rate of degradation, for example, too fast degradation of the scaffold may not be appropriate for the cells to produce enough ECM, and slow degradation may affect cell migration and other processes essential for cell survival [60]. Biodegradability can also be harnessed for controlled drug release, e.g. peptides specific to enzyme degradation [61]. For example, Zisch et al. prepared degradable hydrogel with the use of MMPs degradable peptides crosslinked within the PEG matrix functionalized with a vinyl sulfone [62]. Non-biodegradable hydrogels, on the other hand, are designed to be long-lasting and remain in the body for extended periods. They are typically used in applications where a long-term, durable support structure is needed, such as in contact lenses, wound dressings, or artificial implants. Non-biodegradable hydrogels are not designed to degrade, so they can potentially cause long-term harm to the body if they are not removed or replaced over time [63].

2.5. Ionic charge

Ionic hydrogels, or polyelectrolytes, are a type of hydrogel composed of monomers with specific ionic charges. These can be divided into four distinct categories based on the charge: anionic hydrogels, which contain negatively charged ions; cationic hydrogels, which contain positively charged ions; amphiphilic hydrogels, which contain both positively and negatively charged ions; and neutral hydrogels, which have no net charge. **Anionic** hydrogels include alginic acid, pectin, HA, carrageenan, dextran sulfate, chondroitin sulfate chondroitin. Anionic hydrogels show a definite increase in swelling coefficient as the pH of the environment increases. Anionic hydrogel networks are usually defined as homopolymers of negatively charged acidic or anionic monomers or copolymers of an anionic monomer and a neutral monomer [63]. Chitosan and polylysine, which have a positive charge are examples of cationic hydrogels. **Cationic** polymeric networks could also be derived through modifications such as partial hydrolysis of the existing non-ionic pre-formed polymer networks. Cationic pendant groups in a polymer network on the contrary behavior to anionic pendants give rise to hydrogels, which remain collapsed in the basic environment and swollen in the acidic environment due to the electrostatic repulsion between the positively charged groups [64]. Polyampholytic hydrogel networks are referred to as macromolecules capable of possessing both positively and negatively charged moieties in the polymer network. Subsequently, the group of amphiphilic hydrogels can include, for example, collagen, gelatin, carboxymethylated chitin, fibrin, and starch. The last group of natural hydrogels, which includes such polymers as dextran, agarose, and pullulan, is an example of neutral hydrogels [65].

2.6. Response to stimuli

Hydrogels can be further categorized according to their reaction to external environmental stimuli, such as pH, temperature, or enzyme. Responses may range from a physical change in the material's shape or structural integrity to changes in permeability, solubility, or optical properties. The first group of hydrogels may include **pH**-responsive hydrogels. They change their volume in response to changes in the pH of the surrounding environment. This volume change can be due to changes in the ionization state of acidic or basic functional groups in the hydrogel polymer. Examples of pH-responsive hydrogels include poly (acrylic acid) and poly(methacrylic acid) hydrogels [66,67]. Next, temperature-responsive hydrogels, are a type of hydrogel that undergoes reversible swelling or shrinking in response to changes in temperature. These hydrogels are made up of polymers that have both hydrophilic and hydrophobic parts, allowing them to respond to changes in temperature. At low temperatures, temperature-responsive hydrogels are typically hydrophilic and swollen, while at higher temperatures, they become more hydrophobic and shrink [63]. This behavior change can be exploited for various applications, such as drug delivery, tissue engineering, and biosensors. Enzyme-responsive hydrogels can change their physical or chemical properties in response to the presence or activity of specific enzymes. They can be made from a variety of natural or synthetic materials, including polymers such as PEG, chitosan, and HA [68]. Chemical-responsive hydrogels can undergo reversible changes in their structure, properties, or behavior in response to changes in the chemical environment. The response of these hydrogels can be triggered by various chemical stimuli, such as pH, ionic strength, solvent polarity, or the presence of specific molecules [69].

2.7. Chain composition

Another criterion used to classify hydrogels is the composition of the polymer chain. Homopolymeric hydrogels are composed of a single type of polymer chain. These chains can be either natural or synthetic in origin. These hydrogels can be designed to have a range of properties, such as different degrees of swelling, mechanical strength, and biodegradability, depending on the choice of monomer and the method of synthesis. Homopolymer hydrogels have been used in a variety of applications, such as drug delivery, tissue engineering, and wound healing. Examples of homopolymeric hydrogels include poly(acrylic acid) and PEG hydrogels [70]. Copolymeric hydrogels, e.g. poly (N-isopropylacrylamide-co-acrylic acid) and poly(ethylene glycol)-co-poly(lactic-co-glycolic acid) hydrogels, are composed of two or more different types of polymer chains. These chains can be arranged randomly or in a specific pattern. By varying the composition of the polymer chains, copolymer hydrogels can be designed to have unique properties, such as improved mechanical strength, enhanced biocompatibility, and improved drug release kinetics. Copolymer hydrogels have been used in applications such as drug delivery, tissue engineering, and biosensors. [71]. Hybrid hydrogels are composed of two or more different types of polymer chains in combination with other materials, such as inorganic nanoparticles or proteins. They can have improved mechanical strength, enhanced biocompatibility, and improved drug release kinetics compared to single-network hydrogels. Hybrid hydrogels, i.e. PEG hydrogels with incorporated gold nanoparticles and gelatin-methacrylate hydrogels have been used in applications such as drug delivery, tissue engineering, and biosensors [72]. Composite hydrogels are composed of two or more different types of polymer chains, each forming a distinct phase within the hydrogel. These different phases can have diverse mechanical, swelling, and degradation properties. By combining different materials, composite hydrogels can be designed to have unique properties and functionalities, making them a versatile class of biomaterials. Composite hydrogels, e.g. interpenetrating network (IPN) hydrogels and semi-IPN hydrogels. have a wide range of potential applications in fields such as tissue engineering, drug delivery, biosensors, and wound healing [73]. Crosslinked hydrogels are composed of polymer chains that have been crosslinked to form a 3D network structure. Crosslinking can be achieved through a variety of methods, including chemical, physical, and enzymatic crosslinking. Examples of crosslinked hydrogels include PA hydrogels and chitosan-alginate hydrogels [74].

3. Biology of stem cells and characteristics of MSCs

Stem cells are characterized by a high capacity to divide and differentiate into specific cell types. The basic division includes embryonic stem cells (ESCs) with pluripotent properties and adult stem

cells (ASCs), which distinguish cells with multipotent and unipotent characteristics [75]. ESCs arise from the inner cell mass (ICM), which is a component of the blastocyst, and take part in embryogenesis capable of producing any cell of the body. ASCs are undifferentiated and participate in tissue repair by forming specialized cells under certain conditions. Various signals mediate this process such as cell-to-cell contact, factors secreted by tissues, but also compounds that affect genetic modifications [76]. ASCs exist in niches as either resting or active (during division). Stem cell (SCs) divisions occur at different rates depending on the tissue renewed. Tissues with high rates of division include the epidermis, intestinal epithelium, and blood cells. A low rate of division is shown by cells of the liver, pancreas, or muscle [77]. The pro-regenerative effect of SCs and their differentiation abilities are related to their autocrine and paracrine properties. SCs secrete growth, angiogenic, anti-inflammatory, anti-apoptotic, and pro-proliferative factors, immunomodulators, and compounds responsible for extracellular matrix homeostasis (metalloproteinases, collagens). These molecules can be encapsulated in extracellular vesicles. As mentioned above, SCs are found in almost all organs of the body [78,79]. A description of the key types of SCs for regenerative medicine is presented below. In addition, the advantages and disadvantages of stem cells are shown in Fig. 2.

Mesenchymal stromal cells (MSCs) are a widely studied group of SCs. MSCs belong to the ASCs and can differentiate into multiple lineages. Based on the source of acquisition, they are divided into bone marrow mesenchymal stromal cells (BM-MSCs) and adipose-derived mesenchymal stromal cells (AD-MSCs). The sources specified are readily renewable/treated as waste during medical procedures. In addition, cells can be taken directly from the patient, reducing the risk of transplant rejection by the patient's body [80]. Classification of MSCs according to the International Society for Cellular Therapy includes cell adherence to the culture flask and differentiation into osteocytes, adipocytes, and chondrocytes. Additionally, MSCs should show the presence of the positive surface markers CD73, CD90, and CD105, but not the negative surface markers CD14, CD34, and CD45 [81]. MSCs are normally cultured in vitro in Dulbecco's modified Eagle's medium (DMEM) enriched with fetal bovine serum (FBS) [82].

BM-MSCs account for 0.001–0.01% of bone marrow cells. Isolation is performed using either gradient cell separation or fluorescence/magnetic cell sorting flow cytometry. The methods for obtaining BM-MSCs are simple but quite painful for the patient. Cells show the typical phenotype of surface markers. In addition, early passages, exhibit expression of the stromal antigen 1 (STRO-1) [83]. BM-MSCs present high functional heterogeneity. They participate in physiological tissue regeneration as well as disease states such as osteoporosis, obesity, fractures, and acute myeloid leukemia [84]. Moreover, these cells' major histocompatibility complex class I is expressed at moderate levels, reducing the risk of graft-versus-host disease. BM-MSCs have immunomodulatory effects by interacting with B and T lymphocytes, dendritic cells, or macrophages [85]. Studies have shown that BM-MSCs have the greatest immunosuppressive properties in both paracrine and direct cell-to-cell contact. Further, they secrete large amounts of angiogenesis-stimulating interleukin-8 and vascular endothelial growth factor [86].

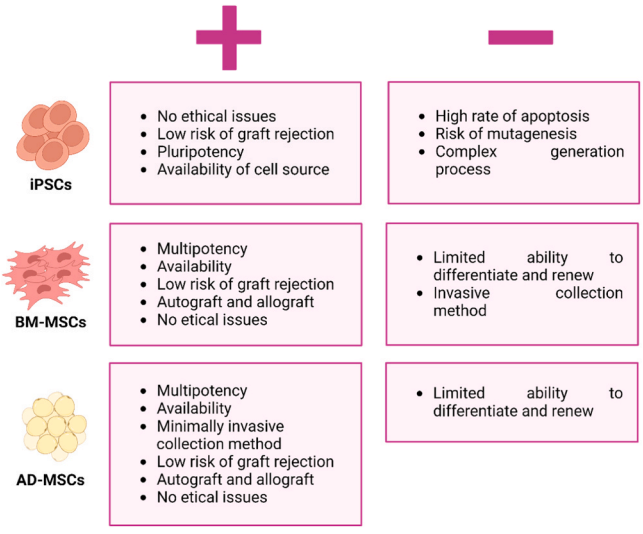

Fig. 2. Potentials and limitations for the main types of stem cells. *Created with BioRender.com.*

AD-MSCs are isolated from adipose tissue as a stromal vascular fraction (SVF), which is enzymatically digested to obtain the target cells. The procedure during which SVF is extracted is performed, in part, to remove excess adipose tissue (liposuction). Obtaining AD-MSCs is therefore possible from unused tissue after the procedure. The characteristics of AD-MSCs are generally common with those described for MSCs. Compared to BM-MSCs, they have an expression of the CD36 marker and lack expression of CD106 [87]. The tissue microenvironment can modulate factors secreted by AD-MSCs. Studies indicate that AD-MSCs are involved in creating an anti-inflammatory and angiogenic phenotype [88]. AD-MSCs secrete factors such as hepatocyte growth factor, transforming growth factor β1, prostaglandin 2, tumor necrosis factor-α, and vascular endothelial growth factor [89]. AD-MSCs present a phenotype similar to BM-MSCs that elicits little immune response due to low levels of MHC class I and lack of MHC class II molecules. The cells also exhibit strong immunomodulatory properties by inhibiting dendritic cell differentiation, NK cells, and lymphocyte proliferation. AD-MSC therapies are used for neurodegenerative and autoimmune disorders and diseases of the skeletal and cartilage systems [90].

Induced pluripotent stem cells (iPSCs) are pluripotent cells that arise as somatic, postmitotic cells (e.g. fibroblasts) reprogrammed by various agents in varial/ nonviral vectors. Transcription factors involved in this process include octamer binding transcription factor 3/4 (OCT3/4), cellular-Myelocytomatosis (c-Myc), (sex determining region Y)-box 2 (SOX2), and Krüppel-like factor 4 (KLF4). Reprogramming cells further leaves many confessions in terms of efficiency, incomplete reprogramming, or genome integration [91,92]. The ability to self-renew and differentiate into all cells of the body makes iPSCs widely used in regenerative medicine. In addition, they can be "personalized" to the patient as well as to the disease. Of concern are undifferentiated iPSCs, which can undergo tumorigenesis. Various elimination strategies are used to avoid this, such as a medium containing a lot of L-alanine, to which non-differentiated human iPSCs are sensitive [93]. iPSCs can be used in diseases associated with genetic mutations, as clustered regularly interspaced short palindromic repeats/ caspase 9 (CRISPR/CAS9) genome editing methods used to "repair" the altered gene in the patient's cells. At the same time, organoids produced from iPSCs can serve as models for studying disease mechanisms and testing new drugs [94].

The use of MSCs for 3D bioprinting is really popular nowadays due to their high pro-regenerative potential, well-known biology, optimized methods of isolation and cell culture, and the lack of ethical concerns or less complex methods of obtaining than for example iPSCs. MSCs were used in the 3D printing of many tissues including bone, cartilage, muscles, cardiovascular tissues, and neural tissues [95].

4. Technical aspects of hydrogels in bioprinting

Bioprinting has become an integral part of tissue engineering, overcoming previous limitations of this technology related to poor control over hierarchical structures and their assembly. In addition, among several methods of controlling biomaterial structure, such as cell-sheet technology, embedding or molding, centrifuge casting, dielectrophoresis, magnetic-force driven cell-motion, micro-fluidics, biospraying, bioprinting is considered the most valuable tissue engineering technique [96]. The significant development of bioprinting research is

mainly due to the limited availability of donors thanks to which lost or damaged organs and tissues could be replaced, e.g. skin, cartilage, bone, heart tissue, or vessels. In addition, 3D-printed objects are increasingly used in clinical practice as models for surgical planning and medical education [97]. In contrast to 2D monolayer cultures, 3D-cell cultures give the in vitro model both a higher level of clinical relevance and biological significance by mimicking the spatial environment [98]. The general scheme of the bioprinting process includes three stages such as pre-bioprinting, bioprinting, and post-bioprinting. Stage one involves obtaining the anatomical structure of the target tissue using an appropriate imaging technique such as CT (computed tomography) and MRI (magnetic resonance imaging), followed by the development of a computer-aided CAD design that includes information about the individual layers in cross-section, which will be further printed in a layer-by-layer process using printing techniques such as extrusion-based, inkjet-based, laser-assisted, and stereolithography bioprinting. The second stage is a computer-assisted robotic bioprinting process. In the final stage, on the other hand, in vivo environments are reproduced to maintain cell viability and proper differentiation [99, 100]. All printing methods have their advantages and disadvantages; however, due to their low cost, compatibility with various bioinks, and scalability, extrusion bioprinting is the most widely used technique [101], especially for biomaterials with high viscosity and high cell density [102]. An example of materials that are widely used in tissue engineering and thus in bioprinting are hydrogels. Their high water content means they can mimic natural tissue and an environment suitable for normal cell proliferation [103]. Of the many biomaterials, polymer hydrogels represent a material with potential application for scaffold fabrication by extrusion. However, for this method, the polymer bioink must have certain properties such as minimum viscosity and crosslinking ability to maintain a certain structure after printing [104].

Bioprinting technology makes it possible to produce personalized, physiologically appropriate tissue reconstructions. However, one of the biggest challenges is the development and fabrication of a bioink [105], which has two main tasks, i.e. biological and structural. One of the main roles of bioink is to provide an appropriate microenvironment for regulating the activity and encapsulation of living cells and remodeling the ECM. Accordingly, appropriate physical, as well as biological properties of the bioink, are crucial. Among the physical properties that the bioink must fulfill are controllable viscosity, printability, shear-thinning rheological behavior, mild gelation conditions, and appropriate mechanical properties. In turn, among the biological properties, it is biodegradability, non-immunogenicity, or bioinstructive properties [106]. Another key property of bioink is the low concentration of endotoxins in it. Banach-Kopeć et al.proposed a novel method to remove endotoxins from chitosan hydrogel, as a potential component of bioink. It was shown that the developed method involving precipitation of chitosan in an alkaline environment at pH 9, washing the precipitate with chloroform and its subsequent sterilization at 121°C, and then carbon dioxide saturation to dissolve the polymer in water allows the removal of significant amounts of endotoxins by up to 97.6% relative to their initial concentration in chitosan. In addition, the purification method does not change this polymer's dynamic viscosity or molecular weight [107]. Moreover, the bioink from a technical point of view must exhibit the ability to deposit in a controlled manner to ensure printing fidelity, known as printability. Physical properties such as viscosity/stiffness, rheological behavior, and printing parameters play a key role here. However, as bioink printability improves, cell viability and bioactivity decrease due to conditions different from those in natural ECM. Therefore, one of the current problems and challenges of many researchers is to overcome the biofabrication window paradigm, i.e. to obtain a material with ideal printability and the highest possible cell viability (Fig. 3) [108].

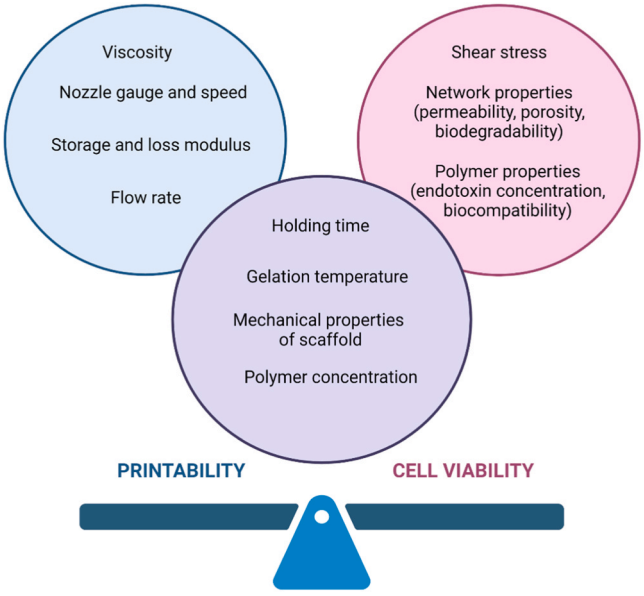

Fig. 3. The main challenge in the bioprinting process is to obtain a material with the highest printability providing good cell viability. Both parameters may be influenced by many factors. *Created with BioRender.com.*

4.1. Rheological factors

During the characterization of hydrogels with their potential use as bioink, their rheology is of great importance [104]. However, the type of polymer used will directly affect the rheological parameters of the bioink. Since most materials do not have the appropriate rheological parameters crucial in bioprinting, it is a good solution to carry out their physical or chemical modifications and create composite materials. An example of such a solution is the chitosan-agarose composite mixture described in patent application no. P.443403. The chitosan hydrogel, which has poor mechanical properties, and agarose, which is characterized by a lack of biological activity, cannot be used in bioink as a single component of bioink. However, their compositions are characterized by good mechanical properties, biological activity, and sol-gel phase transition at 26–36°C without transforming back to sol at 37°C [109]. Rheology characteristics are a powerful platform for analyzing material flow properties of bioinks in particular. Considering that the most important characteristic of biomaterials is their ability to be continuously extruded during needle printing, several basic parameters such as viscosity, shear-thinning rheological behavior, yield stress, gelation rate, and critical concentration must be determined to predict its extrusion performance. Whether a material will be extrudable is determined by its shear-thinning rheological behavior in a dynamic environment. Shear-thinning properties are a key parameter when selecting hydrogels as bioink components behave like non-Newtonian fluids and have stress-relaxation properties. In addition, if the high-viscosity material has adequate shear-thinning properties then through better polymer-polymer interactions it is possible to obtain a print with a good shape retention factor [110]. For the bioink to flow, it is necessary to apply a critical stress which is defined by a parameter called yield stress. The higher this parameter is, the pressure required to exceed the forces of attraction due to intermolecular bonds in the physically cross-linked network is greater which negatively affects cell viability. Additionally, to ensure the best printability, the hydrogel gelation time is important, which should be as short as possible [104, 110]. To meet the first criterion, the study of Liu et al. proposed a direct printing strategy for cell-loaded constructs using a physical bioink based on 3% gelatin methacrylate (GelMA), which under cooling at 21°C shows very good shear-thinning and self-healing properties. The print was further stabilized by its subsequent UV crosslinking [101]. On the

other hand, Wu et al. to improve slight shear-thickening behavior at low shear rates GelMA developed a mixture of 1% alginate, 3% cellulose nanocrystals, and 5% GelMa to change the intermolecular interactions and thus obtained a formulation with strong shear-thinning properties as well as high viscosity [111].

4.1.1. Viscosity

Another physicochemical parameter that determines the potential use of hydrogels in bioprinting is their viscosity, which increases with the concentration as well as the molecular weight of the polymer. Although higher viscosity provides, from a technical point of view, better extrusion of layers that do not collapse, on the other hand, it can lead to head clogging and interruption of the bioprinting. This is why shear thinning, which plays a primary role in bioprinting, is so crucial during extrusion [104]. The effect of viscosity on cell viability has been studied for a GelMA-based bioink. It was shown that during bioprinting, it was crucial to provide lower concentrations of up to 3% GelMA to obtain highly porous and soft constructs. This approach ensured high cell viability and did not limit cell spreading and migration due to an overly cross-linked hydrogel network, which is the case when higher concentrations of this polymer are used [101]. Rheological optimization of the hydrogel affects better printability, shape retention, and cell viability. Mondal et al. in their study developed an alginate and gelatin-based bioink and investigated the effect of rheological parameters on the survival of non-small cell lung cancer (NSCLC) patient-derived xenograft (PDX) cells and lung CAFs co-cultures. Since $< 4\%$ sodium alginate solutions have low viscosity, it is necessary to add another biomaterial such as gelatin to improve rheological properties. Gelatin improves the elastic properties of the hydrogel and also cell adhesion. To achieve good printability, it was necessary to use an alginate solution with concentrations of 3.25 or 3.5% and 4% gelatin, respectively, while achieving high cell viability immediately after extrusion as well as 15 days after printing [112]. To improve the properties of alginate, Temirel et al. also proposed a formulation based on 4% alginate and gelatin. Based on viscosity measurements, they found that the optimal concentration of gelatin in the bioink is 3%, as it improves both the viscosity of the formulation and allows it to be extruded while maintaining its shape. On the other hand, with 4% gelatin in the bioink, too much viscosity made it impossible to use such a formulation in bioprinting. The viscosity of the bioink also affects the porosity of the printed scaffolds. As the concentration of gelatin increased, the percentage of normalized pores increased from 50% to 98% (1–3% gelatin). In addition, the survival rate of embryonic NIH 3T3 mouse fibroblast cells after extrusion was 15% higher in the presence of gelatin in the bioink [113].

4.1.2. Storage and loss modulus

One of the basic mistakes when characterizing hydrogels is to define viscosity as a single parameter. Although the viscosity of the material determines the fidelity of the printed shape, its high value does not always mean that the resulting structure will have high mechanical strength or good printability. Therefore, it is necessary to determine the dynamic modulus divided into a conservative modulus G' and a loss modulus G". It is the ratio of G' to G", or the loss tangent (tanδ) that determines whether a material behaves as a solid or liquid. If the ratio $\tan\delta = G'/G''$ is too high, the ink behaves like a liquid and collapses when printed. On the other hand, if it is too low, the extruded fiber is inhomogeneous. Therefore, the hydrogels included in the bioink must have both high viscosity and an appropriate G' to G" ratio to obtain a construct with good resolution and mechanical strength [114,115]. In their study, Gao et al. investigated the effects of G" and G' modulus and tanδ on the printability of a gelatin-alginate composite. They showed that as the proportion of gelatin in the mixture increased, there was an interruption during extrusion due to increased viscosity, i.e. nozzle plugging. The reason for this phenomenon was the disproportionately high behavioral modulus. On the other hand, when the ratio of alginate was too high they observed the spreading of fractions after deposition which in turn was caused by a disproportionately high loss modulus. In summary, lower tanδ was correlated with higher structural integrity, while higher tanδ was correlated with higher extrusion uniformity. Therefore, during bioink printing, it was important to maintain a trade-off between structural integrity and extrusion homogeneity, which was possible with tanδ in the range of 0.25–0.45. However, as the printability of bioink is affected by several rheological properties, among others, yield stress and the frequency and rate of deformation during extrusion, which need to be taken into account, this parameter can be different depending on the composition of the bioink [114]. The effect of loss modulus and storage on the extrudability of bioink was also explained in the study by Wu et al. For example, a 4% solution of GelMA had a viscosity modulus of G" higher than the elastic modulus of G' at low frequencies of its extrusion while at 8 Hz G' was greater than G". This indicates that GelMA is smoother at low frequencies and cannot be used in bioprinting. The property of slight shear thickening at low shear rates may be due to intermolecular interactions of GelMa when the physical binding energy and thermal energy are similar in their values. In addition, at relatively low shear rates and low viscosity of transient GelMa, coil disentanglement and orientation in the flow direction may occur as the shear rate increases. Only after a certain critical shear rate is exceeded is shear behavior observed. Accordingly, the addition of alginate and cellulose nanocrystals to the formulation, which caused significant changes in intermolecular interactions, contributed to the formulation becoming more like a solid which resulted from the fact that G' was larger than G" over the entire frequency range. The strong shear properties in the shear rate range of 01–1000 s-1 enabled this composite to be used in bioprinting [111]. Another solution is to increase the concentration of GelMA to speed up the gelation process, which occurs very slowly at low polymer concentrations. However, to achieve the highest possible cell viability rate, the use of polymers with high concentrations is not recommended.

4.2. Printability

During the evaluation of the potential use of bioink in bioprinting, its printability is also determined. Obtaining an object identical to the designed one is not always possible due to the refraction or instability of the extruded fibers. As the cell scaffold should precisely mimic the architecture and shape of the organ, it is necessary to achieve the highest possible resolution and shape fidelity [116]. The printability and cell survival are both heavily influenced by equivalent parameters such as printing temperature, polymer concentration, and holding time. For example, a study by Ouyang et al. showed that a higher concentration of gelatin and a lower printing temperature contributed to better printability. In contrast, the highest cell viability was obtained with a lower concentration of this polymer and a higher printing temperature [117]. For example, Butler et al. due to the "biofabrication window" paradigm and the claim that it is necessary to find a compromise between printability and the ability to encapsulate cells while maintaining their viability decided to determine such a relationship for N, O-carboxymethyl chitosan and agarose from neuro2A cells to determine the optimal composition of the bioink [118]. However, the main factor that should determine the trade-off between printability and cell viability should be the use of such a scaffold. In the case of soft tissues, for example, printability will play a lesser role than in the bioprinting of complex organs such as the heart. To characterize the resulting print, and thus the printability of a given bioink, it is necessary to define the following indicators: extrudability, strand printability, integrity factor, irregularity, and pore printability [119]. The printability of gelatin-based and alginate-based bioink has been characterized by Gao et al. Extrusion susceptibility, i.e., the minimum pneumatic pressure required to extrude the material at a fixed flow rate, is co-determined by G' and G". The general trend is that as the compound viscosity increases, the required extrusion pressure increases, and it increases faster when G'

increases. For the gelatin-alginate formulation, higher tanδ was correlated with greater extrusion uniformity obtaining perfect smooth lines at tanδ 0.43. In contrast, it was negatively correlated with structural integrity. As tanδ increased, the collapse of the print was observed. In summary, the tanδ gives the bioink a smoother character and negatively affects shape fidelity [114]. The alginate-gelatin hydrogel bioink was evaluated for printability by assessing the shape of the printed square (Pr). The closer the Pr was to 1, the closer the printed shape was to a square. When the bioink was under-gelation it was Pr< 1, and when over-gelation Pr> 1. With Pr between 0.9 and 1.1, the printed constructs had good mechanical stability [98]. The hydrogel formulation alginate-carboxymethylcellulose was proposed as the basic component of the bioink. The diffusion rate (Drf) and Pr material were evaluated. A filament collapse test was carried out by evaluating the filament collapse at the mid-span of the suspended filament at a distance of 1–6 mm. In addition, the area of fiber collapse (Cf) was evaluated, that is, the percentage of the actual area after the deflection of the suspended fiber with the theoretical area. It was shown that the range of printability for 4% alginate and 4% carboxymethyl cellulose is 0.78–0.92 with a pore size of 2–5 mm. Such a Pr value indicates a nearly square pore geometry and thus good printability. In addition, the same formulation shows a collapse area ratio even when increasing the distance value at a minimum level as well as an almost zero Cf. Quantitative analysis of Dfr showed that the composite exhibited minimal spreading of material [120].

4.3. Properties after gelation

4.3.1. Degradation degree

In addition to printability, bioink must be mechanically robust and have an appropriate degradation profile. The degradation rate of cellular scaffolds is mainly determined by the composition of the bioink and must be tailored to the specific application. In the case of cellular scaffolds used for efficacy testing of drugs, the degree of degradation will not play such an important role. However, in tissue engineering, an optimized 3D scaffold should feature adequate mechanical support during the tissue regeneration period and, on the other hand, degrade in vivo to allow tissue remodeling. In this regard, the ideal degradation rate is considered to be such a rate of tissue regeneration that there is a gradual replacement by new ECM components. The scaffold based on alginate, carboxymethyl cellulose, and nano fibrillated cellulose crosslinked additionally with calcium chloride solution and loaded with hSF human fibroblast cells showed a remaining % weight of about 75% after 14 days of printing, which was not significant for disruption of scaffold integrity. For a given degree of degradation, the bioink has been shown to have the potential for long-term use [121]. The role of scaffolds is to provide both a soft and stable environment throughout the regeneration period. In their study, Wu et al. showed that a balanced process between the enzymatic degradation of GelMA and the generation of ECM influences stable mechanical properties over time [111]. Another scaffold with a modified degradation rate was printed by extrusion through the use of oxidized alginate. With an increase in the proportion of oxidized alginate in the alginate-gelatin formulation, it was possible to obtain softer scaffolds that degraded much faster resulting in higher levels of cellular clustering. The presence of gelatin resulted in scaffolds that supported the chondrogenic differentiation of MSCs for 28 days [122].

4.3.2. Mechanical properties

For a cellular scaffold, to mimic the microenvironment of natural tissue, it must have adequate mechanical properties. For bioinks based on natural hydrogels such as alginate, gelatin, fibrin, hyaluronic acid, collagen, or chitosan, providing a microenvironment with adequate mechanical properties is impossible. Therefore, it is necessary to develop a bioink formulation to improve the low mechanical properties of the other components. To improve the mechanical properties of chitosan, Maturavongsadit et al. added nano cellulose to the bioink, which had a significant effect on improving these properties. With this formulation, it was possible to mimic the bone microenvironment and promote the proliferation and differentiation of osteogenic cells [123]. A study by Solon et al. examined the effect of environmental stiffness on the morphological and physical properties of fibroblasts. It was confirmed that in the range of stiffness of the substrate to which they adhere 1–5kPa, fibroblasts can adjust their average stiffness without forming stress fibers. On the other hand, when the stiffness of the substrate exceeded 5kPa, the cells were softer than the substrate as a result of reaching a limit in the mechanism of strengthening the cytoskeleton of the cells. It is supposed that cells do not have a predetermined intrinsic stiffness and can adjust it to the substrate. The situation is different for MSCs, which show differentiated differentiation into specific cell types depending on the stiffness of the matrix which mimics the stiffness of native tissue [119].

4.4. Challenges

The primary challenge for many researchers is to develop a hydrogel-based bioink formulation that exhibits both high printability and cell viability. To date, research by many authors has mainly focused on the use of alginate (Table 1) as the main component of the bioink, due to its rapid gelation under the influence of Ca2 + ions. Another common approach is the chemical modification of gelatin to GelMA, which crosslinks under UV exposure, thereby increasing the mechanical properties of such a scaffold. However, the crosslinking methods described above are often used only in the final stage of bioprinting. Undoubtedly, the rate of curing of the bioink during its extrusion is an important element determining the success of its application in bioprinting. Therefore, it is necessary to use such crosslinking methods, e.g. chemical or under temperature, which will allow immediate gelation of the system during its extrusion and thus make the curing rate independent only of the G' and G" parameters. An example of such a solution is described in patent application no. P.443403, in which both the mass proportion of chitosan and agarose determine the gelation rate and the temperature of the sol-gel transition [109].

The application of bioprinting offers tremendous opportunities, previously unattainable through previous tissue engineering methods. Despite the enormous potential, there are still many challenges for researchers.

Obviously, the main problem concerns the difficulty of achieving high cell survival rates while keeping the scaffold as printable as possible "biofabrication window". Nevertheless, this challenge is compounded by a number of difficulties that affect almost every stage of biofabrication, that is, from the development of the bioink, meaning a composition with the right composition and thus with specific rheological parameters, to the adaptation of crosslinking methods as well as the bioprinting process itself, concerning the parameters of the bioprinter and the printing process. In view of the above, it is significant that in order to achieve the set goal and thus a specific application use, it is necessary to take into account these three variables [124].

Moreover, using bioink methods, in addition to producing bioinks that will be characterized by adequate printability and high survival rate of the cells contained therein, it is further expected that these scaffolds will provide a suitable environment for the cells, which will undergo a number of post-bioprinting maturation processes within it. This is another challenge that researchers are targeting, that is, the study of cell viability, cell proliferation, the biodegradation of scaffolds and thus their durability over time, as well as the integration of a hierarchical vascular network, which is an interesting approach to the possibility of upscaling the bioprinting of functional tissue and organ constructs for transplantation [125]. Moreover, for a given construct to have an applied use, the solution in question must be simple and reproducible. However, still, in most studies, cell viability is qualitatively assessed for constructs built with only a few layers and mostly covers a short period

Table 1
Examples of bioinks used in conjunction with the bioprinter that have been studied.

Bioink composition	Bioprinter and printing parameters	Physicochemical properties	Cell survival
GelMA	Modified printer Lulzbot TAZ 4, Aleph Objects Nozzle temperature: 21 °C Speed: 400 mm/min	Pore diameter: 35.7–66.7 μm Young's modulus: 1.8–6.9 kPa Resolution: 500 μm Gelation time: 58.0–22.8 min	HUVEC Cell density 4 × 10^6 mL^{-1} Survival rate: After 1 day: 88.7–85.1% After 7 days: 91.0–89.3% [101]
Chitosan Glycerophosphate Hydroxyethylcellulose Nanocrystals of cellulose	Chitosan Glycerophosphate Hydroxyethylcellulose Nanocrystals of cellulose BioX, Cellink, Göteborg Nozzle temperature: 25 °C Extrusion pressure: 12–20 kPa Speed: 2 mm/s	Cell-free bioink viscosity: 106.09–136.29 Pa·s Cell bioink viscosity: 251.88–258.90 Pa·s Compound viscosity at 1 Hz of cell-free bioink: 14, 18 and 16 Pa·s Compound viscosity at 1 Hz of cell bioink: 23, 32 and 42 Pa·s Yield stress: 401.93–536.68 Pa of cell-free bioink 413.18–585.21 ± 61.77 Pa of cellular bioink	MC3T3-E1 Cell density of 5 × 10^6 mL^{-1} Retention of high viability after printing and no significant proliferation after 7 days [123]
Sodium alginate Gelatin	INKREDIBLE Cellink Extrusion pressure: 45 kPa	tan δ < 1 Viscosity at shear rate 1 s-1: 12.10 Pa·s Filament width: 372.59 mm Scaffold stiffness: 1–8 kPa in 12 days	NSCLC PDX (EGFR T790M) Cell density 10 × 10^6 mL^{-1} Survival After printing: 97,51% After 15 days: 94,23% [112]
Gelatin Sodium alginate	Specially designed printer Flow rate: 0.04 and 0.01 mL/m (inner diameter/outer diameter) Feed rate: 6 mm/s Pressure: 15 psi	Young's modulus: 0.5–1.8 kPa Normalized pore count: 50–98%	3T3 NIH mouse fibroblast cells Cell density 2 × 10^6 mL^{-1} Survival rate: After printing: Pure alginate: 70%. Alginate-gelatin: 85%[113]
ADA-Gel oxidized alginate gelatin	Bioplotter RP V2.9, EnvisionTEC GmbH Flow rates: 1 mg/s Printing speeds: approximately 25 mm/s Material temperature: 26 °C Extrusion pressure: 0.12–2.15 bar	tan δ: 0.1 – 0.57	RSC96 and HUVEC Survival rate: After 1 day: more than 90% After 7 days: decrease < 80% (HUVEC) and about 90% (RSC96) [119]
Gelatin Methylcellulose Cross-linked with transglutaminase	RegenHU 3D Discovery, Fribourg Printhead/bioink temperature: 27 °C Flow speed: 12 mm/s Extrusion pressure: 25 kPa	Homogeneity of the filament U= 1.012 Pore ratio: Pr= 0.86 Fidelity of shape: I= 0.93 Stiffness: 5–50 kPa	NIH 3T3 Fibroblasts Survival rate: After 1 day: 86% After 3 days: 68% After 5 days: At crossover points: 33% At others: 61% For monolayer constructs: 82% [127]
Sodium alginate Gelatin	BioBots, BioBots Printing temperature: 25 °C Printing speeds: 5 mm/s Extrusion pressure: 193 kPa	Strand width: 0.31 mm Printing accuracy: 97% Compressive modulus: 48 kPa Parameter optimization index: POI= 88.82	Mesenchymal stem cells (MSCs) from adult sheep adipose tissue Survival rate: 92.3% [126]

of time i.e. up to 7 days after printing [101,123,126,127], rarely exceeding 14 days [112].

From an application point of view, e.g. the use of a scaffold in wound healing, the determination of whether a given system has a potential application should concern much larger constructs, with a hierarchical structure, as well as their longer incubation period. Additionally, determining the biodegradability of scaffolds in the context of cell growth and proliferation is still an often ignored issue. This is another huge challenge regarding the post-biodegradation stage, in which a given scaffold should be designed in such a way that these two processes are interconnected, as only their proper alignment will result in fully functional constructs.

5. Biomedical application of 3D bio-printed hydrogels

3D bioprinting is a rapidly developing method in regenerative medicine. Due to the high precision, the technology allows for the exact reconstruction of the tissue. It is also characterized by reproducibility, which allows for automation and production on a larger scale. In addition, the 3D construct is known to reproduce tissues of similar physiological size and geometry [128]. The most commonly printed tissues include skin, cartilage, and bone described below (Fig. 4).

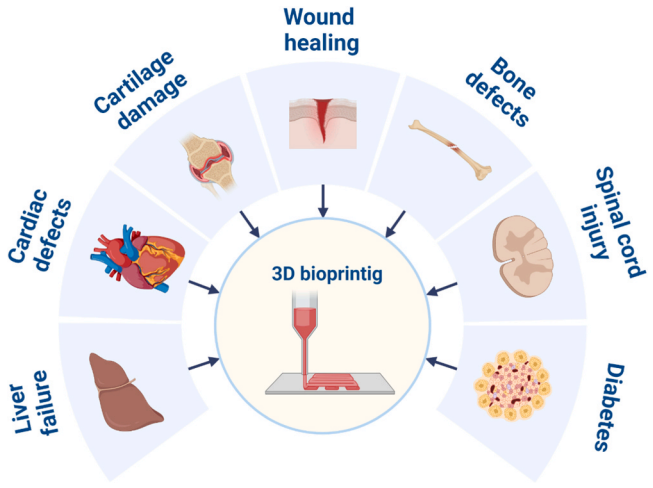

Fig. 4. 3D bioprinting can be utilized in tissue engineering and regenerative medicine in the treatment of various disorders including wound healing, spinal cord injury, and bone or cartilage defects. *Created with BioRender.com.*

5.1. Wound healing

In the context of wound healing, it is important to create a skin construct, recreating its normal anatomical structures and functions through the presence of blood vessels or skin appendages-hair, sebaceous, and sweat glands. The cells used in skin renewal through 3D printing mainly include keratinocytes, fibroblasts and epidermal stem cells, AD-MSCs, BM-MSCs, or iPSCs [129]. 3D bioprinting technology allows the reproduction of a piece of skin of any size and shape, improves the precision of the dressing used, and reduces the need for complex surgical procedures [130].

Jorgensen et al. conducted a study confirming epidermal barrier formation and collagen remodeling by skin constructs in full-thickness wounds. Fibrinogen hydrogel mixed with cells from the epidermis, dermal dermis layer, and subcutaneous tissue was used as a bioink. The cells were imprinted as the different layers of the skin, respectively. The constructs produced in this way were applied to wounds excised from mice. Application of the bioprinted skin resulted in wound closure by day 21 and regeneration throughout the thickness. Histologically, the construct was similar to physiological skin, and the presence of host cells within it was demonstrated [131].

In contrast, Baltazar et al. developed a skin construct containing human fibroblasts, keratinocytes, endothelial cells from umbilical cord blood, endothelial colony-forming cells, and pericytes. The cellular components were suspended in type I collagen. Their transplantation into the damaged dorsal skin of immunodeficient mice resulted in improved vascularization and the maintenance of a blood vessel network 2–4 weeks and epidermal renewal 4 weeks after transplantation. Epidermis renewal occurred through the maturation of keratinocytes in the presence of pericytes [132].

As mentioned above, SCs are also used for 3D bioprinting. Roshangar et al. seeded AD-MSC cells onto printed hydrogel scaffolds formed by a mixture of alginate and collagen. Then applied these scaffolds to damaged rat dorsal skin. Using the scaffolds alone resulted in faster wound healing. On the other hand, using AD-MSCs in the scaffold induced faster epithelialization of the searched area and the formation of a multilayered epidermis [133].

Using MSCs derived from human umbilical cords in a diabetic wound model in mice also improved healing outcomes. The stem cells were 3D printed along with alginate gel. Such constructs reduced inflammation, improved normal tissue regeneration, and induced showed expression of growth factors associated with wound healing (including transforming growth factor beta (TGF- B)) [134]. Furthermore, Abaci et al. reprogrammed endothelial cells into iPSCs, and with 3D bioprinting, produced constructs that formed vascular networks with a fixed micropattern. Administration of the substitutes to wounds in immunodeficient mice promoted the formation of new blood vessels and increased the proliferation of basal keratinocytes [135]. Moreover, application of the alginate hydrogel matrix and human umbilical cord MSCs at the wound site in diabetic mice accelerated regeneration. Increased expression of TGF B, IL33, collagen production, and mast cells were observed [136].

An increasing number of studies are using 3D printing technology to prepare dressings for patients. Armstrong et al. used autologous minimally manipulated homologous adipose tissue in 10 patients with chronic diabetic foot ulcers. 60% of wounds with the printed, fitted dressing were completely covered with epithelium 12 weeks after treatment. No wound site infection or rejection of the constructs was observed [137]. Additionally, the application of the autologous minimally manipulated homologous adipose tissue and fibrin glue was tested. 3D bio-printed scaffolds were used in 10 patients with diabetic foot ulcers at the site of the lesion. In 7/10 patients, the wound was healed after 12 weeks. Also, no scar formation and changes in the structure of adjacent tissues were observed [138] Another type was dressing composed of minimally manipulated extracellular matrix derived from autologous adipose tissue. In 17 patients with the scaffold applied closure of the diabetes wound was observed after about 4 weeks. In the control group of 16 patients, the regeneration process was delayed [139].

5.2. Cartilage and bone defects

Cartilage is built of connective tissue. Cartilage tissue has a very limited regenerative capacity, making various lesions such as osteoarthritis require innovative solutions. Markel Lafuente-Merchan et al. tested nanocellulose alginate-based bioinks to which chondroitin sulfate (CS) and dermatan sulfate (DS) were added and then mixed with mouse MSCs. The supplementation of DS improved the cells' metabolic activity and functionality. Meanwhile, both components improved the expression of genes (SRY-box transcription factor 9 (SOX9), aggrecan (ACAN), collagen type 2 (COL2), collagen type 1 (COL1)) related to MSCs differentiation into cartilage [140]. In contrast, Beketov et al. used a bioink composed of 4% collagen and chondrocytes isolated from the cartilage of conceived rats. The produced scaffolds formed a homogeneous tissue after subcutaneous implantation; collagen was replaced by an extracellular matrix. The cartilage tissue had high levels of glycosaminoglycans (GAG) and COL2 [141]. Sun et al. produced a construct from poly (lactic-co-glycolic acid) and rabbit BM- MSCs with the addition of bone morphogenetic protein 4 and transforming growth factor β3. It was used to evaluate the repair of full-thickness cartilage damage in the knee joint. After application of the scaffold, vitreous cartilage similar in appearance to normal cartilage was formed, rich in GAGs and exhibiting a chondrocyte phenotype (presence of proteoglycan 4 (PRG4) and collagens II, X) [142]. At the same time, 3D constructs are being used in nasal cartilage transplants. Lan et al. utilized a scaffold made from type I collagen and human nasal chondrocytes. It was cultured in vitro and supplemented with TGFβ3 for another 9 weeks and then implanted subcutaneously into nude mice. The printed cartilage retained its original size and shape, had more collagen than control Chydro-Gide, higher GAG content, and increased expression of COL2. However, a decrease in cell viability was observed for the 3D construct [143]. Application of the scaffolds also improves intervertebral disc regeneration. The bioink was a polylactide along with a hydrogel, which was loaded with rat BM-MSCs. The 3D constructs were tested in vitro and in vivo. Cell viability was preserved, the extracellular matrix was maintained, and the scaffolds ensured the deposition of proteoglycans and collagen within the disk space [144].

Bone repair is particularly important in large defects when bone growth and regeneration conditions are not preserved. A key element for bone regeneration is the creation of a microenvironment that promotes osteogenesis [145]. In 2018, a study about a scaffold of gelatin and hydroxyapatite with ascorbic acid and β-glycerophosphate disodium salt hydrate was created. It was electrospinning and then rat BM-MSCs cells were seeded onto it. The prepared constructs were administered to rats with cranial defects. It stimulated the reconstruction of the parietal bone, which after 6 weeks was almost regenerated, without the presence of inflammatory cells. The formed tissue after 12 weeks showed similarity to physiological tissue and had a lamellar structure. In contrast, in the control group, fibrous connective tissue was observed in the areas of bone reconstruction [146]. Tao et al. also used gelatin methacrylate /dextran emulsion printing via digital light processing with BM-MSCs in cranial defects. The constructs provided bone regeneration with a much larger area than controls. Histological staining showed islands of osteoid indicative of bone maturation. Markers of osteogenesis-collagen 1 and osteocalcin were present [147]. An important element in bone regeneration is the porosity of the tissue constructs. Calcium phosphorus-based ceramic scaffolds, such as 3D printed tricalcium phosphate powder with iron ions (FE 3 +) and silicon ions (Si 2 +), can provide this. When administered in the rat distal femur model, it accelerates the formation of new blood vessels and increases the production of type I collagen [148]. Another novel approach is printing cells in situ using Laser-Assisted Bioprinting (LAB). Mesenchymal stromal cells along with hyaluronic acid and collagen have been used in the 3D

printing of a mouse skull vault defect. The disc-shaped printed constructs resulted in even faster bone reconstruction until the mature bone was produced. In the case of the printed rings, bone regenerated exclusively at the periphery of the defect [149]. In situ bioprinting in the calvarial defect model was also carried out with endothelial cells combined with thermosensitive hydrogel to stimulate angiogenesis. At the same time, a light-crosslinked hydrogel combined with BM-MSCs was used as a matrix for bone reconstruction. The constructs formed significantly stimulated angiogenesis and osteogenesis [150].

5.3. Spinal cord injury

Spinal cord injury can be caused by a primary damage (dislocation, extrusion) or arise as a complication of previous injuries (inflammation, swelling, ischemia). It is characterized by permanent damage to nerve cells resulting in inability to renew their function [151].

For successful spinal cord repair, it is necessary to map the structure of the spinal cord with the spatial distribution of neural stem cells (NSC). Liu et al. used a bioink composed of chitosan, hyaluronic acid derivatives, and matrigel mixed with NSCs. The prepared scaffolds are utilized in rats with spinal cord injury. It provided high cell viability, renewed axons, and restored locomotor abilities [152]. In contrast, Zhou et al. used 2 types of cells- BM-MSCs and NSCs. The cells were 3D printed with methacryloyl gelatin hydrogel and then placed at the site of spinal cord hemisection in the rat. Construct reduced inflammation, and scar formation. In addition, it promoted the differentiation of nerve cells and improved the animal's motor abilities [153]. Another study used sodium alginate/gelatin combined with NSCs and oligodendrocytes. Constructs were 3D printed and then implanted in place of complete spinal cord transection in a rat. Scaffolds improved nerve regeneration and enhanced the animals' motor skills. In addition, organized structure of the scaffold ensured regeneration of axons and formation of new neurons [154].

In contrast, Gao et al. developed hydrogels from gelatin methacrylate, hyaluronic acid methacrylate and poly(3,4-ethylenedioxythiophene) Sulfonated Lignin (PEDOT:LS). The aforementioned hydrogels were then 3D printed with NSCs and applied to a rat model of complete spinal cord transection. The scaffolds used promoted the restoration of motor function in rats. The addition of PEDOT:LS increased the conductivity of the hydrogels and enhanced the differentiation ability of NSCs toward neurons [155]. Koffler et al. proposed using continuous microscale projection printing (μCPP) to reproduce the complex structure of the central nervous system. The 3D constructs were created from polyethylene glycol, gelatin methacrylate and neural progenitor cells. They were implanted at the site of a complete transection of the spinal cord in rats. The scaffolds ensured the regeneration of damaged axons and the elongation of those already present below the site of injury, which could lead to the formation of synaptic connections. Six months after transplantation, neural progenitor cells filled the injury site. Immunohistochemical staining did not reveal the presence of the stem state marker nestin and the cell division-associated marker Ki67 which may indicate maturation of the cells [156].

5.4. Other applications

3D bioprinting capabilities are also being applied in other cases, particularly in unavailable, extensive injury or lost biological function renewal. Disease entities studied include cardiac disfunction, spinal cord injury, diabetes or liver failure (Table 2).

Table 2
3D bioprinting as a treatment model in various disease entities.

Disease	Bioink	Results and references
Congenital heart defects	Neonatal human c-kit + progenitor cells laden-cardiac extracellular matrix-gelatin methacrylate	In a rat model of right ventricle failure, the use of the 3D construct resulted in the increased vascular formation, reduced cardiac fibrosis, and limited cardiomyocyte hypertrophy[157]
Cardiac diseases	Extracellular matrix hydrogel with iPSCs differentiated into cardiomyocytes and patient-specific endothelial cells	Embedding the printed construct between two layers of the rat's omentum for 7 days produced elongated cells that could contract[158]
Diabetes	Pancreatic extracellular matrix with hyaluronic acid methacrylate encapsulated with pancreatic islets	The 3D construct was implanted into the subcutaneous connective tissue of diabetic mice, which improved blood glucose levels and increased insulin levels. In addition, it contributed to the formation of new vessels and the expansion of existing networks[30]
Liver failure	Methacrylate gelatin with primary human hepatocytes	When implanted in mice, the constructs stimulated vascularization and maintained normal liver cell function[159]
Liver failure	A mixture of gelatin and alginate along with HepaRG cells	Abdominal administration in mice with liver damage resulted in improved survival in the animals. The cells had functions similar to those exhibited by normal liver cells such as drug metabolism. In addition, vascularization improved[160]
Liver failure	3% alginate hydrogel with induced hepatocyte-like cells derived from mouse embryonic fibroblasts	Scaffolding in a mouse model of liver injury resulted in accelerated cell proliferation and increased albumin expression[161]

6. Clinical need for 3D bio-printed hydrogel constructs – current status and future directions

The field of 3D bioprinting can provide an alternative to missing therapies. It is a rapidly developing method in regenerative medicine because it is based on already-known assumptions and well-known standard 3D printing. Due to the high precision, the technology allows for the exact reconstruction of the tissue. It is also characterized by reproducibility, which allows for automation and production on a larger scale [128]. Personalized medicine is essential for treating many disorders such as large skin defects, atypical fractures, and chronic wounds. The treatment of these dysfunctions poses a massive burden on the economy and a challenge for medical personnel [162,163].

According to Precedence Research, the market for 3D bioprinting has been valued at $2.13 billion in 2022. It is estimated that its value will increase to 8.3 billion dollars in 2023 [164]. In addition to technological development, 3D bioprinting is an important element in the advancement of medicine. It can provide innovative solutions for severe clinical cases such as deep chronic wounds, cartilage defects, or large bone damage. However, further studies involving animals and, the subsequent progression of patients, are needed to confirm the effectiveness of the 3D constructs on a larger scale.Based on the time of complete wound healing, wounds can be classified as acute and chronic. Acute wounds heal in an organized process, divided into three overlapping stages which include inflammatory, proliferative, and remodeling phases [165]. However, the process of wound healing can be affected by different factors, like patients' age, overall health, nutrition, lifestyle (e.g. alcohol intake or smoking), and diseases (e.g. diabetes, vascular problems). Also, the medications (e.g. non-steroidal anti-inflammatory drugs, steroids, chemotherapy) and other treatments that the patient receives, e.g. radiotherapy can have a negative effect on wound healing. All these factors can lead to the formation of chronic wounds, which do not heal in a proper manner and time [166].

Chronic wounds constitute not only medical but also economic burdens, as they consume a big part of medical budgets. For example, Medicare data from 2014 shows that in the U.S. almost 15% of their beneficiaries (8.2 million people) had at least one type of wound (e.g. arterial ulcers, chronic ulcers, diabetic foot ulcers, pressure ulcers, skin disorders, surgical wounds, traumatic wounds or venous ulcers) or associated infections (e.g. diabetic infections, skin infections, surgical infections or venous infections). It was estimated that Medicare had to spend $28.1 - $96.8 billion for wound care [167]. In Europe, 1,5–2 million people is estimated to suffer from acute or chronic wound, and the cost of care for only one type of chronic wound – diabetic ulcers (DFU) predicted to be €6–8 billion per year [168]. Additionally, Canada is spending $509 million on DFU treatment [169].

Taking into account the number of patients and the impact on their lives, the wound healing problems have been named a "silent epidemic' as the effect on patient's quality of life, their families, and health care systems is often not fully recognized [168]. The COVID-19 pandemic also affected wound care. A 40% decrease in the visits to wound care centers in 2020 compared to 2019 has been reported. It can lead to increased hospitalization rates, 30-day readmission, and the necessity to use more acute care services [170].

For extensive burn wounds (Fig. 2) or chronic wounds, basic care methods are insufficient to restore the tissue. In addition, the prolonged healing process is associated with discomfort in the patient's functioning. The use of a 3D bioprinted dressing using hydrogels and stem cells not only provides an environment for tissue regeneration and cell proliferation but also customizes the shape and size of the construct needed [171].

Also, irregular, large bone defects are not amenable to standard treatment. Due to the small number of sites in the body where a bone fragment can be taken without loss of function, hypersensitivity, or disease at the donor site, their therapy is limited. The use of a 3D bioconstruct can restore the connection between the two parts of the bone, provide the proper shape and, without an immune response, stimulate the environment to restore the defect [172,173].

The natural ability to renew tissues and organs is possible in various disease states. However, severe multi-organ damage can only be treated with transplants. Huge shortages in the availability of organs for transplantation often involve the death of the patient [174]. According to the Health Resources & Services Administration, 105,800 people are currently waiting for a transplant, and 17 people die each day due to organ unavailability in the USA [175]. Allografts at times may also lead to immune rejection. 3D bioprinting may find application in whole-organ restoration. Over the years, it has been possible to produce by this method various tissues that provide conditions close to physiological ones in the body. However, the process requires the production of a multicellular construct, enriched with vascular and neural networks. Therefore, a great deal of research is required to ultimately create a functional organ [176].

3D bioprinting may also be helpful in developing complicated in vitro models for drug testing. Currently, there is a big need for precise in vitro models in drug screening. Traditionally 2D cultures do not perfectly reflect conditions in the human body and may lead to misleading results [177]. Interestingly, 3D bioprinting also offers solutions that can be helpful in not only testing but also manufacturing of advanced drugs e.g. vaccines, therapeutics, and delivery systems [10]. What is more, the use of 3D bioprinting enables the creation of tissue constructs to study e.g. pharmacokinetics. Bioprinted organs and tissues can also serve as an alternative for the use of animals in drug testing [178]. It can be especially helpful in the cosmetics industry, where animal testing is forbidden in many countries, e.g. European Union.

The use of 3D bioprinting still has a lot of challenges to overcome, however, its advantages and the huge application potential in medicine cause it to be constantly developed (Fig. 6). 3D bioprinting may be a solution not only to the organ shortage or the need for complicated in vitro models for drug or cosmetics testing but it can also be applied in

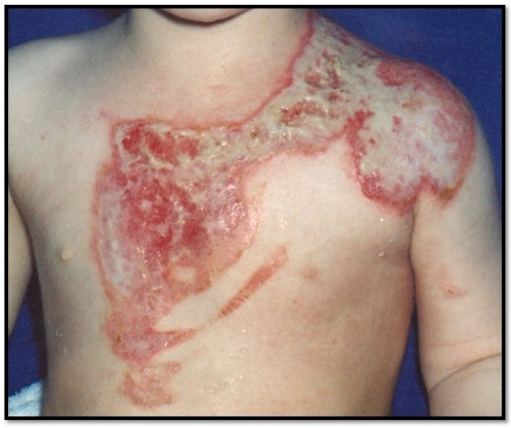

Fig. 5. Patient with a deep burn. Destroy whole skin, waxy tissue is necrosis in the process of liquefaction, red tissue is granulation tissue forming.

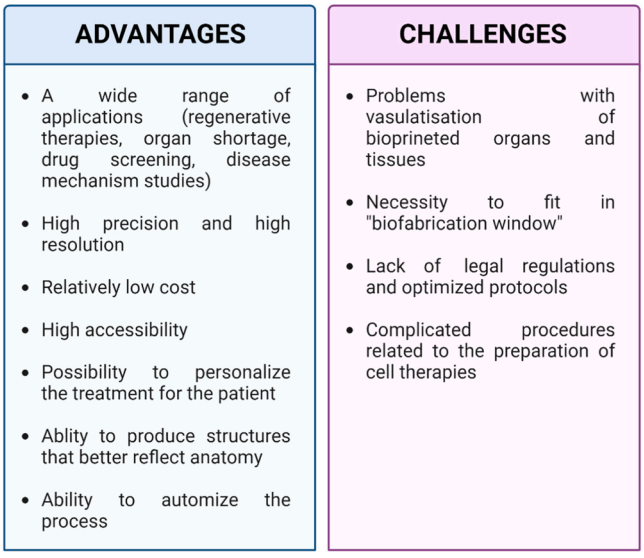

Fig. 6. Advantages of 3D bioprinting and challenges that need to be overcome. *Created with BioRender.com.*

regenerative medicine and drug delivery or studying the mechanisms of diseases. The advantages of extrusion-based bioprinting, which is currently the most widely used method, include high accessibility, low costs, and high printing precision [179]. The use of 3D bioprinting has also advantages over other biofabrication methods. It enables the production of more complex and precise constructs, which better reflect the anatomic structures. It also allows to automize of the process of high precision mass production of these structures. What is more, the use of computer-aided design allows to produce constructs that are tailored to the individual needs of the patient. It also allows to use medical images in the process of biofabrication. Also, the process of layer-to-layer biofabrication makes it easier to control the spatial distribution of cells and scaffolds in the printed tissue [180,181]. However, many challenges need to be overcome. For example, apart from the technical aspects, there is still a problem with the proper vascularization of bioprinted tissues, and the mechanism of the immune response after the implantation of biofabricated constructs needs to be fully evaluated [182].

In conclusion, 3D bioprinting and its modifications hold great promise in tissue engineering, regenerative medicine, and personalized therapy tailored to the individual patient. However, some issues limit the use of these therapies in everyday clinical practice. First, because the application of 3D bioprinting in clinics is still quite new, currently there

are no legal regulations and validated, optimized protocols that allow its use without safety concerns. Additionally, as cell-based products, 3D bioprinted constructs for the preparation may require sterile conditions and GMP facilities equipped with cell culture hoods and qualified personnel. This, in turn, may translate into a high single cost of such therapy and complicated procedures of their application in the market. However, this issue should not eliminate such therapies from the treatment of patients, because even if a given therapy is expensive, but it will help the patient more effectively and significantly shorten the treatment time, the final cost of treatment for a single patient may be much lower than the long-term use of less effective therapy. An innovative method that may significantly expand the possibilities of the medical sector is the four-dimensional (4D) printing technique, which allows for the reconstruction and maturation of 3D bioprinted tissues. Due to its low affordability and difficult manufacturing technique, it requires further analysis and detailed research.

3D bioprinting may be a solution for many diseases like chronic wounds, and cartilage or bone defects. Looking ahead, bioprinted organs may revolutionize the field of transplantation and help to solve the problem of organ shortage. It may not only shorten time to help waiting time for the organ but also help patients who, due to legal regulations, have little chance of receiving an organ and those who reject this form of treatment for religious or ethical reasons.

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CRediT authorship contribution statement

Milena Deptuła: Conceptualization, Writing –Writing – review and editing, Visualization, Funding acquisition, Supervision. **Małgorzata Zawrzykraj:** Conceptualization, Writing – original draft and preparation, Writing – review and editing, Visualization. **Justyna Sawicka:** Writing – original draft and preparation, Visualization, Funding acquisition. **Adrianna Banach- Kopeć:** Writing – original draft and preparation. **Robert Tylingo:** Writing – review and editing. **Michał Pikuła:** Writing – review and editing, Funding acquisition, Supervision. **Milena Deptuła and Małgorzata Zawrzykraj:** Contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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