UPDATES FOR THE DEVELOPMENT OF 3D BIOPRINTING FROM DECELLULARIZED SCAFFOLDS: A NARRATIVE REVIEW

ATUALIZAÇÕES NO DESENVOLVIMENTO DA BIOIMPRESSÃO 3D A PARTIR DE ESTRUTURAS DESCELULARIZADAS: UMA REVISÃO NARRATIVA

Marcelo Costa Araújo[1], Maria Eduarda Arantes da Cunha[2], Camila Botelho Miguel[2], Giovana Figueiredo Maciel[2], Carlos Antônio Trindade da Silva[1], Carlo José Freire Oliveira[1], Siomar de Castro Soares[1], Wellington Francisco Rodrigues[1,2]

[1]Curso de Pós-graduação em Medicina Tropical e Infectologia, Universidade Federal do Triângulo Mineiro. [2]Laboratório Multidisciplinar de Evidência Científica, Centro Universitário de Mineiros, Mineiros, GO.
wellington.frodrigues@hotmail.com

ABSTRACT

Regenerative medicine has significantly advanced in the past two decades, with tissue engineering playing a key role in expanding application possibilities and solutions for medical challenges. Decellularization of tissues and organs has accelerated bioengineering efforts, enhancing repair strategies for various injuries. This technique's adaptability has led to its application across multiple tissue types, with certain methods demonstrating greater efficiency and potential for clinical integration. This study aims to document adjustments in decellularization techniques, key advances, and applications within tissue and organ repair. Using a retrospective and descriptive approach, this narrative review addresses both general and specific aspects of decellularization. Key topics include the underlying principles, primary techniques, advancements, applications, and future perspectives of decellularization methods. We expect this review to solidify the main evidence supporting tissue and organ repair strategies via decellularization and to provide insights or generate questions for further research, contributing to the enhancement of quality of life through more targeted primary studies.

KEYWORD: Regenerative Medicine, 3D Bioprinting, Tissue Engineering, Decellularization.

RESUMO

A medicina regenerativa avançou significativamente nas últimas duas décadas, com a engenharia de tecidos desempenhando um papel fundamental na expansão das possibilidades de aplicação e na resolução de desafios médicos. A descelularização de tecidos e órgãos acelerou os esforços em bioengenharia, aprimorando as estratégias de reparo para diversos tipos de lesões. A adaptabilidade dessa técnica

permitiu sua aplicação em múltiplos tipos de tecidos, sendo que alguns métodos demonstraram maior eficiência e potencial para integração clínica. Este estudo tem como objetivo documentar os ajustes nas técnicas de descelularização, principais avanços e aplicações no reparo de tecidos e órgãos. Utilizando uma abordagem retrospectiva e descritiva, esta revisão narrativa aborda tanto os aspectos gerais quanto específicos da descelularização. Os principais tópicos incluem os princípios básicos, as técnicas primárias, os avanços, as aplicações e as perspectivas futuras dos métodos de descelularização. Espera-se que esta revisão solidifique as principais evidências que sustentam as estratégias de reparo de órgãos e tecidos por meio da descelularização e forneça insights ou gere perguntas para novas pesquisas, contribuindo para a melhoria da qualidade de vida através de estudos primários mais direcionados.
PALAVRAS-CHAVE: Medicina Regenerativa, Bioimpressão 3D, Engenharia de Tecidos, Descelularização.

INTRODUCTION

Bioprinting represents a frontier in medical treatment, offering unprecedented solutions for tissue repair and regeneration by enabling the replacement of damaged cells with healthy ones, with minimal harm to patients or other living organisms. Notably, bioprinting promises to reduce the reliance on animal models in drug testing, alleviating ethical concerns, cutting costs, and enhancing the translational relevance of preclinical findings to human health[1].

The advent of bioprinting dates back to the early 2000s, when conventional printers were adapted to deposit cellular layers in petri dishes. Initial iterations of bioprinting involved layering cells in a 2D format, progressively evolving toward three-dimensional composites. This development led to innovations such as "inkjet bioprinting," where cellular spheroids were strategically positioned to merge in culture, producing thicker and more complex tissue structures[2]. The establishment of Organovo in 2007 marked a pivotal moment, as the company embarked on the commercial production of bioprinted tissues, including pioneering efforts in kidney tissue fabrication[3].

In 2011, Cyfuse Biomedical of Japan introduced a distinct bioprinting technology using the Kenzan method, which relies on arrays of micro-needles for precise cellular pairing, contrasting with scaffold-based cell layering. This method

enables the successive addition of photopolymers, creating detailed constructs applicable to human healthcare[4]. Consequently, bioprinting has rapidly advanced to applications in surgical design, facial reconstruction, bone prosthesis development, and even the replication of organ-specific tissues[5].

Despite this progress, bioprinting remains challenged by the need for effective scaffolds, or structures, that support cellular adhesion and development within bioengineered tissues. Decellularization, one key approach, entails the removal of cellular contents from tissues, preserving only the extracellular matrix (ECM) to facilitate in vivo biocompatibility and immune compatibility[6]. However, substantial debate persists regarding the optimal methodologies for scaffold preparation to ensure homogeneous cell distribution and ECM integration, which are crucial for achieving functional tissue regeneration[7].

This study aims to elucidate the advancements and persistent challenges within 3D bioprinting, particularly the development of biocompatible scaffolds and the refinement of bioink definitions for constructing viable, functional tissues. By assessing the current technologies and addressing the limitations that restrict bioprinting's potential, this work seeks to advance understanding in both human and veterinary regenerative medicine.

DEVELOPMENT

Bioprinting techniques vary depending on tissue requirements and may involve a combination of methods to achieve optimal results.

Inkjet-based bioprinting functions similarly to a conventional 2D inkjet printer. This contactless technique positions droplets of bioink, with each droplet containing between cells. These droplets are manipulated via electrical currents and magnetic fields for precise placement. There are two primary methods within this approach: continuous inkjet printing (CIJ) and drop-on-demand (DOD) printing. CIJ offers a higher droplet generation rate, but it requires fluid recycling, which raises contamination risks. Conversely, DOD only releases bioink as needed,

enhancing cleanliness and reducing waste[7]. Heat or mechanical compression is often used to eject inkjet droplets, though the piezoelectric inkjet system is an alternative. In this system, piezoelectric crystals in the ink chamber vibrate under an electric pulse, forcing droplets through the nozzle. While the piezoelectric method is cost-effective and avoids thermal damage, nozzle clogging may occur, impacting droplet consistency and cell viability. Localized heating has been shown to have minimal effects on cell integrity even at temperatures approaching 300°C when exposure is brief[8].

Laser-assisted bioprinting (LAB) utilizes a process adapted from laser-induced forward transfer (LIFT) to precisely deposit cells and biomaterials onto substrates. LAB is capable of handling highly viscous materials and complex bioinks. A pulsed infrared laser targets a donor ribbon layered with an absorbent metal coating, heating the layer until the bioink droplets are vaporized and transferred to the target substrate[9]. This technique minimizes contamination risk, supports various substrate viscosities, and maintains structural integrity, though gravitational settling of cells and fabrication times remain challenging considerations[10].

Extrusion-based bioprinting is the most common approach due to its versatility and ability to handle a wide range of bioink viscosities. Bioinks are extruded via pressurized syringes, enabling controlled deposition onto the substrate. Cross-linking methods such as UV exposure, thermal application, and enzymatic action are commonly used to stabilize the printed constructs. With extrusion-based systems, variables such as platform positioning, air pressure, and extrusion speed can be adjusted for enhanced accuracy and structure complexity[11]. Multiple printing heads are often incorporated to facilitate simultaneous multi-material printing, reducing cross-contamination risks[12].

While 3D bioprinting has revolutionized tissue engineering, challenges persist in achieving vascularization and cellular viability for in vivo applications. Although skin bioprinting has been tested both in vitro and in vivo, limited thickness and the lack of native tissue vascularization pose significant limitations.

For example, in a study utilizing gelatin-chitosan (PGC) hydrogel as bioink, collagen fibers were successfully printed and integrated onto the dorsal surface of a mouse, maintaining cellular viability and supporting vascular integration for up to 11 days[13]. Similarly, it was demonstrated successfully in vivo bioprinting using human cells derived from biopsies. Their approach allowed for a rapid printing process, yet further advances are needed to match the complexity of natural skin layers[14]. Cardiac tissue printing has garnered attention due to the prevalence of heart disease. Successfully printed a cardiac model with two ventricles, using gelatin bioink and calcium chloride under electrical stimulation, laying the groundwork for further developments in cardiac bioprinting[15].

Scaffolds are essential to bioprinting, providing structural support for cellular organization. Decellularization, a promising method, involves various physical, chemical, and biological processes to clear cellular contents while retaining extracellular matrix (ECM) integrity. Physical methods include controlled mechanical forces or ultrasound, though they risk ECM damage if not carefully regulated[16]. Chemical decellularization typically employs hypertonic or hypotonic solutions, acids, or enzymes to catalyze cell lysis and ECM preservation, though residual chemicals may interfere with ECM properties[17].

Perfusion protocols, such as those applied to rat hearts, have been successful in removing DNA and intracellular proteins, preserving the 3D structure required for recellularization[18]. However, residual material evaluation remains crucial for toxicity avoidance, and qualitative verification methods, including histological and fluorescent staining, are used to confirm scaffold purity[19]. Sterilization methods include acid and gamma-ray treatments, although ECM structural degradation can result. The use of antibiotics at 4°C or lower temperatures is debated, with preference for fresh tissue preservation to minimize structural compromise[20].

Recent studies highlight the application of decellularized scaffolds in cartilage and corneal reconstruction. In corneal engineering, decellularized human corneal scaffolds have shown promise for anterior surface reconstruction, displaying optimal transmission properties and biocompatibility[21,22]. Other

innovations include hybrid bioinks that combine alginate with ECM from decellularized tissues, which demonstrate enhanced cell viability and differentiation capabilities[23].

One of the primary advantages of decellularization is the preservation of the native extracellular matrix (ECM), which maintains essential biochemical signals for cellular adhesion, proliferation, and differentiation. ECM provides an environment that closely mimics in vivo conditions, creating an ideal niche for cell homing and tissue-specific responses[24]. This bioactive environment is critical in promoting tissue regeneration, as it influences angiogenesis, stem cell differentiation, and immune modulation. Unlike synthetic scaffolds, which may lack bioactivity or generate immune responses, decellularized ECM scaffolds exhibit superior biocompatibility and reduced immunogenicity, making them highly favorable for clinical applications[25].

Decellularization processes also facilitate the creation of scaffolds with complex architectures that retain the microvascular structures necessary for vascularization. This is particularly advantageous in the regeneration of organs such as the liver and heart, where vascularization is a limiting factor in achieving functional recellularization[26]. Advances in perfusion decellularization techniques have demonstrated success in removing cellular debris while preserving vascular pathways, as observed in decellularized heart-lung and kidney scaffolds[27]. The preservation of these pathways not only enhances nutrient diffusion but also supports the integration of transplanted cells.

Despite these advantages, several limitations and challenges remain. One significant drawback of decellularization techniques is the potential for ECM damage during the removal of cellular components. Harsh chemical treatments, for instance, can degrade key ECM proteins such as collagen and elastin, affecting the mechanical properties and bioactivity of the scaffold[28]. Additionally, residual chemical agents used in the decellularization process may induce cytotoxicity or interfere with cell viability during recellularization[24]. As a result, optimizing

decellularization protocols to balance effective cell removal with minimal ECM damage remains a key area of ongoing research.

Emerging strategies aim to overcome these limitations by integrating novel processing techniques. For example, supercritical carbon dioxide extraction has been shown to effectively decellularize tissues while preserving ECM integrity, reducing cytotoxic residues compared to traditional methods[26]. Another promising approach involves the development of decellularized tissue-specific hydrogels, which are derived from ECM components and offer tunable mechanical and biochemical properties tailored to specific tissue types[29]. These hydrogels have demonstrated improved cell proliferation and differentiation, particularly in organoid and assembloid models, where the 3D microenvironment plays a critical role in mimicking native tissue conditions.

In terms of applications, decellularized bone scaffolds have shown exceptional promise in orthopedic tissue engineering due to their capacity to replicate the native bone microstructure and support osteogenesis[30]. Studies indicate that decellularized bone matrices can be processed into various forms, including hydrogels, powders, and electrospun scaffolds, each tailored to specific clinical needs[30]. Additionally, advancements in decellularization techniques for tracheal scaffolds have demonstrated the feasibility of creating biocompatible and mechanically robust constructs suitable for airway repair, with reduced preparation times and enhanced cell-seeding efficiency[31].

Looking forward, the combination of decellularized ECM with advanced bioinks and 3D bioprinting technologies presents exciting possibilities for organ regeneration. Hybrid approaches that combine decellularized ECM components with synthetic or natural polymers can offer enhanced mechanical strength and bioactivity, addressing current limitations in scaffold design[25]. Furthermore, integrating vascularization strategies, such as endothelial cell pre-seeding and pro-angiogenic factor incorporation, can significantly improve the success rates of in vivo applications[24].

In conclusion, while decellularization offers numerous advantages in preserving the native microenvironment and promoting cellular functionality, its limitations underscore the need for continued innovation. Optimizing protocols, reducing cytotoxicity, and integrating decellularized scaffolds with bioactive materials are key directions for future research. As these advancements unfold, they will likely accelerate the clinical translation of bioprinted tissues and organs, bridging the gap between laboratory achievements and real-world applications in regenerative medicine.

The use of decellularized cardiac tissues presents a notable application, wherein the developmental state of cardiac progenitors is achieved under specific conditions, enabling functional heart tissue formation[32]. Decellularized osteochondral ECM scaffolds have shown potential for bone-cartilage interface regeneration, with collagen, silk fibroin, fibrin, and keratin among the commonly employed natural bioinks[33]. These constructs support osteoblast, chondrocyte, and mesenchymal stem cell integration, fostering cellular migration, proliferation, and differentiation needed for osteochondral tissue development[34].

Decellularized scaffolds derived from various organs and tissues have demonstrated significant potential in regenerative medicine, providing mechanical support, biochemical cues, and a microenvironment conducive to tissue repair[35]. In liver tissue engineering, decellularized scaffolds have been utilized to recreate liver specific architectures, facilitating hepatocyte attachment and vascular integration, both of which are essential for restoring liver function. Recent studies have shown that combining decellularized liver scaffolds with stem cell derived hepatocytes enhances cellular repopulation efficiency and improves liver-specific functionality[36].

In neural tissue engineering, decellularized peripheral nerve matrix hydrogels (DNM-G) have gained attention due to their ability to provide a neuroinductive environment for axonal regeneration. These scaffolds not only support myelination and neural network formation but can also be functionalized through mechanical and chemical modifications to tailor their properties[37]. Studies

show that DNM-G hydrogels improve the outgrowth and survival of dorsal root ganglion neurons when compared to conventional collagen gels, making them promising candidates for clinical applications in peripheral nerve injuries[37].

In cartilage repair, decellularization techniques have been advanced to produce highly bioactive scaffolds that address osteoarthritis and other degenerative conditions. Post-decellularization modifications, such as cross-linking with bioactive molecules or mechanical reinforcement, have been shown to improve scaffold integration and enhance chondrocyte viability[38]. These improvements in mechanical and biochemical properties are crucial for maintaining the functional integrity of cartilage implants.

Supercritical fluid-based decellularization, a recent innovation, has shown significant advantages over traditional decellularization methods. By preserving key ECM components, this technique enhances the bioactivity and mechanical properties of the resulting scaffolds, making them more effective in applications such as bone and vascular tissue regeneration[39]. In bone tissue engineering, for instance, decellularized bone matrices have been processed into hydrogels and bioinks, which mimic the structural and biochemical characteristics of native bone, promoting osteogenesis and angiogenesis[30].

Plant-based decellularization has emerged as a novel strategy in tissue engineering due to the inherent vascular-like structures of plants. These structures can be repurposed into bioactive scaffolds with nanofibrous architectures that promote cell attachment, differentiation, and tissue remodeling[40]. Recent advances in decellularization of plant tissues, coupled with biofabrication techniques, have resulted in scaffolds suitable for applications in bone, cardiac, and vascular tissue regeneration.

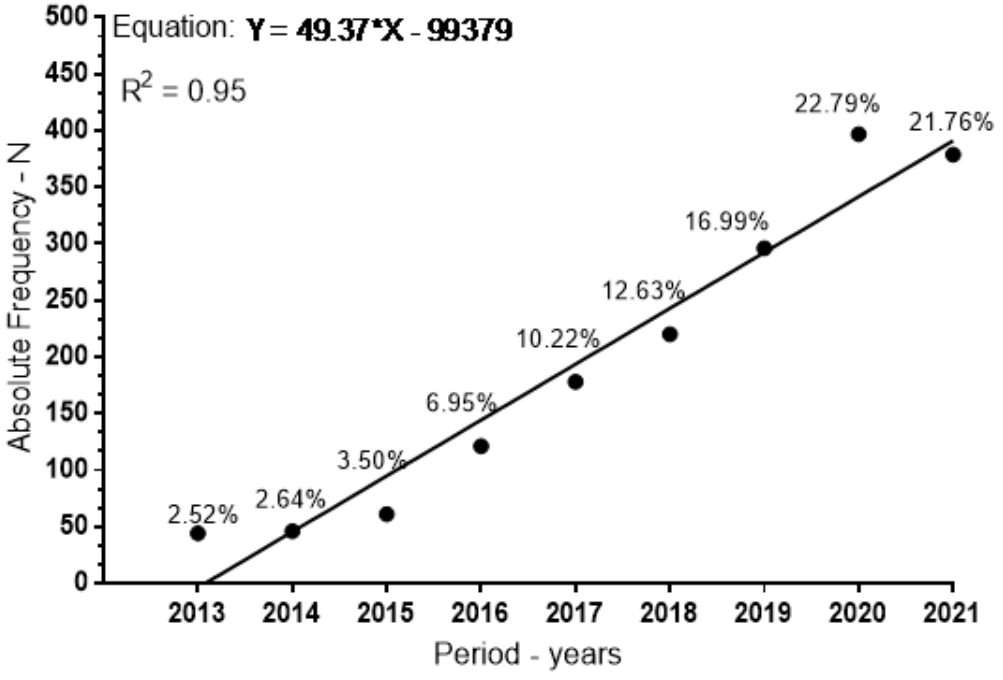
Decellularized skeletal muscle ECM scaffolds represent another promising application, particularly in the context of musculoskeletal repair. They provide structural and biochemical cues necessary for muscle regeneration and can be further processed into bioinks or hydrogels for 3D bioprinting applications. Studies

have shown that these scaffolds effectively support satellite cell attachment and differentiation, which are key processes in muscle regeneration[41].

Overall, decellularized scaffolds offer versatility in regenerative medicine, from supporting cell attachment and differentiation to facilitating vascularization and functional restoration. Their integration with stem cells and functionalization using bioactive molecules continues to expand their applications across various tissues, such as skin, cartilage, heart, and liver[35]. As technological innovations progress, the combination of decellularized ECM with bioinks and 3D printing is expected to bridge the gap between preclinical research and clinical applications, ultimately enhancing the scope of tissue engineering.

The trajectory of bioprinting research indicates significant potential for translating laboratory findings into clinical applications. Data from the Medical Subject Headings (MeSH) on "bioprinting" show a notable increase in publications from 2013 onward, correlating positively with the growth of dedicated research teams and the diversification of applications[42]. However, a downturn observed from 2020 to 2021 likely reflects a shift in research focus during the COVID-19 pandemic[43]. Despite this growth, the transition from preclinical studies to clinical applications remains a critical challenge due to limited understanding of the biological mechanisms underlying cell-scaffold interactions and the optimization of decellularized materials for different tissue types[35]. Addressing these limitations through innovations in scaffold design, bioinks, and recellularization protocols is essential for bioprinting to meet its full potential in solving complex healthcare challenges through functional bioprinted tissues and organs.

Figure 1. Linear regression model for scientific production associated with the MeSH term “bioprinting.” Data were obtained from a PubMed/MEDLINE search covering the period from 2013 to 2021. A linear regression analysis was applied to generate the line equation and assess trends in study frequency.



The second indicator (2) was constructed by applying a method designed to increase the consistency of evidence. The descriptors “bioprinting” and “Models, Animal” were used, including synonyms for “Models, Animal” to broaden the search. Data were obtained from the PubMed/MEDLINE database on 03/18/2021, using the following search string: (bioprinting[MeSH Terms]) AND ((((((((((((Models, Animal[MeSH Terms])) OR (Animal Model[MeSH Terms])) OR (Animal Models[MeSH Terms])) OR (Model, Animal[MeSH Terms])) OR (Laboratory Animal Models[MeSH Terms])) OR (Animal Model, Laboratory[MeSH Terms])) OR (Animal Models, Laboratory[MeSH Terms])) OR (Laboratory Animal Model[MeSH Terms])) OR (Model, Laboratory Animal[MeSH Terms])) OR (Models, Laboratory Animal[MeSH Terms])) OR (Experimental Animal Models[MeSH Terms])) OR (Animal Model, Experimental[MeSH Terms])) OR (Animal Models, Experimental[MeSH Terms]))

OR (Experimental Animal Model[MeSH Terms])) OR (Model, Experimental Animal[MeSH Terms])) OR (Models, Experimental Animal[MeSH Terms])).

This search retrieved 21 studies, of which 11 were selected based on eligibility criteria—specifically, studies using complex biological models with non-human animals to develop bioprinting assessments. Additionally, one study was included as it involved a model with human cells in *in vitro* evaluations (Table 1).

Bioprinting technologies, through the spatially defined gradients of immobilized biomolecules, can be tailored to guide cell growth and differentiation into various lineages[26,44,45]. However, a major challenge arises when translating these preclinical findings to human applications due to the variability in cellular behavior, tissue remodeling dynamics, and scaffold degradation rates observed across different species and experimental conditions[41]. Advanced biomaterials, such as bionic hydrogels encapsulating microspheres, provide controlled release systems that emulate the extracellular matrix (ECM), creating microenvironments that support cell survival within printed scaffolds[44]. These biomaterials can regulate inflammatory responses and offer protection against bacterial infections, potentially enhancing the success of bioprinted tissues for clinical applications[44].

In preclinical in vivo models, most studies focused on bone-cartilage repair[47-49] and cardiac tissue engineering[49,50]. These applications underscore the potential of bioprinting in regenerative medicine, particularly in tissue regeneration and the acceleration and improvement of tissue repair under various pathological conditions. However, challenges such as the lack of vascularization, limited cell viability, and immune rejection in large animal models highlight the importance of developing standardized protocols and incorporating dynamic bioreactor systems during preclinical validation[39].

Emerging strategies, such as combining decellularized scaffolds with stem cell-based organoids, aim to overcome some of these limitations by promoting better integration and functional tissue development[29]. In addition, the use of organ-on-chip platforms may provide a valuable bridge between preclinical testing and

clinical translation by enabling real-time monitoring of cell-scaffold interactions under physiologically relevant conditions[41].

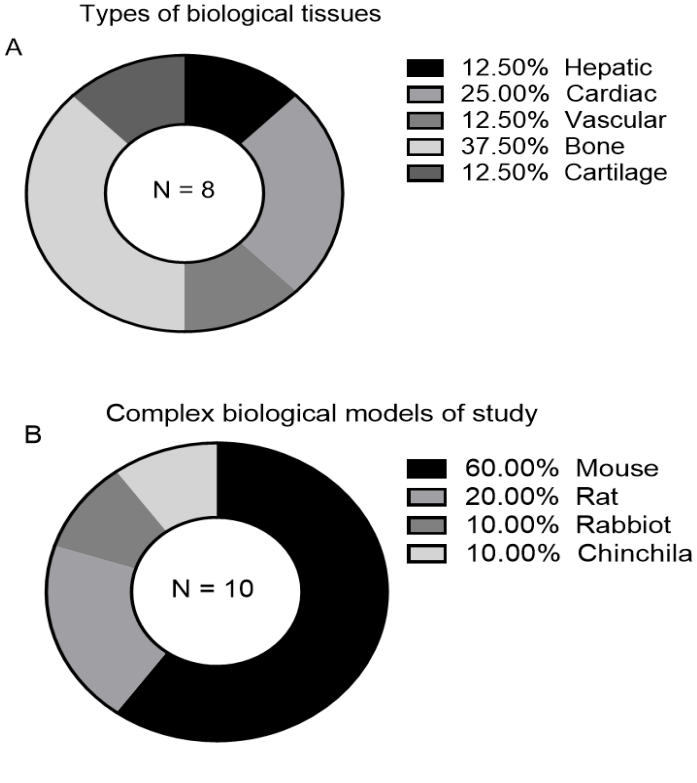
Table 1. Overview of studies applying bioprinting in complex non-human models, including animal and in vitro assessments.

Study/Problem	Animal model	Thecnology /Method	Conclusion/Desfecho
Liver Transplantation[1]	Mouse	Extrusion	Generate human liver tissues as the alternative transplantation donors for treatment of liver diseases
Therapeutic angiogenesis[6]	Mouse (C57BL/6)	Hdrogel microspheres encapsulating VEGF-overexpressing	Induce angiogenesis in limb ischemia
Protocol/3D Bioprinted Patch in a Murine Model of Myocardial Infarction[9]	Mouse	Bioprinted patch	Application of a bioprinted patch onto the infarcted area of the heart
Tissue regeneration[11]	Mouse (NOG)	Laser-Assisted Bioprinting	Tissue regeneration and the acceleration and enhancement of vascularization
Drug release[13]	Mouse	Water soluble chitosan	Drug release
Osseointegration of titanium implants[14]	Not in vivo (Human cells)	Extrusion	Prepares the way for osteogenic coatings to be directly manufactured on the implant surface and packaged for surgery
Bone defect[15]	Rat	Nanocomposite scaffolds	Accelerate the bone healing in critical bone defects
Repair tympanic membrane perforations[16]	Chinchilla	Acellular grafts to treat TMP based on endoscopic imaging	Technology could be transferred to other medical pathologies and be used to rapidly scan internal organs
Cartilage repair[51]	Not in vivo (mimicked rabbit knee)	Anisotropic hydrogels micropatterns	New perspective for cartilage regeneration and repair
Muscle defect injuries[39]	Rat	Supporting polycaprolactone	accelerated muscle function restoration
Pediatric craniofacial reconstruction[17]	Rabbit	Direct-write micro-printer system	Candidate as a safe, efficacious pediatric bone tissue engineering strategy
Model of cardiac tissue[52]	Mouse	Extruded	Support the integration of the engineered cardiac tissue with host's vasculature

Relative frequencies for the types of biological tissues and animal species studied in association with bioprinting within biological models are presented

(Figure 2). The analysis revealed a predominance of studies focused on bone disorders (37.50%), followed by cardiac (25.00%), hepatic, vascular, and cartilage-related conditions (each at 12.50%). Cumulatively, bone and cartilage disorders account for 50% of all studies, while cardiac and vascular alterations represent over a third of the evaluations (37.50%) (Figure 2a). Regarding the types of non-human biological models used, 60% of the studies involved mice, followed by 20% with rats, and 10% each for rabbits and chinchillas (Figure 2b).

Figure 2. Frequencies of bioprinting studies in complex non-human biological models categorized by type of biological tissue and animal species. Data were extracted from the PubMed/Medline database using the descriptors “bioprinting” and “models, animal.”
(A) Relative frequency of bioprinting studies stratified by type of biological tissue evaluated. (B) Relative frequency of bioprinting studies stratified by animal species studied.



.

The third indicator was established by applying a method to ensure consistency in the evidence. The descriptor “bioprinting” was used to identify

studies that allow for the visualization of indicators related to bioprinting in complex human biological models. The survey was conducted on 02/18/2022, consulting the PubMed/Medline, Embase, and Cochrane databases, which are associated with health sciences literature. A filter for randomized clinical trials was applied, yielding a total of 19 studies (PubMed/Medline = 6 studies; Embase = 5; Cochrane = 8).

Upon reviewing the eligibility criteria, 6 studies were identified, including 5 clinical evaluations and 1 conceptual study discussing potential applications of bioprinting (Table 2). The identified studies showed that bioprinting has been used and/or considered in direct human interventions for a range of medical needs: treating diabetic foot wounds[53], enhancing the teaching-learning process in applied medical education[54], assisting patients in understanding thyroid disease and consenting to necessary clinical interventions[55], alleviating symptoms for patients with moderate to severe dry eye (study still in progress), addressing dysfunctions in male reproductive tissues (observational study: results not disclosed), and in surgical applications for elbow fractures[56] (Table 2). In each case, a 3D printing method was either utilized or referenced. Conventional techniques served as comparators or controls for the bioprinting interventions, as detailed in Table 2. Across all clinical intervention studies, results indicated that bioprinting methods outperformed traditional approaches (Table 2).

Table 2. Summary of studies involving bioprinting applications in complex human biological models. Each study includes information on the application field, specific intervention using bioprinting techniques, comparators used, and observed outcomes. Bioprinting interventions were shown to provide improved effectiveness and patient outcomes in areas such as wound care, clinical education, informed consent processes, management of dry eye symptoms, and surgical procedures.

Application	Intervention	Comparator	Outcome
Diabetic foot wounds[53]	Minimally manipulated extracellular matrix prepared from autologous homologous adipose tissue by using 3D bioprinting.	Standard wound care	Better effectiveness in wound reduction and re-epithelialization.
Clinical education for the interns[54]	3D-printing craniocerebral models	Oral teaching and imaging data	Improved the learning efficiency for the clinical interns.
Improving a patient's understanding and satisfaction during the informed consent process[55]	Personalized 3D-printed thyroid model that characterizes a patient's individual thyroid lesion.	Without a 3D-printed model of their thyroid lesion	Personalized 3D-printed models showed significant improvement in general knowledge, benefits and risks of surgery, and satisfaction of the patientes.
Elbow fractures[56]	3D printing-assisted surgery	Conventional surgery	The 3D group showed shorter surgical duration, lower blood loss and higher elbow function score

FINAL CONSIDERATIONS

The field of tissue regeneration and bioprinting has undergone remarkable progress, driven by the high demand for organ and tissue transplantation. Although bioprinting technology remains costly, ongoing efforts toward the democratization of research and the development of more affordable, accessible solutions hold

promise. Investment in this area is critical, given its potential to improve health outcomes and enhance the quality of life globally.

Despite the promising advancements, many challenges persist, particularly in translating in vitro findings into in vivo clinical applications. Results observed in controlled laboratory environments often diverge from those obtained in human trials, underscoring the complexity of replicating biological conditions in engineered systems. As such, technical improvements across a wide array of tissue types remain essential, especially since different biological tissues exhibit varied responses to bioprinting and decellularization techniques. The absence of standardized, application-specific protocols further complicates the consistent reproduction of results.

One of the most pressing obstacles is the limited understanding of the underlying biological and technological mechanisms that govern 3D bioprinting and decellularization. Advancing knowledge in this domain is crucial to overcoming current limitations. For example, studies aimed at enhancing vascularization, maintaining structural integrity, and improving coagulation compatibility within bioengineered tissues could substantially improve the functionality of bioprinted constructs, bringing the technology closer to clinical applications (Figure 3).

Figure 3. Bioprinting Techniques and Applications illustrates key bioprinting methods in clinical practice: organ/prosthesis printing (top right), skin graft bioprinting (center), and three main techniques—inkjet (droplet deposition), extrusion (continuous deposition), and laser-assisted (cell transfer).

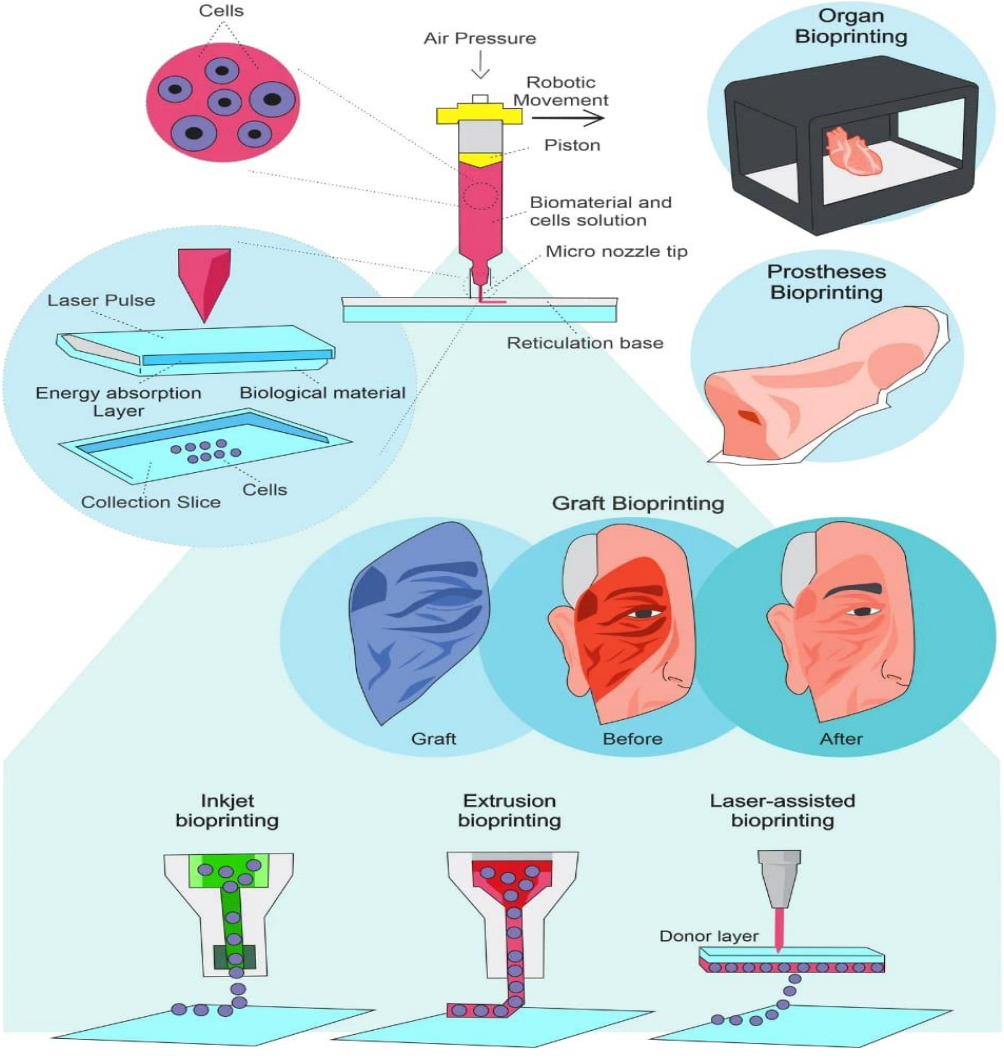


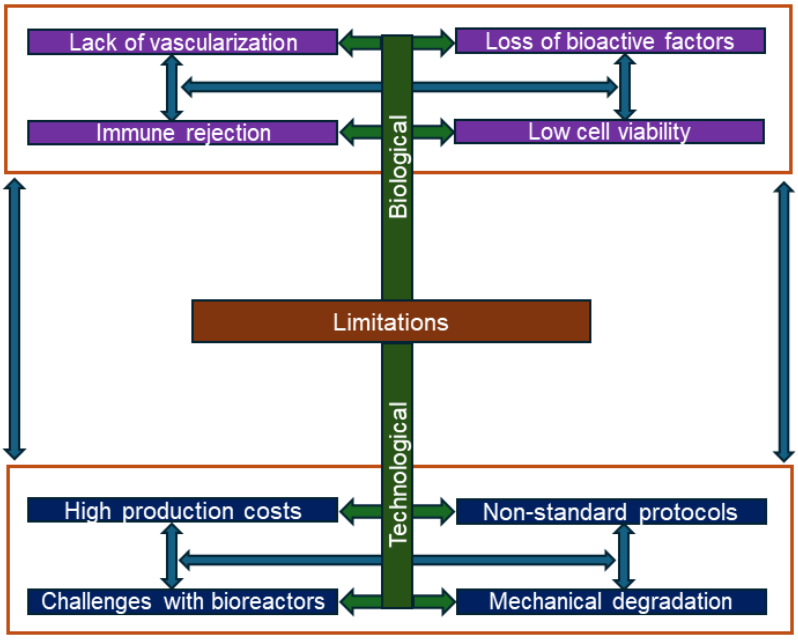
Figure 4 further illustrates the key limitations faced by decellularization and bioprinting techniques, categorized into two major groups: biological and technological limitations. On the biological side, challenges include insufficient vascularization, immune responses, the loss of bioactive factors during decellularization, and low cellular viability during recellularization. These issues limit the successful integration and performance of bioengineered tissues in host

environments. On the technological front, factors such as high production costs, lack of standardized protocols, mechanical property degradation, and difficulties with bioreactor optimization remain major obstacles to large-scale application.

Addressing these challenges requires an integrated approach, with ongoing innovations in biorreactors, hybrid bioinks, and the molecular characterization of cell-matrix interactions playing pivotal roles. By bridging the gap between biological and technological limitations, future advancements can optimize decellularization protocols and bioprinting methods, thereby accelerating their transition from experimental setups to clinical settings. Continued interdisciplinary research is necessary to refine current methods, reduce costs, and establish reproducible models that meet the demands of diverse clinical applications.

With sustained efforts, bioprinting and decellularization technologies have the potential to transform the landscape of regenerative medicine. As researchers strive to optimize both biological and technical components, the path toward viable, functional, and accessible bioengineered tissues becomes increasingly attainable.

Figure 4. Limitations of Bioprinting and Decellularization summarizes major challenges: Biological issues (poor vascularization, immune responses, loss of bioactive factors, low cell viability) and technological obstacles (high costs, non-standardized protocols, reduced mechanical strength, bioreactor optimization difficulties).



CONFLICT(S) OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

AUTHORS' CONTRIBUTIONS

Conceptualization, W.F.R., C.B.M., and C.J.F.O.; data curation, W.F.R., C.B.M., G.F.M., and M.E.A.C.; investigation, W.F.R., C.B.M., G.F.M., M.E.A.C., and M.C.A.; formal analysis, W.F.R., C.B.M., C.J.F.O., M.C.A., M.E.A.C., and S.C.S.; funding acquisition, M.C.A., S.C.S., and C.J.F.O.; methodology, W.F.R., C.B.M., G.F.M., C.A.T.S., and M.E.A.C.; supervision, W.F.R., C.B.M., and C.J.F.O.; writing—original draft, W.F.R., C.B.M., G.F.M., C.A.T.S., and M.E.A.C.; writing—review and editing, W.F.R., C.B.M., G.F.M., C.J.F.O., M.C.A., and S.C.S.

ACKNOWLEDGMENT(S)

Wellington Francisco Rodrigues expresses his gratitude to the Science, Technology, and Innovation Development Grant program by FAPEMIG (identifier RED-00181-23) for the scholarship provided, which has been instrumental in supporting this research. Camila Botelho Miguel extends her appreciation to CNPq for the senior postdoctoral fellowship (Process No. 102630/2024-0) awarded through Call 32-2023, under the supervision of Carlo José Freire Oliveira. Their generous support has been invaluable in enabling the advancement of this work.

REFERENCES

1. Yang H, Sun L, Pang Y, Hu D, Xu H, Mao S, Peng W, Wang Y, Xu Y, Zheng YC, Du S, Zhao H, Chi T, Lu X, Sang X, Zhong S, Wang X, Zhang H, Huang P, Sun W, Mao Y. Three-dimensional bioprinted hepatorganoids prolong survival of mice with liver failure. Gut. 2021;70(3):567–574. https://doi.org/10.1136/gutjnl-2019-319960.

2. Breathwaite EK, Weaver JR, Murchison AC, Treadwell ML, Odanga JJ, Lee JB. Scaffold-free bioprinted osteogenic and chondrogenic systems to model osteochondral physiology. Biomedical Materials (Bristol, England). 2019;14(6):065010. https://doi.org/10.1088/1748-605X/ab4243.

3. Tasoglu S, Demirci U. Bioprinting for stem cell research. Trends in Biotechnology. 2013;31(1):10–19. https://doi.org/10.1016/j.tibtech.2012.10.005.

4. Premaratne ID, Toyoda Y, Celie KB, Brown KA, Spector JA. Tissue Engineering Models for the Study of Breast Neoplastic Disease and the Tumor Microenvironment. Tissue Engineering Part B, Reviews. 2020;26(5):423–442. https://doi.org/10.1089/ten.TEB.2019.0347.

5. Potjewyd G, Moxon S, Wang T, Domingos M, Hooper NM. Tissue Engineering 3D Neurovascular Units: A Biomaterials and Bioprinting Perspective. Trends in Biotechnology. 2018;36(4):457–472. https://doi.org/10.1016/j.tibtech.2018.01.003.

6. Shen J, Ji Y, Xie M, Zhao H, Xuan W, Yin L, Yu X, Xu F, Su S, Nie J, Xie Y, Gao Q, Ma H, Ke X, Shi Z, Fu J, Liu Z, He Y, Xiang M. Cell-modified bioprinted microspheres for vascular regeneration. Materials Science & Engineering C, Materials for Biological Applications. 2020;112:110896. https://doi.org/10.1016/j.msec.2020.110896.

7. Phang SJ, Arumugam B, Kuppusamy UR, Fauzi MB, Looi ML. A review of diabetic wound models-Novel insights into diabetic foot ulcer. Journal of Tissue Engineering and Regenerative Medicine. 2021;15(12):1051–1068. https://doi.org/10.1002/term.3246.

8. Sharma P, Wang X, Ming C, Vettori L, Figtree G, Boyle A, Gentile C. Considerations for the Bioengineering of Advanced Cardiac In Vitro Models of Myocardial Infarction. Small (Weinheim an der Bergstrasse, Germany). 2021;17(15). https://doi.org/10.1002/smll.202003765.

9. Roche CD, Gentile C. Transplantation of a 3D Bioprinted Patch in a Murine Model of Myocardial Infarction. Journal of Visualized Experiments (JoVE). 2020;(163):10.3791/61675. https://doi.org/10.3791/61675.

10. Castillo-Henríquez L, Castro-Alpízar J, Lopretti-Correa M, Vega-Baudrit J. Exploration of Bioengineered Scaffolds Composed of Thermo-Responsive Polymers for Drug Delivery in Wound Healing. International Journal of Molecular Sciences. 2021;22(3):1408. https://doi.org/10.3390/ijms22031408.

11. Osafune K. Regenerative treatments for kidney diseases: The closest and fastest strategies to solving related medical and economic problems. Artificial Organs. 2021;45(5):447–453. https://doi.org/10.1111/aor.13943.

12. Kérourédan O, Ribot EJ, Fricain JC, Devillard R, Miraux S. Magnetic Resonance Imaging for tracking cellular patterns obtained by Laser-Assisted Bioprinting. Scientific Reports. 2018;8(1):15777. https://doi.org/10.1038/s41598-018-34226-9.

13. Marimuthu T, Kumar P, Choonara YE. Visible light-curable water-soluble chitosan derivative, chitosan hydrogel, and preparation method: a patent evaluation of US2019202998A1. Expert Opinion on Therapeutic Patents. 2021;31(5):351–360. https://doi.org/10.1080/13543776.2021.1903433.

14. McBeth C, Lauer J, Ottersbach M, Campbell J, Sharon A, Sauer-Budge AF. 3D bioprinting of GelMA scaffolds triggers mineral deposition by primary human osteoblasts. Biofabrication. 2017;9(1):015009. https://doi.org/10.1088/1758-5090/aa53bd.

15. Liu B, Li J, Lei X, Cheng P, Song Y, Gao Y, Hu J, Wang C, Zhang S, Li D, Wu H, Sang H, Bi L, Pei G. 3D-bioprinted functional and biomimetic hydrogel scaffolds incorporated with nanosilicates to promote bone healing in rat calvarial defect model. Materials Science & Engineering C, Materials for Biological Applications. 2020;112:110905. https://doi.org/10.1016/j.msec.2020.110905.

16. Kuo CY, Wilson E, Fuson A, Gandhi N, Monfaredi R, Jenkins A, Romero M, Santoro M, Fisher JP, Cleary K, Reilly B. Repair of Tympanic Membrane Perforations with Customized Bioprinted Ear Grafts Using Chinchilla Models. Tissue Engineering Part A. 2018;24(5-6):527–535. https://doi.org/10.1089/ten.TEA.2017.0246.

17. Wang MM, Flores RL, Witek L, Torroni A, Ibrahim A, Wang Z, Liss HA, Cronstein BN, Lopez CD, Maliha SG, Coelho PG. Dipyridamole-loaded 3D-printed bioceramic scaffolds stimulate pediatric bone regeneration in vivo without disruption of craniofacial growth through facial maturity. Scientific Reports. 2019;9(1):18439. https://doi.org/10.1038/s41598-019-54726-6.

18. Lin X, Chen J, Qiu P, Zhang Q, Wang S, Su M, Chen Y, Jin K, Qin A, Fan S, Chen P, Zhao X. Biphasic hierarchical extracellular matrix scaffold for osteochondral defect regeneration. Osteoarthritis Cartilage. 2018;26(3):433–444. https://doi.org/10.1016/j.joca.2017.12.001.

19. Dai W, Sun M, Leng X, Hu X, Ao Y. Recent Progress in 3D Printing of Elastic and High-Strength Hydrogels for the Treatment of Osteochondral and Cartilage Diseases. Frontiers in Bioengineering and Biotechnology. 2020;8:604814. https://doi.org/10.3389/fbioe.2020.604814.

20. Marques CF, Diogo GS, Pina S, Oliveira JM, Silva TH, Reis RL. Collagen-based Bioinks for Hard Tissue Engineering Applications: a Comprehensive Review. Journal of Materials Science: Materials in Medicine. 2019;30:32. https://doi.org/10.1007/s10856-019-6234-x.

21. Nulty J, Burdis R, Kelly DJ. Biofabrication of Prevascularised Hypertrophic Cartilage Microtissues for Bone Tissue Engineering. Frontiers in Bioengineering and Biotechnology. 2021;9:661989. https://doi.org/10.3389/fbioe.2021.661989.

22. Shavandi A, Silva TH, Bekhit AA, Bekhit AE-D. Keratin: Dissolution, Extraction and Biomedical Application. Biomaterials Science. 2017;5:1699–1735. https://doi.org/10.1039/c7bm00411g.

23. Echave MC, Hernáez-Moya R, Iturriaga L, Pedraz JL, Lakshminarayanan R, Dolatshahi-Pirouz A, Taebnia N, Orive G. Recent advances in gelatin-based therapeutics. Expert Opin Biol Ther. 2019;19(8):773–779. https://doi.org/10.1080/14712598.2019.1610383.

24. Neishabouri A, Soltani Khaboushan A, Daghigh F, Kajbafzadeh AM, Majidi Zolbin M. Decellularization in Tissue Engineering and Regenerative Medicine: Evaluation, Modification, and Application Methods. Frontiers in Bioengineering and Biotechnology. 2022;10:805299. https://doi.org/10.3389/fbioe.2022.805299.

25. Zhang X, Chen X, Hong H, Hu R, Liu J, Liu C. Decellularized extracellular matrix scaffolds: Recent trends and emerging strategies in tissue engineering. Bioactive Materials. 2021;10:15–31. https://doi.org/10.1016/j.bioactmat.2021.09.014.

26. Hsieh DJ, Srinivasan P, Yen KC, Yeh YC, Chen YJ, Wang HC, Tarng YW. Protocols for the preparation and characterization of decellularized tissue and organ scaffolds for tissue engineering. Biotechniques. 2021;70(2):107–115. https://doi.org/10.2144/btn-2020-0141.

27. Taylor DA, Kren SM, Rhett K, Robertson MJ, Morrissey J, Rodriguez OE, Virk H, Chacon-Alberty L, Curty da Costa E, Mesquita FCP, Sampaio LC, Hochman-Mendez C. Characterization of perfusion decellularized whole animal body, isolated organs, and multi-organ systems for tissue engineering applications. Physiological Reports. 2021;9(12):e14817. https://doi.org/10.14814/phy2.14817.

28. Yang J, Dang H, Xu Y. Recent advancement of decellularization extracellular matrix for tissue engineering and biomedical application. Artificial Organs. 2022;46(4):549–567. https://doi.org/10.1111/aor.14126.

29. Moura BS, Monteiro MV, Ferreira LP, Lavrador P, Gaspar VM, Mano JF. Advancing tissue decellularized hydrogels for engineering human organoids. Adv Funct Mater. 2022;32(29):2202825. https://doi.org/10.1002/adfm.202202825.

30. Amirazad H, Dadashpour M, Zarghami N. Application of decellularized bone matrix as a bioscaffold in bone tissue engineering. Journal of Biological Engineering. 2022;16(1):1. https://doi.org/10.1186/s13036-021-00282-5.

31. Wang Z, Sun F, Lu Y, Pan S, Yang W, Zhang G, Ma J, Shi H. Rapid preparation of decellularized trachea as a 3D scaffold for organ engineering. International Journal of Artificial Organs. 2021;44(1):55–64. https://doi.org/10.1177/0391398820924041.

32. Shoueir KR, El-Desouky N, Rashad MM, Ahmed MK, Janowska I, El-Kemary M. Chitosan Based-Nanoparticles and Nanocapsules: Overview, Physicochemical Features, Applications of a Nanofibrous Scaffold, and Bioprinting. International Journal of Biological Macromolecules. 2021;167:1176–1197. https://doi.org/10.1016/j.ijbiomac.2020.11.072.

33. Critchley S, Sheehy EJ, Cunniffe G, Diaz-Payno P, Carroll SF, Jeon O, Alsberg E, Brama PAJ, Kelly DJ. 3D printing of fibre-reinforced cartilaginous templates for the regeneration of osteochondral defects. Acta Biomater. 2020;113:130–143. https://doi.org/10.1016/j.actbio.2020.05.040.

34. Natarajan ABM, Sivadas VPD, Nair PDPD. 3D-printed Biphasic Scaffolds for the Simultaneous Regeneration of Osteochondral Tissues. Biomedical Materials. 2021;16:054102. https://doi.org/10.1088/1748-605X/ac14cb.

35. Chai Z, Li Z. Applications of decellularized extracellular matrix for regenerative medicine. In: Sheikh FA, editor. Engineering Materials for Stem Cell Regeneration. Springer, Singapore. 2021. https://doi.org/10.1007/978-981-16-4420-7_23.

36. Toprakhisar B, Verfaillie CM, Kumar M. Advances in recellularization of decellularized liver grafts with different liver (stem) cells: towards clinical applications. Cells. 2023;12:301. https://doi.org/10.3390/cells12020301.

37. Liu S, Rao Z, Zou J, Chen S, Zhu Q, Liu X, Bai Y, Liu Y, Quan D. Properties regulation and biological applications of decellularized peripheral nerve matrix hydrogel. ACS Applied Bio Materials. 2021;4(8):6473–6487. https://doi.org/10.1021/acsabm.1c00616.

38. Nouri Barkestani M, Naserian S, Uzan G, Shamdani S. Post-decellularization techniques ameliorate cartilage decellularization process for tissue engineering applications. Journal of Tissue Engineering. 2021;12:2041731420983562. https://doi.org/10.1177/2041731420983562.

39. Kim BS, Kim JU, So KH, Hwang NS. Supercritical fluid-based decellularization technologies for regenerative medicine applications. Macromolecular Bioscience. 2021;21(8):e2100160. https://doi.org/10.1002/mabi.202100160.

40. Arslan Y, Paradiso A, Celiktas N, Erdogan T, Yesil-Celiktas O, Swieszkowski W. Bioinspired microstructures through decellularization of plants for tissue engineering applications. European Polymer Journal. 2023;198:112415. https://doi.org/10.1016/j.eurpolymj.2023.112415.

41. Philips C, Terrie L, Thorrez L. Decellularized skeletal muscle: A versatile biomaterial in tissue engineering and regenerative medicine. Biomaterials. 2022;283:121436. https://doi.org/10.1016/j.biomaterials.2022.121436.

42. Hong N, Yang GH, Lee J, Kim G. 3D bioprinting and its in vivo applications. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2018;106(1):444–459. https://doi.org/10.1002/jbm.b.33826.

43. Ong CS, Yesantharao P, Huang CY, Mattson G, Boktor J, Fukunishi T, Zhang H, Hibino N. 3D bioprinting using stem cells. Pediatric Research. 2018;83(1-2):223–231. https://doi.org/10.1038/pr.2017.252.

44. Lee SJ, Lee JH, Park J, Kim WD, Park SA. Fabrication of 3D Printing Scaffold with Porcine Skin Decellularized Bio-Ink for Soft Tissue Engineering. Materials (Basel). 2020;13(16):3522. https://doi.org/10.3390/ma13163522.

45. Shi L, Hu Y, Ullah MW, Ullah I, Ou H, Zhang W, Xiong L, Zhang X. Cryogenic free-form extrusion bioprinting of decellularized small intestinal submucosa for potential applications in skin tissue engineering. Biofabrication. 2019;11(3):035023. https://doi.org/10.1088/1758-5090/ab15a9.

46. De Santis MM, Alsafadi HN, Tas S, Bölükbas DA, Prithiviraj S, Da Silva IAN, Mittendorfer M, Ota C, Stegmayr J, Daoud F, Königshoff M, Swärd K, Wood JA, Tassieri M, Bourgine PE, Lindstedt S, Mohlin S, Wagner DE. Extracellular-matrix-reinforced bioinks for 3D bioprinting human tissue. Adv Mater. 2021;33(3):e2005476. https://doi.org/10.1002/adma.202005476.

47. Isaeva EV, Beketov EE, Demyashkin GA, Yakovleva ND, Arguchinskaya NV, Kisel AA, Lagoda TS, Malakhov EP, Smirnova AN, Petriev VM, Eremin PS, Osidak EO, Domogatsky SP, Ivanov SA, Shegay PV, Kaprin AD. Cartilage formation in vivo using high concentration collagen-based bioink with MSC and decellularized ECM granules. Int J Mol Sci. 2022;23(5):2703. https://doi.org/10.3390/ijms23052703.

48. Kiani M, Movahedin M, Halvaei I, Soleimani M. Formation of organoid-like structures in the decellularized rat testis. Iranian Journal of Basic Medical Sciences. 2021;24(11):1523–1528. https://doi.org/10.22038/IJBMS.2021.58294.12948.

49. Cai A, Zheng Z, Müller-Seubert W, Biggemann J, Fey T, Beier JP, Horch RE, Frieß B, Arkudas A. Microsurgical Transplantation of Pedicled Muscles in an Isolation Chamber—A Novel Approach to Engineering Muscle Constructs via Perfusion-Decellularization. Journal of Personalized Medicine. 2022;12(3):442. https://doi.org/10.3390/jpm12030442.

50. Polisetti N, Schmid A, Schlötzer-Schrehardt U, Maier P, Lang SJ, Steinberg T, Schlunck G, Reinhard T. A decellularized human corneal scaffold for anterior corneal surface reconstruction. Scientific Reports. 2021;11(1):2992. https://doi.org/10.1038/s41598-021-82678-3.

51. Sun Y, Wu Q, Zhang Y, Dai K, Wei Y. 3D-bioprinted gradient-structured scaffold generates anisotropic cartilage with vascularization by pore-size-dependent activation of HIF1α/FAK signaling axis. Nanomedicine. 2021;37:102426. https://doi.org/10.1016/j.nano.2021.102426.

52. Maiullari F, Costantini M, Milan M, Pace V, Chirivì M, Maiullari S, Rainer A, Baci D, Marei HE, Seliktar D, Gargioli C, Bearzi C, Rizzi R. A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. Scientific Reports. 2018;8(1):13532. https://doi.org/10.1038/s41598-018-31848-x.

53. Kesavan R, Sheela Sasikumar C, Narayanamurthy VB, Rajagopalan A, Kim J. Management of diabetic foot ulcer with MA-ECM (minimally manipulated autologous extracellular matrix) using 3D bioprinting technology – an innovative approach. International Journal of Lower Extremity Wounds. 2024;23(1):161–168. https://doi.org/10.1177/15347346211045625.

54. Cheng H, Dai X, Wu B, Ye L, Wang H, Li S, Wang W. 3D-printed craniocerebral model promotes the interests and education efficiency for medical interns in the neurosurgery department. International Journal of Clinical and Experimental Medicine. 2020;13(12):9302–9310.

55. Seok J, Yoon S, Ryu CH, Kim SK, Ryu J, Jung YS. A personalized 3D-printed model for obtaining informed consent process for thyroid surgery: a randomized clinical study using a deep learning approach with mesh-type 3D modeling. Journal of Personalized Medicine. 2021;11(6):574. https://doi.org/10.3390/jpm11060574.

56. Yang L, Grottkau B, He Z, Ye C. Three-dimensional printing technology and materials for treatment of elbow fractures. International Orthopaedics. 2017;41(11):2381–2387. https://doi.org/10.1007/s00264-017-3627-7.