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REVIEW ARTICLE

Blood-derived biomaterials for tissue graft biofabrication by solvent-based extrusion bioprinting

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Abstract

This article provides an overview of the different types of blood-derived biomaterials that can be used as solvent additives in the formulation of inks/bioinks for use in solvent extrusion printing/bioprinting. We discuss the properties of various blood sub-products obtained after blood fractionation in terms of their use in tailoring ink/bioink to produce functional constructs designed to improve tissue repair. Blood-derived additives include platelets and/or their secretome, including signaling proteins and microvesicles, which can drive cell migration, inflammation, angiogenesis, and synthesis of extracellular matrix proteins. The contribution of plasma to ink/bioink functionalization relies not only on growth factors, such as hepatocyte growth factor and insulin growth factors, but also on adhesive proteins, such as fibrinogen/fibrin, vitronectin, and fibronectin. We review the current developments and progress in solvent-based extrusion printing/bioprinting with inks/bioinks functionalized with different blood-derived products, leading toward the development of more advanced patient-specific 3D constructs in multiple medical fields, including but not limited to oral tissues and cartilage, bone, skin, liver, and neural tissues. This information will assist researchers in identifying the most suitable blood-derived product for their ink/bioink formulation based on the intended regenerative functionality of the target tissue.

Keywords: Blood-derived products; 3D printing; Solvent-based extrusion; Bioprinting; Functionalized bioinks; Plasma; Tissue grafts/implants

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1. Introduction

In the context of therapeutic tissue engineering (TE) and regenerative medicine (RM), biofabrication entails the automated production of complex living and non-living biologically relevant products manufactured from living cells, native matrices, biomaterials, and molecules using different devices[1,2]. Here, we focus on one of the most commonly used technologies, solvent-based extrusion (SBE) (bio)printing. In this context, solvents containing printable biomaterial composites (chiefly hydrogels) and optional additives are extruded through a nozzle in a layer-by-layer additive manner using

mechanically, piston, or pneumatically driven tools. Once deposited, their shape is maintained by gelification of the ink/bioink by means of induced physicochemical reactions. There are two modalities, ink vs. bioink, that differ in the presence of cells in the extruded biomaterial. Primarily, solvent-based extrusion three-dimensional (3D) printing entails the building of scaffolds with tailored geometry but without cells[3–5]. To create living constructs, 3D printing can be followed by cell seeding. However, depending on the geometry of the printout, the access of cells to the core of the construct can be problematic. In contrast, SBE bioprinting uses bioinks (i.e., cell-laden inks), thus implementing the concurrent biofabrication of the scaffold and cellular components for the additive manufacturing of living tissue structures[6]. Composite inks/bioinks can be formulated by adding blood-derived products to printable hydrogels. When platelets are added to the solvent, the distinction between ink and bioink is blurred, as platelets are considered anucleated cells. Although platelets lack genomic DNA, they contain various RNA biotypes, including coding mRNAs, and the translational machinery needed to translate them into proteins. Thus, just like cells, they can react to environmental stimuli granting biological complexity to printable biomaterials[7]. However, platelets lack other cellular attributes, such as growth and replication capacities. In the clinical context, regulatory authorities have classified autologous platelet-rich plasma (PRPs) as "non-standardized medicinal products" instead of advanced therapies. Therefore, printable biomaterials loaded with platelets would have different regulatory considerations than printable biomaterials loaded with cells.

Bioprinting technologies are used in biomedical research for several purposes, such as creating disease models[8], drug screening, basic cell biology, or the creation of functional implants with structural organization. Due to injuries, disease, surgery, and other reasons, a large number of patients with tissue defects need graft implantation. Both SBE approaches, 3D printing and bioprinting, can be explored in therapeutic tissue engineering and regenerative medicine to create mature competent tissue grafts that integrate within the host tissue once they are implanted. The medical need for tissue grafts is particularly important when tissue defects exceed a critical size. Scalable methods include the development of engineering strategies[9] and the creation of microtissue building blocks (with fewer limitations in nutrient transport) that could fuse to generate a competent implant, either *in vitro* or with the use of the body as a bioreactor. Other challenges involve reproducing the vasculature and metabolic state of the organ. In one instance, microtissue building blocks were bioprinted and cultured chondrogenically to create a competent tissue graft, and the process of microtissue maturation and fusion *in vitro* was guided using a 3D-printed polymer framework[9].

Ink and bioink formulations are at the core of these technologies, requiring bioink components to interact in non-trivial ways. Several (bio)printed tissue grafts, such as nerve grafts[10], blood vessels and vascular networks[11], tracheal implants[12], liver[13], bone[14], cartilage[15], vascular[16], and parathyroid grafts[17], have been implanted into animals to study their functionality (Figure 1). However, the clinical translation of these products faces notable challenges for several reasons, mainly related to predictability. First, due to sub-optimal experimental models, there is no full understanding of the physiological complexity involved, including the dynamic integration of multiple components (i.e., biomaterials, different cell phenotypes, and a large array of signaling proteins) and their interactions with the host tissue/organ. Many of the critical mechanisms of tissue repair rely on the close interplay between cells and the dynamic tissue microenvironment through molecular signaling[18].

Therefore, to fulfill as many requirements as possible regarding the predictability of bioprinted scaffolds, an utmost need is the correct functionalization of the bioink with signaling molecules. For example, Sun *et al.*[19] bioprinted functionalized scaffolds with transforming growth factor beta 3 (TGF-b3) and connective growth factor (CTGF) mixed with bone marrow stromal cells (BMSCs) for intervertebral disc (IVD) regeneration. In another example, a 3D-printed polycaprolactone microchamber was coated with platelet-derived growth factors (PDGFs) and bone morphogenetic protein 2 (BMP-2), and spheroids containing adipose stromal cells (ASCs) were cultured within the microchambers for dual growth factor delivery in bone regeneration[20]. In another example, methacrylated hyaluronic acid (MeHA) combined with collagen bioink was loaded with nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), and Schwann cells[21].

However, the functionalization of bioinks with single/dual growth factors does not approach the immense complexity of cell communication and competent tissue biofabrication. Alternatively, biomaterials can be functionalized with tailored blood-derived products to transform inert biomaterials into reactive (stimuli-response) biomaterials, drawing inspiration from physiological repair mechanisms in which hemostasis (blood clot formation, fibrin formation) is the starting point, and platelet degranulation and secretome release trigger the regenerative cascade[18].

This article describes the different types of blood-derived biomaterials that can be used in solvent-based extrusion

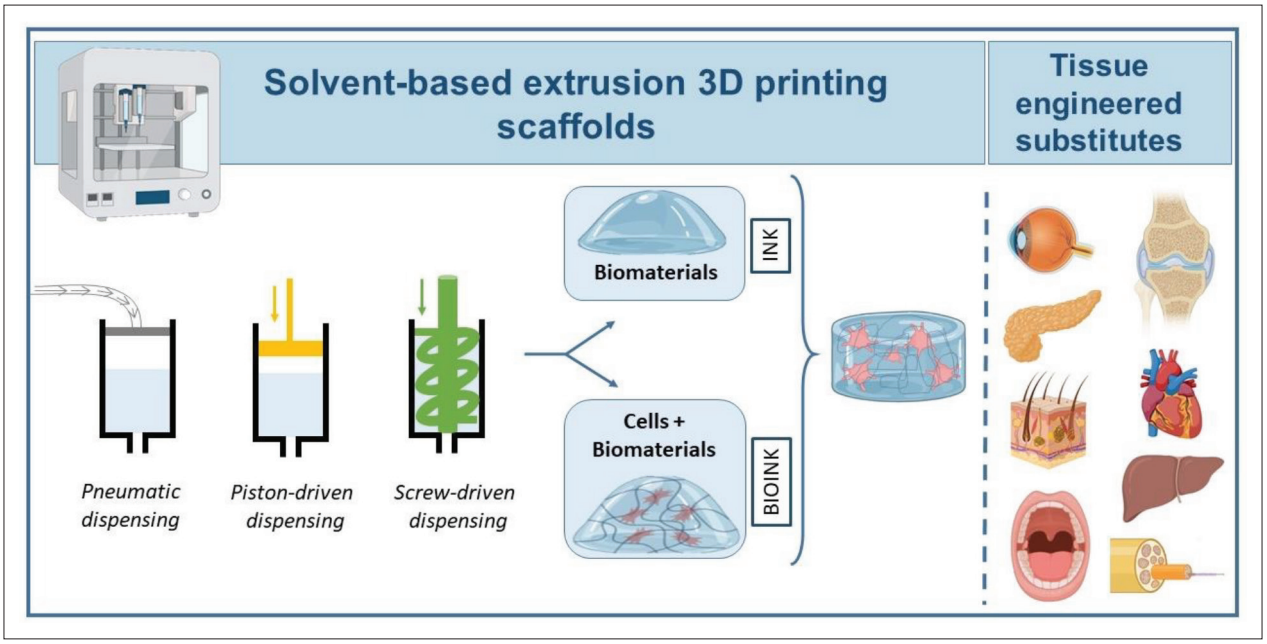

Figure 1. Solvent-based extrusion 3D printing/bioprinting for tissue grafts.

printing/bioprinting and discusses the potential of various blood sub-products obtained after blood fractionation for tailoring (bio)ink properties for printability and anticipated functionality. We also review the current progress of bioprinting using bioinks functionalized with different blood-derived products, leading toward the development of more advanced patient-specific 3D constructs.

2. Blood-derived biomaterials as bioink components

In this section, we will discuss the properties of blood-derived products in regenerative medicine, considering that most of our understanding originated in transfusion science. In fact, whole blood (versus plasmapheresis) is the most common type of blood donation and is often efficiently fractionated into several components, i.e., red cell concentrates (45%), platelet concentrates (PCs; 1%), and plasma (fresh-frozen plasma [FFP]; 55%), to be transfused according to specific patients' needs[22]. In addition, approximately 30% of the plasma recovered from whole blood donations is used to produce plasma-derived medicinal products, which include cryoprecipitates that can be further processed by blood technological companies to prepare immunoglobulins, fibrinogen, or coagulation factors, such as factor VIII, factor XIII, and von Willebrand factor.

2.1. Terminology

The terminology for blood-derived products differs slightly between the transfusion and regenerative medicine contexts. The therapeutic use of autologous PRP at the point of care, for managing musculoskeletal conditions, difficult to heal wounds, or other ambulatory conditions, relies on the availability of Conformité Européenne (CE)-marked manufacturing devices (commonly centrifuges) and kits. These medical devices and associated protocols guarantee appropriate sterility, feasibility, and compliance with legal requirements. However, these medical devices, intended for clinical routine, are diverse, thereby obtaining PRP in different compositions. In the context of autologous PRP therapies, the different terminologies are contingent on cellular composition, e.g., the presence or absence of leukocytes distinguishes leukocyte-rich and platelet-rich plasma (L-PRP and pure PRP, respectively), and on the enrichment of platelets relative to peripheral blood[23]. On the other hand, platelet concentrates (PCs) in transfusion have higher concentrations of platelets than pure PRP (in regenerative medicine), but both are leukocyte-depleted to prevent immunological reactions, particularly for allogeneic uses. In general, bioinks are prepared from blood bank products that do not meet quality control requirements or are outdated for transfusion purposes. For example, it is known that PCs can be stored for up to 5 days at room temperature under gentle agitation. Thereafter, room temperature storage results in progressive loss of platelet discoid shape, hindering circulation and functionality as a hemostatic agent. At this point, expired PCs are a source material for additional manipulations to produce sub-products for use in regenerative medicine[24]. For instance, platelet lysate can replace bovine serum

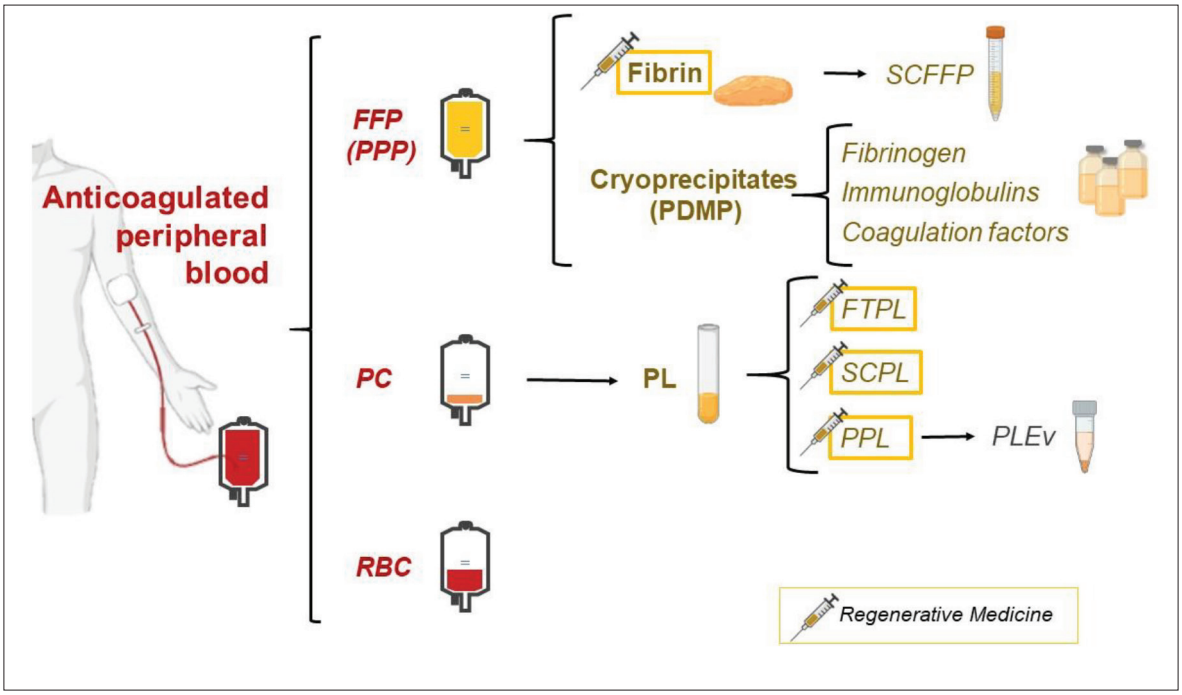

Figure 2. Types of blood-derived products. Abbreviations: FFP, fresh-frozen plasma; PPP, platelet-poor plasma; PC, platelet concentrate; RBC, red blood cell concentrate; SDFFP, serum-converted fresh-frozen plasma; PL, platelet lysate; FTPL, frozen thawed platelet lysate; SCPL, serum-converted platelet lysate; PPL, platelet pellet lysate; PLEv, platelet extracellular vesicles.

to expand cells for transplantation[24]. In addition, biofabrication/bioprinting technologies can be upgraded by introducing platelet lysate into the bioink formulation, thereby harnessing the unique features of the platelet secretome[25]. The bioink composition determines the functional characteristics of the construct. A bioink is designed not only to deliver cell/biological molecules but also to function as a system to support cellular function and organization or to trigger communication between various cellular compartments *in vivo*. The addition of the platelet secretome, rich in neurotrophic factors and anti-oxidative and anti-inflammatory molecules, can upgrade the functionality of biomaterial inks[26]. Blood-derived products reliant on the properties of the platelet secretome include platelet lysate, frozen-thawed platelet lysate, serum-converted platelet lysate, and platelet pellet lysate[27,28]. On the other hand, platelet-poor plasma (PPP), frozen-thawed PPP, serum-derived PPP, and fibrinogen can be obtained after platelet and/or fibrinogen depletion and therefore confer different biological properties on the final bioink formulation (Figure 2). Because bioinks contain cells from allogeneic donors, in some instances, heat treatment at 56°C for 30–60 min is performed to inactivate the immunological components (C3, C4, and other major proteins in the complement pathways and immune system). Moreover, proteolytic enzymes such as thrombin are inactivated. However, the stability of growth factors and cytokines is often compromised by these treatments. A potential procedure to avoid this drawback is lyophilization, which allows the availability of ready-to-use bioink components[29].

2.2. Platelet-rich plasma

2.2.1. Platelet secretome

Information regarding both the physiology of platelets and the properties of plasma is crucial to take advantage of these cells for tissue regeneration[30,31]. Platelets are anucleated cells originating from the partitioning of the megakaryocyte cytoplasm in the bone marrow. They enter the blood stream, where they circulate at a concentration of 250,000 plt/μL for approximately 10 days before being destroyed in the spleen. Traditionally, they have been best known for their role in hemostasis. However, less than 1/10 of the circulating platelets are needed to fulfill this function[24].

Thus, what other roles do platelets perform in regenerative medicine, and what advantages could they provide in bioprinting technologies?

The platelet proteome contains approximately 5200 proteins, of which a large pool is stored in the 50–80 alpha granules present per platelet[32]. Alpha granules are dense vesicles (200–500 nm) containing growth factors and cytokines, antioxidants, and adhesion molecules

synthesized by megakaryocytes (their parent giant cells located in the bone marrow, spleen, and lungs) before platelet segmentation and release into the blood stream. In addition, these molecules can be captured from the peripheral blood by endocytosis through the open canalicular system. Furthermore, each platelet contains 5–8 dense granules, which are 200–300 nm in diameter and contain 5-hydroxytryptamine (5-HT), adenosine diphosphate (ADP), adenosine triphosphate (ATP), Ca^{2+}, and pyrophosphates[33]. Several studies have analyzed the proteomes of different blood derivatives and established the link with healing functions[34].

Furthermore, platelet extracellular vesicles (pEVs) have captured recent attention as a sub-platelet therapy[35,36]. Essentially, platelets have a propensity to generate extracellular vesicles, with a yield of 10–160 pEVs/platelet; thus, in typical clinical-grade PC containing 4–7 × 10^{11} platelets, approximately 10^{13}–10^{14} pEVs can be found. Platelet vesicle heterogeneity is dictated by the activation stimulus that triggers their formation: microvesicles (0.1–1 μm diameter) shed from the platelet membrane and exosomes[37,38].

2.2.2. Functional attributes and biological mechanisms

The functionality of a bioink depends on the kinetics of cytokine release, which may cause changes in cell behavior (e.g., differentiation of cells, expression of particular genes). These changes should be beneficial, and the fabrication process must be designed such that the changes are predictable and the environment of the cell is not changed beyond healthy regenerative bounds. Moreover, the use of blood-derived biomaterials must not sacrifice the structural goals for the bioprinted scaffold. The functional priorities during bioink formulation must be determined according to the application. For example, the bioprinting of wound dressings does not require research focused on the long-term stability of bioprinted constructs under cell culture conditions[39]. Instead, functional properties such as the ability to recruit defined populations of immune cells (without eliciting an unresolved inflammatory response) and the ability to drive vascular ingrowth and innervation should be explored[40]. In contrast, the effectiveness of bioprinted cartilage implants relies not only on the biological properties but also on the mechanical stability. In the context of tissue graft biofabrication, two paramount objectives are controlling the immune system to prevent graft rejection and promote integration.

Specific evaluation of printouts for tissue grafting purposes is focused on several functional attributes: maintaining the cell phenotype and function, shaping the immune response to graft implantation, and driving the maturation and integration within the host tissue by promoting cell trafficking and angiogenesis. These can be considered as prerequisites for cross-platform validation, *in vitro* and *in vivo*. Adding platelets to the bioink can influence the immunomodulatory properties of the tissue-engineered graft as an approach to reduce graft rejection. Platelets secrete a broad array of chemotactic proteins with the potential to recruit cells from the vasculature to the graft and activate them to initiate tissue repair. Chemokines are low-molecular-weight proteins (8–10 kDa) that trigger signaling by binding to CXCR1 and CXCR2 receptors expressed by immune cells, including neutrophils and monocytes/macrophages. Moreover, the polarization status of monocytes/macrophages can be shaped toward M2 in the presence of platelets[41]. Furthermore, platelets are key regulators of angiogenesis, releasing both promoters of vascular development, such as basic fibroblastic growth factor (bFGF), endothelial growth factor (EGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and anti-angiogenic proteins, including but not limited to thrombospondin (TSP) and platelet factor 4 (PF-4). These are a few examples of platelet contributions within a tissue-healing microenvironment.

2.3. Fresh-frozen plasma

Another blood component frequently used in printing/bioprinting is FFP. This biomaterial is available in larger volumes than PCs but contains few signaling proteins. However, its stability is a major asset, as plasma frozen at -18°C within 8 h of collection can be stored for up to 7 years at -65°C. Plasma is made of 91%–92% water combined with 8%–9% solid materials, including pro-coagulant factors, predominantly fibrinogen, which is responsible for hemostasis. Additional plasmatic proteins, such as albumin and globulin, help to maintain the colloidal osmotic pressure at approximately 25 mmHg. Electrolytes, sodium, potassium, bicarbonate, chloride, and calcium ions maintain pH; immunoglobulins help fight infections. The complex formulation of plasma could contribute exceptionally to the design of bioink, tailoring its mechanical, biochemical, and immunological properties depending on the targeted tissue or biological mechanism.

Another of the main advantages of plasma products is the two physical configurations of the material: liquid or gel after the addition of exogenous activators. On the one hand, liquid plasma facilitates mixture with other biomaterials; on the other hand, gel-like consistency gives it a complex fibrillary internal structure, which acts as its own "smart" support matrix that allows the release of biomolecules into the microenvironment. Calcium ions (Ca^{2+}) and thrombin are some of the most commonly used activators to promote the internal network structure of

plasma-based hydrogels. The concentration and the process temperature directly affect the rheological properties, i.e., viscosity. From a rheological point of view, plasma as a liquid material presents a complex shear modulus (G*) close to zero, increasing in consistency when it is mixed with activators such as thrombin or calcium solutions. The clotting of plasma occurs with the conversion of fibrinogen into fibrin. Plasma hydrogels present a non-linear viscoelastic response under shear deformation.

2.4. Fibrinogen/fibrin

Fibrinogen is an abundant blood glycoprotein (concentration 2.0–4.0 g/L), produced by hepatocytes in the liver. It is involved in hemostasis and clot formation. Briefly, upon platelet activation, plasma prothrombin converts into thrombin (in the presence of Ca^{2+}) and cleaves soluble fibrinogen, releasing fibrinopeptides A and B. In doing so, thrombin transforms plasmatic fibrinogen into insoluble fibrin networks with large pores and high permeability. Fibrinogen/fibrin is a crucial component of most blood-derived products but removed in serum-converted fresh-frozen plasma and serum-converted platelet lysates (Figure 2).

Alternatively, purified fibrinogen is prepared by cryoprecipitation or chemical precipitation and embodies commercial preparations of fibrin glue or fibrinogen adhesives. The latter also includes thrombin and $CaCl_2$ to be mixed at the point of care for tissue sealing. The resulting fibrin networks are composed of highly branched fibers and are less prone to lysis than plasma clots.

3. Progress in plasma-functionalized bioinks

The use of 3D printing/bioprinting in tissue grafting will only grow as new bioinks match the complexity and dynamic nature of healing mechanisms. In this section, we will discuss how SBE printing/bioprinting platforms can benefit from the broad family of blood-derived products and how these products, depending on the specific formulation, can influence printability and rheology and confer biologically relevant properties on the construct. Thus, the ideal bioink should have several properties: (i) good printability, (ii) non-toxicity and no immunological reactions, (iii) good mechanical stability after curing, (iv) good biodegradability, (v) mimicry of the *in vivo* microenvironment, and (vi) the support and promotion of cellular activities (i.e., proliferation, migration, or differentiation).

The main bioink component for solvent extrusion is a shear-thinning hydrogel that can flow during extrusion and protect cells from shear stresses. Hydrogels are hydrophilic physically and chemically crosslinked polymers. They possess a high water content, which provides a suitable microenvironment for soluble protein retention (growth factors, chemokines, and cytokines), gradient formation, and diffusion to influence cell behavior and tissue repair/regeneration. When mixing biomaterials with blood-derived products, the retention/release of healing factors depends on the plasma formulation and specific characteristics (functional groups) and processing of the hydrogel, i.e., sterilization for clinical translation and crosslinking reactions to maintain the stability of the tissue constructs.

Knowledge about the anatomy and physiology of the target tissue guides the functionalization strategy and the choice of inductive tissue components, specific cell phenotypes, or cell aggregates to be loaded within the hydrogels. This systematic review reveals that some blood biomaterials, such as fibrinogen, are used as inductive elements and to enhance printability, while others are used for printable hydrogel functionalization. In some instances, blood-derived biomaterials are included in the bioink formulation, used as a cell carrier in the bioprinting system, or added to the bioprinted scaffold. Table 1 summarizes research organized by target tissue, and Table 2 lists studies involving multipurpose bioinks.

In any case, the use of blood-derived biomaterials in bioprinting platforms has been preliminarily explored to meet the specific demands of both soft and hard tissues, but these approaches are still in the early stages of research and far from providing therapeutic solutions. In fact, most of the research is in technology readiness level 2–3 (TRL 2–3), and only one study includes a proof of concept (TRL4) (reviewed in Perez-Valle, Del Amo and Andia, 2020). *In vivo* studies are needed to assess the integration and function of the construct in animal models mimicking the clinical problem.

Several additives should be included in bioink formulations to fulfill printability requirements and match the mechanical properties of the target tissue. For example, alginate is an anionic polysaccharide and a common ingredient in many bioinks because of its low immunological profile. It is often combined with other hydrophilic polymers (11 out of 21 studies, 52%), such as poly(ethylene glycol) (PEG) for cartilage[43], gelatin (protein) for dermal tissue[44], methacrylated gelatin (GelMA) for islet organoids[45], or agarose (polysaccharide) for cardiac tissue[46] (Table 1). However, these bioinert hydrogels cannot create biomimetic tissue unless they are provided with RGD domains for cell attachment and functionalized with cytokines and growth factors to boost cell activities.

Table 1. 3D bioprinting of specific tissue constructs using bioinks functionalized with blood-derived biomaterials

Author (year); *Journal*	Ink formulation. GelMA/PRP	Modality/cell phenotype. Printing/BMSC	Blood-derived product	Evaluation of the output/biological aspects. Proliferation, migration and differentiation. In vivo M2 infiltration in a rabbit osteochondral lesion.
Bone				
Ahlfeld *et al.* (2020); *ACS Applied Materials & Interfaces*[47]	3 w/v% alginate and 9 w/v% methylcellulose, a pasty bioink (plasma-Alg-MC) CPC (calcium phosphate cement)	Bioprinting/ MSCs	FFP	Proliferation Migration Differentiation Bone rigidity
Cao *et al.* (2023); *Int J Bioprint* [48]	Three bioinks: GA/PCL, PRP-GA/PCL, and PRP-GA@laponite/PCL GA composite hydrogel: GelMA/AlgMA (5:1)	Bioprinting/ rat BMSC	Rat PRP	*In vitro*: Mechanical properties, cell proliferation-osteogenic differentiation, macrophage polarization. *In vivo*: Subcutaneous implantation of bone grafts to assess vascularization, enhanced vascularization with PRP-GA&laponite/PCL; implantation of bone grafts in rat femoral condyle defects, X-ray and micro-CT scanning showed better regeneration with PRP-GA@laponite/PCL
Hao *et al.* (2023) ; *Int J Bioprint* [49]	PCL/β-TCP/PRP	No cells	Autologous PRP (4 mL)	Case report: tumor resection and surgical implantation of a personalized 3D-printed scaffold coated with PRP. Seven-month follow-up showed good bone implant integration as assessed by X-ray and CT.
Wei *et al.* (2019); *Bioactive Materials* [50]	Silk fibroin, HA, gelatin, TCP 3D scaffold printing coated with PRP Sterilization for 2 h using UV and 70% ethanol	3D printing / hASCs seeded post-printing	PRP, prepared "in house," with high platelet count and high concentration of VEGF, PDGF-AA, bFGF, TGF-β3	PRP coating of SE 3D-printed scaffolds, improved ADSC growth and proliferation, late-stage gene expression of differentiation factors (OCN and OPN). Pre-clinical *in vitro* data for bone construct manufacturing and transplantation.
Cartilage				
de Melo *et al.* (2019); *Adv Funct Mater* [43]	PEG 20%–alginate 2.5% hydrogel containing 0.25% photoinitiator and supplemented with 1 U mL^{-1} thrombin to allow for fast fibrin crosslinking. PEG was crosslinked by UV light exposition (200 mW cm^{-2}) for 80 s, and alginate was crosslinked by soaking the hydrogel in a 0.1 mol L^{-1} $CaCl_2$ solution for 30 min Fibrinogen containing MSC spheroids	Printing bioprinting / MSCs spheroids	Fibrinogen Thrombin (#)	Spheroid viability Chondrogenic differentiation Engineering cartilage-like tissue (impresora cellink)
Jiang *et al.* (2021); *Acta Biomater* [51]	GelMA/PRP	printing/BMSC	PRP	Proliferation, migration and differentiation. In vivo M2 infiltration in a rabbit osteochondral lesion.
Irmak and Gümüşderelioglu (2020); *Biomedical Materials* [52]	GelMA/PRP GelMA-dependent photoactivated PRP Stimulated by PAC (polychromatic light source)	Bioprinting/ATDC5 murine chondrocyte cell line	PRP	Printability (rheology) degradation, study of platelet activation, release kinetics of TGF-β, PDGF-B, bFGF Cell viability, proliferation, morphology Creation of cartilage constructs for sustained release of growth factor ECM cartilage production, histology, immunohistochemistry Photoactivated GelMA/PRP construct: sustained growth factor release for 35 d

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Table 1. ***Continued***

Author (year); *Journal*	Ink formulation. GelMA/PRP	Modality/cell phenotype. Printing/BMSC	Blood-derived product	Evaluation of the output/biological aspects. Proliferation, migration and differentiation. In vivo M2 infiltration in a rabbit osteochondral lesion.
Li *et al.* (2020); *Tissue Engineering* [53]	Two components mixed 1:1: • 80% PEG • 10% Silk fibroin dissolved in 12.5%, 25% or 50% PRP Crosslinking with PEG 400	Rabbit chondrocytes	PRP double-spinning Plt 8× (from New Zealand rabbits) TGF-β1, PDGF-AB	Mechanical and degradation tests/rheology Cell proliferation, viability, histology, immunohistochemical analyses, qRT-PCR, COL-II, COL-I, COL-X, ACAN
Kumar *et al.* (2019) ; *ACS Biomater Sci Eng* [54]	1 mL of the bioink: 990 µL of fibrinogen solution (60 mg/mL), 155 mg of gelatin-furfuryl at 25°C Heat it at 37 °C for 1 h with stirring, add Rose Bengal (10 µL) Polymerization: 50 units/mL thrombin, and 80 mM $CaCl_2$	Human-induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) Human CM AC16 cell lines (ATCC)	Fibrinogen Thrombin (#)	SEM, swelling assay, rheology, Fourier transform infrared spectroscopy (FTIR), biocompatibility Cardiac cell-laden constructs To validate the applicability of this scaffold toward mimicking cardiac tissue
Cardiac and vascular grafts				
Freeman *et al.* (2019); *Acta Biomater* [55]	Gelatin Fibrinogen crosslinked with thrombin	Rotary bioprinting / neonatal human dermal fibroblasts	Fibrinogen (#)	Tissue engineered vascular grafts, mechanical testing, histology
Maiullari *et al.* (2018); *Scientific Reports* [56]	Alginate, PEG Alginate and polyethylene glycol monoacrylate-fibrinogen (PF) $CaCl_2$ and UV crosslinking	HUVEC iPSC-CM coaxial needle system	Fibrinogen (#)	Creation of constructs with various cell distributions. Janus cell organization was the most efficient for effective vessel-like generation. Subcutaneous implantation in mice; histology after 2 weeks
Wang *et al.* (2018); *Acta Biomater* [57]			Fibrin (#)	Functional contractile cardiac tissue constructs
Zou *et al.* (2020); *Mater Sci Engineering C* [46]	Alginate 20 mg/mL Agarose 6–24 mg/mL PRP 20% PVA sacrificial scaffold	HUVEC H9c2	PRP	Rheology, FTIR, SEM, cell viability, proliferation, collagen Mechanical testing Construction of a hollowed Valentin-shaped heart with microfluid channel networks (3D vessel-like structures)
Corneal ulcers				
Frazer *et al.* (2020); *Trans Vis Sci Tech* [58]	Fibrinogen 0.2, 2 , 5 mg/mL Thrombin 1, 5, 10 U/mL 10% hPL	Corneal epithelial cells	hPL, 3 batches from pooled donors (blood bank)	Tested 9 bioink variations (with different fibrinogen concentration Differences in cell proliferation and wound healing
You *et al.* (2022); *Trans Vis Sci Tech* [59]	Fibrinogen 0.2, 2, 5 mg/mL Thrombin 1, 5, 10 U/mL 10% hPL	No cells	hPL, fibrinogen	*In situ* 3D printing corneal full-thickness perforations in rabbits

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Dermal patches				
Albanna *et al.* (2019); *Scientific Reports* [60]	Fibrin Collagen	Human fibroblasts Human keratinocytes	Fibrinogen from bovine plasma and thrombin (#)	*In situ* bioprinting Murine full-thickness excisional wound Porcine full-thickness wound model
Cubo *et al.* (2017); *Biofabrication* [61]	Human plasma + 30 mg fibrinogen + 200 µL tranexamic acid + $CaCl_2$ 1% w/vol	Human fibroblasts mixed with ink Keratinocytes seeded on top	FFP (30 mg fibrinogen/13 mL FFP)	Bioengineered skin 17 days maturation in *vitro* Immunodeficient mice, full thickness wound model, biofabricated skin grafted in the wound
Cheng *et al.* (2021); *Biofabrication* [62]	Hyaluronic acid: fibrin 1:1	UC-MSCs	Fibrinogen (from bovine plasma) Thrombin (#)	*In situ* handheld deposition Full-thickness burn injuries
Del Amo *et al.* (2021); *Biomedicines* [63]	Alginate/PRP Alginate/PPP	Human dermal fibroblasts	PRP PPP	Dermal patch
Del Amo *et al.* (2022); *Int J Mol Sci* [39]	Adipose ECM/PPP	Human dermal fibroblasts	PRP PPP	Dermal dressing
Diaz-Gomez *et al.* (2022); *Carbohydrate Polymers* [64]	CMC	3D printing	PRP (scaffold coating)	Swelling, rheology, growth factor release MSC proliferation and differentiation Ovo angiogenesis Full-thickness wounds in diabetic rat model
Cheng *et al.* (2021); *Biofabrication* [62]	Hyaluronic acid: fibrin 1:1	UC-MSCs	Fibrinogen (from bovine plasma) Thrombin (#)	Full-thickness burn wound in pigs *In situ* bioprinting
Zhao *et al.* (2022); *Materials Today Bio* [44]	Alginate-gelatin hydrogel	Human dermal fibroblasts Epidermal stem cells	PRP, double spinning citrated blood 10^{12} platelet/µL (human and rat) mixed with ALG (0, 2, 5 and 10%)	*In situ* extrusion (in rat full-thickness wounds) Infiltration of immune cells, polarization of macrophages, collagen deposition and remodeling, angiogenesis, blood perfusion Multicomponent bioink development Integration of 5% PRP in the bioink improved bioactivity *in vitro* and *in vivo*
Liver				
Taymour *et al.* (2021); *Scientific Reports* [65]	3% ALG, 9% MC autoclaved Fibrin supplemented (20 mg/mL) Tisseel 5 w/v% Matrigel	Human hepatocellular carcinoma in Matrigel-supplemented bioink NIH 3T3 mouse fibroblast cell line	FFP (blood bank), alginic acid dissolved in the plasma before adding 9% MC	Coaxial extrusion, core shell printing Two compartments made with two differently modified bioinks and different cell viability, morphology, proliferation, metabolic activity, and functionality (albumin secretion) (with and without Matrigel) algMC with fibrin or with plasma in the fibroblast ink improved cellular interactions and expression of hepatic marker proteins
Neural tissues				
de Melo *et al.* (2021); *J Vis Exp* [66]	GelMA	Murine cortical astrocytes	Fibrinogen (#)	Fabrication of 3D neural-like tissue rich in astrocytes
England *et al.* (2017); *Bioprinting* [67]	Hyaluronate	Schwann cells	Fibrin factor XIII (#)	Preclinical studies for nerve regeneration

Table 1. ***Continued***

Author (year); *Journal*	Ink formulation. GelMA/PRP	Modality/cell phenotype. Printing/BMSC	Blood-derived product	Evaluation of the output/biological aspects. Proliferation, migration and differentiation. In vivo M2 infiltration in a rabbit osteochondral lesion.
Tao *et al.* (2020); *Adv Funct Mater* [68]	PEGDA, GelMA	Schwan cells HUVEC DLP printer	Platelets (fluorescent marker) Encapsulated intact platelets within hydrogel	Proliferation of Schwann cells. Peripheral nerve repair, fabrication of functional nerve conduits, bridging a 10-mm gap in sciatic nerve in rats Reduction of muscle fiber atrophy. No changes in re-myelination and neural protein expression
Oral tissues				
Han *et al.* (2019); *J Tissue Engineering* [69]	Fibrin	Dental pulp stem cells	Fibrin (#)	Biofabrication of dentin pulp complexes
Yi *et al.* (2022); *Regenerative Biomaterials* [70]	Alginate 4%/gelatin 8%	Human gingival fibroblasts Release kinetics of PDGF-AB, TGF-β1, VEGF, EGF, FGF, and PDGF-AA	Anti-coagulated blood to prepare PRF single spinning	*In vitro*: Viability of cell proliferation, gene expression of COL1 and COL-III *In vivo*: Nude mice, histology performed 1, 2, 4 and 8 weeks post-implantation showing reduced inflammation and enhanced angiogenesis
Pancreas				
Duin *et al.* (2022); *Biomedicines* [71]	3% alginate (dissolved in bovine serum albumin or platelet lysate (PL) or FFP)+ 9% MC PBS-algMC, BSA-algMC, PL-algMC, FFP-algMC	Pancreas neonatal porcine islet-like cell clusters macroencapsulation	Alginate was dissolved in either PL (n = 20 donors) FFP (n = 5 donors)	Comparison of bioink formulations: viability, proliferation, a trend for higher cell viability with PL-algMC and FFP-algMC Insulin release in response to glucose stimulation over 4 weeks No relevant differences in functionality between bioinks
Zhu *et al.* (2023); *Colloids and Surfaces B: Biointerfaces* [45]	GelMA ECM	Islet organoid	PRP	

Table 1 shows the state of the art of extrusion bioprinting using bioinks doped with blood-derived product. The constructs aim to mimic both hard/semi-hard and soft tissues including bone, cartilage, cardiac tissue, cornea, skin, liver, pancreas, neural tissues, and oral tissues. We have considered both situations, such as when blood-derived biomaterial is part of the bioink formulation or is added later to the bioprinted construct.

Abbreviations: ADSC, adipose stem cells; Alg, alginate; AlgMA, Gelatin Alginate (composite hydrogel); bFGF, basic fibroblast growth factor; BSA, bone serum albumin; CMC, carboxymethyl cellulose (CMC), CT, computed tomography; DLP, digital light processing; ECM, extracellular matrix; EGF, epidermal growth factor; FFP, fresh-frozen plasma; FGF, fibroblast growth factor; GA, alginate methacrylamide; GelMA, methacrylated gelatin; HA, hyaluronic acid; hASCs, human adipose stromal cells; hPL, Human platelet lysate; HUVECs, human umbilical vein endothelial cells; MC, methylcellulose; MSCs, mesenchymal stem cells; OCN, osteocalcin; OPN, osteopontin; PBS, phosphate-buffered saline; PCL, polycaprolactone; PDGF, platelet-derived growth factor; PEGDA, poly(ethyleneglycol) diacrylate; PEG, poly(ethylene glycol); PL, platelet lysate; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SE, standard error; SEM, scanning electron microscopy; TCP, tricalcium phosphate; TGF-β, transforming growth factor beta; UC-MSCs, mesenchymal stem cells from umbilical cords; UV, ultraviolet; VEGF, vascular endothelial growth factor.
(#) indicates commercially available lyophilized fibrin.

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Table 2. Multipurpose applications

Author (year); *Journal*	Ink formulation	Cell phenotype	Blood-derived product	Characterization of bioink formulation/cytocompatibility
Dani *et al.* (2021); *Gels* *[72]	30 mg/mL ALG dissolved in plasma and added with MC (90 mg/mL)	Immortalized MSCs	FFP (blood bank), $n = 5$ donors	Cell viability using different mixing methods Mixing success: Homogenous mixing of highly viscous biomaterial inks and cell suspensions
Faramazi *et al.* (2018); *Adv Healthcare Mat*	Alginate 1% PRP 50 U/mL	MSCs	PRP	Printability, rheology, cell migration
Mendes *et al.* (2020); *Biofabrication*[73]	HUink = Cellulose nanocrystals (18% wt) Mixed with PL (67–160 mg/mL) (+ thrombin + $CaCl_2$)	hASCs Comparison of HUink with GelMA and with ALG	PL ($n = 12$ donors), platelet concentrate >10^6/µL, freeze (-196°C)/thawing (37°C) dialysis	Printability, bioprinting performance, biofunctionality Versatile bioink
Min *et al.* (2021); *Biofabrication* [74]	PLMA-based hydrogels Methacrylated photo-polymerization of PL PCL/PLMA (PCL frame and cells encapsulated in PLMA) ALG:PLMA Gelatin:PLMA	GFP-hMSCs RFP-HUVEC	3D-printable PL (PLMA)-based hydrogel, which consists of PL from whole blood of humans	Tissue engineering: Bone growth, cartilage repair wound healing
Somasekharan *et al.* (2020); *Bioengineering* [75]	ADA Gelatin PRP Covalent crosslinking in the presence of borax	L929 mouse fibroblast cell line	PRP (750 g, 5 min)	Characterization of ADA-gel-PRP bioink formulation: Printability, cell viability, cytotoxicity, hemolysis, histocompatibility, ISO10993

Another strategy in bioink development consists of developing formulations optimal for extrusion bioprinting but without tissue specifications.

We consider both situations, such as when blood-derived biomaterial is part of the bioink formulation or is added later to the bioprinted construct.

Abbreviations: ADA, alginate di-aldehyde; Alg, alginate; hASCs, human adipose stromal cells; FFP, fresh-frozen plasma; GFP, green fluorescent protein; hMSCs, human mesenchymal stem cells; HUVECs, human umbilical vein endothelial cells; MC, methylcellulose; MSCs, mesenchymal stem cells; RFP, red fluorescent protein; PCL, polycaprolactone; PL, platelet lysate; PLMA, platelet lysate methacrylated; PRP, platelet-rich plasma.

* Development of mixing units developed using computer-aided design (CAD) and 3D printing.

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To improve the functionality of a multipurpose alginate:methylcellulose (3% ALG:9% MC) ink, FFP was added in four studies[47, 65, 71, 72]. The tissue specificity of this multipurpose ink (3% ALG, 9% MC) was achieved with specific additives, such as calcium phosphate cement (CPC) for bone[47] and/or choosing the cell phenotype or cell aggregates and tissue inductive biomaterials. For example, after being supplemented with CPC and loaded with mesenchymal stem cells (MSCs), this ink was investigated for bone biofabrication[47]. Using a coaxial bioprinting system, Taymour *et al.*[65] created different liver compartments to examine the cellular interactions between human hepatocellular carcinoma and mouse fibroblasts encapsulated in Matrigel and FFP, respectively. In addition to specific cytokines, such as insulin-like growth factor 1 (IGF-I) and hepatocyte growth factor (HGF), FFP delivers fibronectin, which provides RGD domains for cell attachment (anchorage). In contrast, PC (or PRP) is richer than FFP in signaling proteins. Notwithstanding the molecular richness of the platelet secretome, a comparison between alginate dissolved in platelet lysate or FFP showed no relevant differences. When pancreas porcine islet-like cell clusters were encapsulated in bovine serum albumin (BSA)-algMC (control), FFP-algMC, or PL-algMC (3% alg 9% MC), the results showed higher cell viability in the constructs containing FFP or platelet lysate, but there was no difference in the restoration of insulin secretion over 4 weeks *in vitro*[71]. These outcomes may indicate a lack of availability of cytokines by encapsulated cells, potentially due to high hydrogel concentrations (3% ALG: 9% MC).

Bioprinting research in the development of tissue substitutes involves soft and hard tissue constructs. Generally, soft (e.g., liver, pancreas) and hard (e.g., bone) or semi-hard (e.g., cartilage) tissues have different biological and structural complexity in terms of cellularity and phenotype variety and functional requirements. Soft tissues, such as neural or endocrine tissues, can have a more complex biology, while hard and semi-hard tissues have to meet demanding mechanical requirements to fulfill their function.

3.1. Hard and semi-hard tissues

3.1.1. Bone

Two studies bioprinted bone with different strategies[50, 72], but both used bone mineral (CPC and tricalcium phosphate [TCP], respectively) to exploit their osteoconductive properties. The different strategies using blood derivatives illustrate the breadth of the possibilities. In one case, a multipurpose bioinert biomaterial composite (ALG/MC) was supplemented with FFP before bioprinting to add biological properties to the MSC-loaded bioink, enhancing cell proliferation, migration, and differentiation[47]. In the other case, Wei *et al.*[50] used a very different strategy; they 3D-printed a composite scaffold (silk fibroin, hyaluronic acid [HA], gelatin, TCP), which was subsequently coated with PRP prior to adipose stem cell (ADSC) seeding. In the printed construct, PRP promoted cell adhesion, proliferation, survival, and some aspects of differentiation toward the osteogenic lineage. Thus, PRP was considered a useful osteoconductive and osteoinductive supplement. The former was attributed to the fibrinogen/fibrin component and the latter to the platelet and plasma secretome. Recently, a personalized bone graft was implanted in a young oncologic patient; the bone implant was manufactured with PCL/β-TCP/PRP[49]. After 7 months, the bone implant showed promising integration based on observation from X-ray and CT images. Although anecdotic, this is a pioneer report showing the clinical feasibility of graft bioprinting. Bone vascularization is crucial for long-term survival of the graft and bone remodeling. A bone graft created with PRP-GA@Lap/PCL has shown regenerative properties in preclinical examination[48].

3.1.2. Cartilage

Because of the increasing clinical demand and the intrinsic lack of healing resources (avascular, aneural, and alymphatic), cartilage biofabrication is a major challenge in tissue engineering. Cartilage constructs must mimic the mechanical properties/requirements of the host tissue, e.g., knee articular cartilage. GelMA is a hydrogel with printable properties and the ability to support ADSC encapsulation. The addition of human platelet lysate (hPL) to GelMA enhanced viscosity and ADSC spreading, proliferation, and osteogenic differentiation in a concentration-dependent manner[76]. Three studies investigated the possibility of manufacturing cartilage-like tissue by adding blood derivatives to PEG/ALG[43], GelMA[52], or PEG/silk fibroin composite[53]. However, the technology readiness level was low (TRL3), and the results were limited to the cell proliferation, viability, and histology in the constructs. De Melo *et al.*[43] used a combined strategy: first, to mimic the mechanical properties of the cartilage extracellular matrix (ECM), they printed 20% PEG/2.5% ALG scaffolds with strong mechanical properties and cartilage-like tissue resilience and elasticity. In parallel, to mimic the pericellular cartilage, they prepared human mesenchymal stem cell (hMSC) spheroids using fibrinogen (microaggregating cells in micrometer-sized spheroids) with a microwell technique. PEG/ALG gel served as the supporting bath, and thrombin was added to the supporting bath to trigger fibrin polymerization. Fibrin showed diffusive capacity and a fast permeation rate, favoring optimal cell viability and the chondrogenic-like differentiation of hMSC spheroids. Likewise, 80% PEG was combined with silk fibroin supplemented with PRP (8× enrichment) to confer

biological activities and loaded with rabbit chondrocytes[53]. Its *in vitro* results pointed to efficient expression of cartilage ECM molecules, which was also confirmed in immunohistological analyses, with promising results in mechanical and degradation tests. Subsequent mixing with PEG optimized the printability. The maturation of the cell-laden bioprinted constructs and the histological and biochemical (sulfated glycosaminoglycan [sGAG]) characterization revealed cartilage-like properties in pre-clinical *in vitro* research.

The preliminary accomplishments in the creation of gel-MA/PRP constructs could be explained by the sustained release of growth factors for 35 days[52]. In these studies, constructs were kept in *in vitro* environments but in any case staffed with bioreactors. Irmak and Gümüşderelioglu optimized GelMA conjugation with PRP via platelet integrin receptors to provide optimal rheological properties to PRP for printability[52]. Printouts fabricated with this composite hydrogel, loaded with a murine chondrocyte cell line, showed chondrogenic properties *in vitro*. In addition, the release of growth factors was slow and sustained, and as a result, the constructs showed cartilage-like properties on histologic evaluations.

3.2. Soft tissues

3.2.1. Cardiac muscle

A major limitation in treating cardiac injury is the failure of current therapies to induce myocardial regeneration and cardiomyogenesis. One possible avenue is to engineer cardiac tissue via 3D bioprinting. As platelet-rich fibrin (PRF) has already been found to deliver competent cells to the injured myocardium[77–79], this function provides the initial hypothesis for adding PRF to bioink formulations.

To fabricate cardiac patches, Kumar *et al.*[54] optimized a previously developed bioink based on a mixture of gel-furfuryl, hyaluronic acid (HA), and Rose Bengal by incorporating fibrinogen. They crosslinked the composite using a two-step process, first irradiation with visible light followed by thrombin/$CaCl_2$ crosslinking of fibrinogen/fibrin[54]. Adding fibrinogen to their former bioink formula provoked a change in scaffold patterns (herringbone pattern) coupled to a reduction in porosity, resulting in improved elastic behavior and mechanical stiffness compatible with the fabrication of cardiac patches. Moreover, induced pluripotent stem cells (iPSCs), cardiomyocytes (CMs), and cardiac fibroblasts printed within the fibrin-gelatin construct could withstand extrusion printing and subsequent dual crosslinking and showed cell growth and proliferation. In addition to viable cell behavior, they revealed heterocellular coupling between excitable CMs and non-excitable cardiac fibroblasts via connexin-43 (Cx43), leading to ECM secretion. Their interest in fibrinogen was derived from studies involving the use of patient PRF to deliver CM for cardiac tissue injuries.

CM spatial orientation is paramount for unidirectional contraction, and cell viability is essential to build cardiac tissue patches. Maiullari *et al.*[56] took advantage of the 3D bioprinting potential to create constructs with various cell organizations (IPSCs-CM and human umbilical vein endothelial cells [HUVECs]) and specific cell orientations. Using microfluidics, the flow of two different bioinks was controlled, and constructs with different cell distributions were created and tested for optimal functionality and the formation of blood vessel-like structures. Constructs were cultured for 2 weeks and matured in vascularized functional tissues. Proof of concept was achieved after subcutaneous implantation in mice.

To solve the shortage of organ donors, a novel organ biofabrication method has been proposed[46]. To meet the challenge of biofabricating a large structure with hollow interiors that could support cell metabolism within the structure, Zou *et al.*[46] used polyvinyl alcohol (PVA) as a sacrificial scaffold with a bioink made of alginate/agarose combined with 20% PRP and loaded with HUVECs and H9c2 cells. In doing so, they showed the potential of the multichannel structure for nutrient delivery.

3.2.2. Cornea

Corneal disease leading to visual impairment is a common problem in ophthalmology that imposes significant economic and social burdens. The cornea is avascular, and transparency is a main attribute. Bioprinting corneas could help to minimize these problems. Few studies have been performed, and the goal was not to bioprint the full cornea but to heal corneal defects by *in vivo* deposition of the bioink. A major requirement of bioink and bioprinted constructs is maintaining transparency. Frazer *et al.*[58] optimized the bioink composition and the concentrations of fibrinogen and platelet lysate(PL) in terms of transparency preservation and wound-healing properties and examined *ex vivo* bioprinting precisely within a corneal defect as a proof of concept. Similarly, You *et al.*[59] used 3D-bioprinted grafts with hPL and fibrinogen to repair corneal full thickness perforations in rabbits.

3.2.3. Skin

Full-thickness skin defects occur due to multiple circumstances, large-scale burns, traumatic injuries, pressure, vascular and diabetic ulcers. *In situ* bioprinting directly delivers cells and biomaterials after scanning the morphological features of the wound after debridement. The successful treatment of non-healing wounds is an open research area, as no effective solutions have yet been

found. Accurate deposition of healing components within the tissue defect is a shared goal in the treatment of corneal ulcers and skin ulcers. In this context, *in situ* bioprinting has been performed using fibrinogen mixed with collagen and loaded with fibroblasts and keratinocytes in murine and porcine excisional wound models[60]. Similarly, *in situ bioprinting* was performed in full-thickness burn wounds in pigs using fibrinogen mixed with HA loaded with MSCs from umbilical cords (UC-MSCs)[62]. Likewise, *in situ* extrusion bioprinting was performed in a rat full-thickness wound using PRP combined with an alginate-gelatin hydrogel loaded with dermal and epidermal cells[44]. Cubo *et al.*[61] pioneered skin bioprinting and developed the concept of bioink functionalization with plasma. They created full-thickness wounds in immunodeficient mice and tested the feasibility and efficacy of bioprinting human plasma combined with additional fibrinogen and loaded with dermal cell. However, these pioneering studies used FFP as an additive (PPP, plasma without platelets). Whether PRP or PPP is more effective in wound healing bioprinting was examined in two studies[39, 63]. The creation of advanced dermal patches using bioprinting technologies with PRP as a means to engineer the biological environment of the wound was reported by Del Amo *et al.*[39]. They formulated different bioinks, i.e., ALG/PRP and adipose ECM/PRP. Both bioinks were compared with PPP bioinks to further assess the specific properties of the platelet secretome in the modulation of inflammation.

3.2.4. Liver and pancreas

The liver lobule consists of blood vessels, ducts, and canals surrounded by sheets of hepatic cells. Generating functional liver constructs is the first step toward the fabrication of liver patches. Coaxial bioprinting using hepatocytes and fibroblasts showed good cell viability using alginate: MC supplemented with FFP. These biomaterials lack cell binding sites and nutrients and thus should be supplemented with blood-derived additives to generate biologically active models. In a preliminary study, Taymour *et al.*[65] stressed the importance of tailoring the microenvironment with blood additives for improved cellular interactions and expression of hepatic marker proteins. However, Duin *et al.*[71] reported no advantages from adding blood-derived platelet lysate, FFP, or albumin to neonatal porcine pancreatic islet-like cell clusters encapsulated in alginate/MC in terms of cell viability or functional reaction to glucose stimulation.

3.2.5. Neural tissues

For large nerve defects, a tissue-engineered graft (conduit) is needed to fill the gap and guide axonal elongation and remyelination. Autograft transplantation is the gold standard to provide a favorable molecular environment. Tao *et al.*[68] fabricated neural conduits using a digital light processing (DLP) printer and filled the conduits with intact platelets encapsulated within poly(ethyleneglycol) diacrylate (PEGDA) and GelMA. This construct promoted the sustained release of cytokines and prevented burst release. The results showed increased thickness and numerous layers of myelin sheath, improved axonal elongation, and increased proliferation of Schwann cells after implantation in a 10-mm gap in a sciatic rat nerve model.

3.2.6. Oral tissues

The gold standard for oral tissue defects is the use of autologous tissue grafting. However, oral mucosa availability is limited, and morbidity at the donor site is another drawback of the procedure. A gingival mucosa patch was bioprinted by using alginate:gelatin combined with PRF loaded with gingival fibroblasts. *In vitro* studies revealed high cell viability and high ECM protein expression by encapsulated cells. Moreover, when the constructs were implanted subcutaneously in nude mice, they showed excellent biocompatibility and suppression of inflammation[70].

Natural organs and tissues are much larger than engineered tissues and contain a branching vascular network that perfuses the entire organ, ensuring that all cells are close to blood vessels with adequate nutrient and oxygen supply. As the field of biofabrication and tissue engineering struggles with this major limitation, advances in the fabrication of multicellular building blocks can help to meet the clinical demand for tissue grafts.

4. Concluding remarks

Biofabrication technologies to replace tissue sections and activate the regenerative cascade in pathological conditions are not yet available. The major challenges include providing the precise molecular signals that drive the cells to new tissue formation. Blood-derived products provide the potential to innovate ink/bioink formulations for enhanced construct functionalities, and it is timely to review the status of engineered tissue constructs using functionalized bioinks.

Bioinks can be developed by adding platelet lysates or serum-converted platelet-rich plasma, both of which offer a large pool of hundreds of signaling proteins for enhanced construct functionalities. On the other hand, plasma with scarce platelets, named PPP, and FFP are available in larger volumes and provide bioinks with adhesive proteins and few growth factors, providing a complex fibrillary environment to support cell activities under different clinical conditions. Research on engineered tissue constructs using blood-functionalized

bioinks has been conducted in bone, cartilage, cardiac tissue, cornea, dermal, liver and pancreas, neural tissues, and oral tissues. Solvent extrusion bioprinting technologies are likely to benefit from the incorporation of blood derivatives into bioinks. However, a deeper understanding of the role of blood derivatives in tissue repair and remodeling is needed to refine bioink formulations in ways that reproduce the complex biology and functionality of host tissues.

To fulfill as many requirements as possible with good predictability, biomaterials can be functionalized with tailored blood-derived products, resulting in the transformation of inert biomaterials into reactive (stimuli-response) biomaterials, inspired by physiological repair mechanisms in which hemostasis (blood clot formation, fibrin formation) is the starting point, and platelet degranulation and secretome release trigger the regenerative cascade.

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Conflict of interest

The authors declare no conflict of interest.

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References

1. Groll J, Boland T, Blunk T, *et al.,* 2016, Biofabrication: Reappraising the definition of an evolving field. *Biofabrication*, 8(1): 013001.

https://doi.org/10.1088/1758-5090/8/1/013001

2. Mironov V, Trusk T, Kasyanov V, *et al.*, 2009, Biofabrication: A 21st century manufacturing paradigm. *Biofabrication*, 1: 022001.

https://doi.org/10.1088/1758-5082/1/2/022001

3. Zhang B, Cristescu R, Chrisey DB, *et al.*, 2020, Solvent-based extrusion 3D printing for the fabrication of tissue engineering scaffolds. *Int J Bioprint*, 6: 28–42.

https://doi.org/10.18063/IJB.V6I1.211

4. Dębski T, Kurzyk A, Ostrowska B, *et al.*, 2020, Scaffold vascularization method using an adipose-derived stem cell (ASC)-seeded scaffold prefabricated with a flow-through pedicle. *Stem Cell Res Ther*, 11: 1–14.

https://doi.org/10.1186/S13287-019-1535-Z/FIGURES/7

5. Lee J, Park D, Seo Y, *et al.*, 2020, Organ-level functional 3D tissue constructs with complex compartments and their preclinical applications. *Adv Mater*, 32: 2002096.

https://doi.org/10.1002/ADMA.202002096

6. Deo KA, Singh KA, Peak CW, *et al.*, 2020, Bioprinting 101: Design, fabrication, and evaluation of cell-laden 3D bioprinted scaffolds. *Tissue Eng - Part A*, 26: 318–338.

https://doi.org/10.1089/TEN.TEA.2019.0298/ASSET/IMAGES/LARGE/TEN.TEA.2019.0298_FIGURE6.JPEG

7. Supernat A, Popęda M, Pastuszak K, *et al.*, 2021, Transcriptomic landscape of blood platelets in healthy donors. *Sci Rep*, 11: 15679.

https://doi.org/10.1038/s41598-021-94003-z

8. Nieto D, Jiménez G, Moroni L, *et al.*, 2022, Biofabrication approaches and regulatory framework of metastatic tumor-on-a-chip models for precision oncology. *Med Res Rev*, 42: 1978–2001

https://doi.org/10.1002/MED.21914

9. Burdis R, Chariyev-Prinz F, Browe DC, *et al.*, 2022, Spatial patterning of phenotypically distinct microtissues to engineer osteochondral grafts for biological joint resurfacing. *Biomaterials*, 289: 121750.

https://doi.org/10.1016/J.BIOMATERIALS.2022.121750

10. Zhang Q, Nguyen PD, Shi S, *et al.*, 2018, 3D bio-printed scaffold-free nerve constructs with human gingiva-derived mesenchymal stem cells promote rat facial nerve regeneration. *Sci Rep,* 8: 1–11.

https://doi.org/10.1038/s41598-018-24888-w

11. Chen EP, Toksoy Z, Davis BA, *et al.*, 2021, 3D bioprinting of vascularized tissues for in vitro and in vivo applications. *Front Bioeng Biotechnol*, 9, 1–22.

https://doi.org/10.3389/fbioe.2021.664188

12. Kandi R, Sachdeva K, Choudhury SD, *et al.*, 2023, A facile 3D bio-fabrication of customized tubular scaffolds using solvent-based extrusion printing for tissue-engineered tracheal grafts. *J Biomed Mater Res Part A*, 111: 278–293.

https://doi.org/10.1002/JBM.A.37458

13. Moss SM, Schilp J, Yaakov M, *et al.*, 2022, Point-of-use, automated fabrication of a 3D human liver model supplemented with human adipose microvessels. *SLAS Discov*, 27: 358–68

https://doi.org/10.1016/j.slasd.2022.06.003

14. Nulty J, Freeman FE, Browe DC, *et al.*, 2021, 3D bioprinting of prevascularised implants for the repair of critically-sized bone defects. *Acta Biomater*, 126: 154–169.

https://doi.org/10.1016/J.ACTBIO.2021.03.003

15. Liang Q, Ma Y, Yao X, *et al.*, 2022, Advanced 3D-printing bioinks for articular cartilage repair. *Int J Bioprint,* 8: 15–30.

https://doi.org/10.18063/ijb.v8i3.511

16. Yeung E, Inoue T, Matsushita H, *et al.*, 2020, In vivo implantation of 3-dimensional printed customized branched tissue engineered vascular graft in a porcine model. *J Thorac Cardiovasc Surg*, 159: 1971–1981.e1.

https://doi.org/10.1016/J.JTCVS.2019.09.138

17. Yu HW, Kim BS, Lee JY, *et al.*, 2021, Tissue printing for engineering transplantable human parathyroid patch to improve parathyroid engraftment, integration, and hormone secretion in vivo. *Biofabrication*, 13(3): 1-10.

https://doi.org/10.1088/1758-5090/ABF740

18. Del Amo C, Perez-valle A, Atilano L, *et al.*, 2022, Unraveling the signaling secretome of platelet-rich plasma: Towards a better understanding of its therapeutic potential in knee osteoarthritis. *J Clin Med,* 11(3): 473.

19. Sun B, Lian M, Han Y, *et al.*, 2021, A 3D-bioprinted dual growth factor-releasing intervertebral disc scaffold induces nucleus pulposus and annulus fibrosus reconstruction. *Bioact Mater*, 6: 179.

https://doi.org/10.1016/J.BIOACTMAT.2020.06.022

20. Lee J, Seok JM, Huh SJ, *et al.*, 2020, 3D printed micro-chambers carrying stem cell spheroids and pro-proliferative growth factors for bone tissue regeneration. *Biofabrication*, 13: 015011.

https://doi.org/10.1088/1758-5090/ABC39C

21. Ngo TB, Spearman BS, Hlavac N, *et al.*, 2020, Three-dimensional bioprinted hyaluronic acid hydrogel test beds for assessing neural cell responses to competitive growth stimuli. *ACS Biomater Sci Eng*, 6: 6819–6830.

https://doi.org/10.1021/ACSBIOMATERIALS.0C00940/SUPPL_FILE/AB0C00940_SI_001.PDF

22. Horstman EE, Tormey CA, 2022, Plasma products for transfusion: An overview. *Ann Blood*, 7: 1–13.

https://doi.org/10.21037/AOB-22-7

23. Dohan Ehrenfest DM, Andia I, Zumstein MA, *et al.*, 2014, Classification of platelet concentrates (platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Ligaments Tendons J*, 4: 3–9.

24. Burnouf T, Agrahari V, Agrahari V, 2019, Extracellular vesicles as nanomedicine: Hopes and hurdles in clinical translation. *Int J Nanomedicine,* 14: 8847–8859.

https://doi.org/10.2147/IJN.S225453

25. Ebner-Peking P, Krisch L, Wolf M, *et al.*, 2021, Self-assembly of differentiated progenitor cells facilitates spheroid human skin organoid formation and planar skin regeneration. *Theranostics*, 11: 8430–8447.

https://doi.org/10.7150/THNO.59661

26. Hermida-Nogueira L, Barrachina MN, Morán LA, *et al.*, 2020, Deciphering the secretome of leukocyte-platelet rich fibrin: Towards a better understanding of its wound healing properties. *Sci Rep*, 10: 1–11.

https://doi.org/10.1038/s41598-020-71419-7

27. Kardos D, Hornyák I, Simon M, *et al.*, 2018, Biological and mechanical properties of platelet-rich fibrin membranes after thermal manipulation and preparation in a single-syringe closed system. *Int J Mol Sci*, 19(11): 1–14.

https://doi.org/10.3390/ijms19113433

28. Widyaningrum R, Burnouf T, Nebie O, *et al.*, 2021, A purified human platelet pellet lysate rich in neurotrophic factors and antioxidants repairs and protects corneal endothelial cells from oxidative stress. *Biomed Pharmacother*, 142: 112046.

https://doi.org/10.1016/j.biopha.2021.112046

29. Andia I, Perez-valle A, Amo Del C, *et al.*, 2020, Freeze-drying of platelet-rich plasma: The quest for standardization. *Int J Mol Sci*, 21: 1–20.

https://doi.org/10.3390/ijms21186904

30. Nurden AT, Nurden P, Sanchez M, *et al.*, 2008, Platelets and wound healing. *Front Biosci*, 13: 3532–3548.

https://doi.org/10.2741/2947/PDF

31. Lee HM, Shen EC, Shen JT, *et al.*, 2020, Tensile strength, growth factor content and proliferation activities for two platelet concentrates of platelet-rich fibrin and concentrated growth factor. *J Dent Sci*, 15: 141–146.

https://doi.org/10.1016/J.JDS.2020.03.011

32. Battinelli EM, Thon JN, Okazaki R, *et al.*, 2019, Megakaryocytes package contents into separate α-granules

that are differentially distributed in platelets. *Blood Adv*, 3: 3092–3098.

https://doi.org/10.1182/BLOODADVANCES.2018020834

33. Ambrosio AL, Di Pietro SM, 2016, Storage pool diseases illuminate platelet dense granule biogenesis. *Platelets*, 28: 138–146.

https://doi.org/10.1080/09537104.2016.1243789

34. Miroshnychenko O, Chalkley RJ, Leib RD, *et al.*, 2020, Proteomic analysis of platelet-rich and platelet-poor plasma. *Regen Ther*, 15: 226–235.

https://doi.org/10.1016/j.reth.2020.09.004

35. Johnson J, Wu YW, Blyth C, *et al.*, 2021, Prospective therapeutic applications of platelet extracellular vesicles. *Trends Biotechnol*, 39: 598–612.

36. Burnouf T, Goubran HA, 2022, Regenerative effect of expired platelet concentrates in human therapy: An update. *Transfus Apher Sci*, 61(1): 103363.

https://doi.org/10.1016/J.TRANSCI.2022.103363

37. Vajen T, Benedikter BJ, Heinzmann ACA, *et al.*, 2017, Platelet extracellular vesicles induce a pro-inflammatory smooth muscle cell phenotype. *J Extracell Vesicles*, 6: 1322454.

https://doi.org/10.1080/20013078.2017.1322454/SUPPL_FILE/ZJEV_A_1322454_SM7826.PDF

38. Saumell-Esnaola M, Delgado D, García Del Caño G, *et al.*, 2022, Isolation of platelet-derived exosomes from human platelet-rich plasma: biochemical and morphological characterization. *Int J Mol Sci*, 23: 2861.

https://doi.org/10.3390/IJMS23052861

39. Del Amo C, Fernández-San Argimiro X, Cascajo-Castresana M, *et al.*, 2022, Wound-microenvironment engineering through advanced-dressing bioprinting. *Int J Mol Sci*, 23(5), 1–19.

https://doi.org/10.3390/ijms23052836

40. Delila L, Wu YW, Nebie O, *et al.*, 2020, Extensive characterization of the composition and functional activities of five preparations of human platelet lysates for dedicated clinical uses. *Platelets*, 32(2): 259–272.

https://doi.org/101080/0953710420201849603

41. Uchiyama R, Toyoda E, Maehara M, *et al.*, 2021, Effect of platelet-rich plasma on M1/M2 macrophage polarization. *Int J Mol Sci*, 22: 2336.

https://doi.org/10.3390/IJMS22052336

42. Perez-Valle A, Del Amo C, Andia I, 2020, Overview of current advances in extrusion bioprinting for skin applications. *Int J Mol Sci*, 21: 1–28.

https://doi.org/10.3390/ijms21186679

43. de Melo BAG, Jodat YA, Mehrotra S, *et al.,* 2019, 3D printed cartilage-like tissue constructs with spatially controlled mechanical properties. *Adv Funct Mater*, 29: 1–26.

https://doi.org/10.1002/adfm.201906330

44. Zhao M, Wang J, Zhang J, *et al.*, 2022, Functionalizing multi-component bioink with platelet-rich plasma for customized in-situ bilayer bioprinting for wound healing. *Mater Today Bio,* 16: 100334.

https://doi.org/10.1016/J.MTBIO.2022.100334

45. Zhu B, Wang D, Pan H, *et al.*, 2023, Three-in-one customized bioink for islet organoid: GelMA/ECM/PRP orchestrate pro-angiogenic and immunoregulatory function. *Colloids Surf B Biointerfaces,* 221: 113017.

https://doi.org/10.1016/J.COLSURFB.2022.113017

46. Zou Q, Grottkau BE, He Z, *et al.*, 2020, Biofabrication of valentine-shaped heart with a composite hydrogel and sacrificial material. *Mater Sci Eng C*, 108: 110205.

https://doi.org/10.1016/j.msec.2019.110205

47. Ahlfeld T, Cubo-Mateo N, Cometta S, *et al.*, 2020, A novel plasma-based bioink stimulates cell proliferation and differentiation in bioprinted, mineralized constructs. *ACS Appl Mater Interfaces*, 12: 12557–12572.

https://doi.org/10.1021/acsami.0c00710

48. Cao B, Lin J, Tan J, *et al.*, 2023, 3D-printed vascularized biofunctional scaffold for bone regeneration. *Int J Bioprint,* 9(3): 702.

https://doi.org/10.18063/IJB.702

49. Hao Y, Cao B, Deng L, *et al.*, 2023, The first 3D-bioprinted personalized active bone to repair bone defects: A case report. *Int J Bioprint,* 9: 654.

https://doi.org/10.18063/IJB.V9I2.654

50. Wei L, Wu S, Kuss M, *et al.*, 2019, 3D printing of silk fibroin-based hybrid scaffold treated with platelet rich plasma for bone tissue engineering. *Bioact Mater*, 4: 256–260.

https://doi.org/10.1016/j.bioactmat.2019.09.001

51. Jiang G, Li S, Yu K, *et al.,* 2021, A 3D-printed PRP-GelMA hydrogel promotes osteochondral regeneration through M2 macrophage polarization in a rabbit model. *Acta Biomater*, 128: 150–162.

https://doi.org/10.1016/J.ACTBIO.2021.04.010

52. Irmak G, Gümüşderelioglu M, 2020, Photo-activated platelet-rich plasma (PRP)-based patient-specific bio-ink for cartilage tissue engineering. *Biomed Mater*, 15: 065010.

https://doi.org/10.1088/1748-605X/ab9e46

53. Li Z, Zhang X, Yuan T, *et al.*, 2020, Addition of platelet-rich plasma to silk fibroin hydrogel bioprinting for cartilage regeneration. *Tissue Eng - Part A*, 26: 886–895.

https://doi.org/10.1089/ten.tea.2019.0304

54. Anil Kumar S, Alonzo M, Allen SC, *et al.*, 2019, A visible light-cross-linkable, fibrin-gelatin-based bioprinted

construct with human cardiomyocytes and fibroblasts. *ACS Biomater Sci Eng*, 5: 4551–4563.

https://doi.org/10.1021/acsbiomaterials.9b00505

55. Freeman S, Ramos R, Alexis Chando P, *et al.*, 2019, A bioink blend for rotary 3D bioprinting tissue engineered small-diameter vascular constructs. *Acta Biomater*, 95: 152–164.

https://doi.org/10.1016/J.ACTBIO.2019.06.052

56. Maiullari F, Costantini M, Milan M, *et al.,* 2018, A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. *Sci Rep*, 8: 1–15.

https://doi.org/10.1038/s41598-018-31848-x

57. Wang Z, Lee SJ, Cheng H-J, *et al.*, 2018, 3D bioprinted functional and contractile cardiac tissue constructs. *Acta Biomater*, 70: 48–56.

https://doi.org/10.1016/j.actbio.2018.02.007

58. Frazer H, You J, Chen Z, *et al.*, 2020, Development of a platelet lysate–based printable, transparent biomaterial with regenerative potential for epithelial corneal injuries. *Transl Vis Sci Technol*, 9: 1–12.

https://doi.org/10.1167/TVST.9.13.40

59. You J, Frazer H, Sayyar S, *et al.*, 2022, Development of an in situ printing system with human platelet lysate-based bio-adhesive to treat corneal perforations. *Transl Vis Sci Technol*, 11(6): 26.

https://doi.org/10.1167/tvst.11.6.26

60. Albanna M, Binder KW, Murphy SV, *et al.,* 2019, In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds. *Sci Rep*, 9(1): 1856.

https://doi.org/10.1038/s41598-018-38366-w

61. Cubo N, Garcia M, Del Cañizo JF, *et al.*, 2017, 3D bioprinting of functional human skin: Production and in vivo analysis. *Biofabrication,* 9: 1–12.

https://doi.org/10.1088/1758-5090/9/1/015006

62. Cheng RY, Eylert G, Gariepy J-M, *et al.*, 2021, Handheld instrument for wound-conformal delivery of skin precursor sheets improves healing in full-thickness burns. *Biofabrication,* 12(2): 025002.

https://doi.org/10.1088/1758-5090/ab6413

63. Del Amo C, Perez-Valle A, Perez-Garrastachu M, *et al.*, 2021, Plasma-based bioinks for extrusion bioprinting of advanced dressings. *Biomedicines*, 9: 1023.

https://doi.org/10.3390/biomedicines9081023

64. Diaz-Gomez L, Gonzalez-Prada I, Millan R, *et al.*, 2022, 3D printed carboxymethyl cellulose scaffolds for autologous growth factors delivery in wound healing. *Carbohydr Polym*, 278: 118924.

https://doi.org/10.1016/J.CARBPOL.2021.118924

65. Taymour R, Kilian D, Ahlfeld T, *et al.,* 2021, 3D bioprinting of hepatocytes: Core-shell structured co-cultures with fibroblasts for enhanced functionality. *Sci Rep*, 11: 5130.

https://doi.org/10.1038/S41598-021-84384-6

66. de Melo BAG, Cruz EM, Ribeiro TN, *et al.*, 2021, 3D bioprinting of murine cortical astrocytes for engineering neural-like tissue. *J Vis Exp*., 173: e62691

https://doi.org/10.3791/62691

67. England S, Rajaram A, Schreyer DJ, *et al.*, 2017, Bioprinted fibrin-factor XIII-hyaluronate hydrogel scaffolds with encapsulated Schwann cells and their in vitro characterization for use in nerve regeneration. *Bioprinting*, 5: 1–9.

https://doi.org/10.1016/J.BPRINT.2016.12.001

68. Tao J, Liu H, Wu W, *et al.*, 2020, 3D-printed nerve conduits with live platelets for effective peripheral nerve repair. *Adv Funct Mater*, 30: 2004272.

https://doi.org/10.1002/ADFM.202004272

69. Han J, Kim DS, Jang H, *et al.*, 2019, Bioprinting of three-dimensional dentin–pulp complex with local differentiation of human dental pulp stem cells. *J Tissue Eng*, 10: 1–10.

https://doi.org/10.1177/2041731419845849

70. Yi K, Li Q, Lian X, *et al.,* 2022, Utilizing 3D bioprinted platelet-rich fibrin-based materials to promote the regeneration of oral soft tissue. *Regen Biomater*, 9: rbac021.

https://doi.org/10.1093/rb/rbac021

71. Duin S, Bhandarkar S, Lehmann S, *et al.*, 2022, Viability and functionality of neonatal porcine islet-like cell clusters bioprinted in alginate-based bioinks. *Biomedicines*, 10: 1420.

https://doi.org/10.3390/biomedicines10061420

72. Dani S, Ahlfeld T, Albrecht F, *et al.*, 2021, Homogeneous and reproducible mixing of highly viscous biomaterial inks and cell suspensions to create bioinks. *Gels*, 7: 227.

https://doi.org/10.3390/GELS7040227

73. Mendes BB, Gómez-Florit M, Hamilton AG, *et al.*, 2020, Human platelet lysate-based nanocomposite bioink for bioprinting hierarchical fibrillar structures. *Biofabrication*, 12: 015012.

https://doi.org/10.1088/1758-5090/ab33e8

74. Min SJ, Lee JS, Nah H, *et al.*, 2021, Development of photo-crosslinkable platelet lysate-based hydrogels for 3D printing and tissue engineering. *Biofabrication*, 13: 044102.

https://doi.org/10.1088/1758-5090/AC1993

75. Somasekharan LT, Kasoju N, Raju R, *et al.*, 2020, Formulation and characterization of alginate dialdehyde, gelatin, and platelet-rich plasma-based bioink for bioprinting applications. *Bioengineering*, 7: 1–12.

https://doi.org/10.3390/bioengineering7030108

76. Kirsch M, Birnstein L, Pepelanova I, *et al.*, 2019, Gelatin-methacryloyl (GelMA) formulated with human platelet lysate supports mesenchymal stem cell proliferation and differentiation and enhances the hydrogel's mechanical properties. *Bioeng*, 6: 76.

https://doi.org/10.3390/BIOENGINEERING6030076

77. Wendel JS, Ye L, Tao R, *et al.*, 2015, Functional effects of a tissue-engineered cardiac patch from human induced pluripotent stem cell-derived cardiomyocytes in a rat infarct model. *Stem Cells Transl Med*, 4: 1324–1332.

https://doi.org/10.5966/SCTM.2015-0044

78. Roura S, Soler-Botija C, Bagó JR, *et al.*, 2015, Postinfarction functional recovery driven by a three-dimensional engineered fibrin patch composed of human umbilical cord blood-derived mesenchymal stem cells. *Stem Cells Transl Med*, 4: 956–966.

https://doi.org/10.5966/SCTM.2014-0259/-/DC1

79. Bellamy V, Vanneaux V, Bel A, *et al.*, 2015, Long-term functional benefits of human embryonic stem cell-derived cardiac progenitors embedded into a fibrin scaffold. *J Hear Lung Transplant*, 34: 1198–1207.

https://doi.org/10.1016/j.healun.2014.10.008