3D Bioprinting of Polycaprolactone Hybrid Construct in Bone Tissue Engineering

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https://doi.org/10.5109/7395507

出版情報：Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 11, pp.123-130, 2025-10-30. International Exchange and Innovation Conference on Engineering & Sciences
バージョン：

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Abstract: *Due to limitations and disadvantages of current therapies to replace or restore bone tissue, researchers are getting interested in discovering new interventions. Bioprinting is one aspect of tissue engineering which has been emerging in recent years to build three-dimensional (3D) constructs containing biological cells and shows promising potential to overcome the challenges of current interventions for replacing and restoring bone tissue function. In bone tissue engineering, 3D bioprinted constructs are usually printed with thermoplastic polymer and cell-laden hydrogels, which is termed as hybrid constructs. This hybrid construct mimics the native bone, where it comprises a stiff, acellular structural component and is surrounded by a softer matrix with a varying amount of cells. Polycaprolactone (PCL), a thermoplastic polymer, is the most commonly used polymer in biomedical and tissue engineering applications for bone and cartilage repair due to its non-toxic, biodegradable, and biocompatible properties. This paper will discuss on 3D bioprinting in bone tissue engineering applications, where the explanation will be based on the introduction of bioprinting, the materials used, the potential of cell sources for printing bone scaffolds and a previous study on printing PCL hybrid scaffolds.*

Keywords: 3D bioprinting, Bone tissue engineering, PCL, Hydrogels, Hybrid construct

1. INTRODUCTION

Autologous and allograft bone grafts are current treatments utilized to repair or restore skeletal tissue malfunction resulting from trauma, damage, illness, or ageing. However, they have limitations and disadvantages that may be harmful. Despite being the gold standard for bone replacement, autologous bone graft is expensive and limited in quantity, worse, it can result in donor site morbidity [1]. In addition, allograft bone graft may have possibilities of immune rejection and introduction of infections or diseases [1]. Tissue engineering, the field that might address this issue, is an interdisciplinary field that combines medicine and engineering, which can construct biological substitutes to repair and replace diseased or damage tissues and restore tissue functionality. Tissue engineering involves a combination of cells, engineering, and materials to generate artificial tissues and organs [2]. In bone tissue engineering, strategies involve developing scaffolds from natural or synthetic materials for cell transplantation and guiding new bone growth. [3].

Conventional techniques to fabricate scaffolds, such as electrospinning, sol-gel, phase separation, freeze drying, solvent casting and particle leaching, gas foaming, powder-forming, usually require post-processing cell seeding mechanism [4]. Unfortunately, this seeding mechanism is usually reported with insufficient cell seeding and non-uniform and uncontrollable cell distribution on the inside and inner composition of the scaffold, where the cell usually attaches on the surface of the scaffold [5, 6, 7]. To overcome this problem, new fabrication technique termed as three-dimensional (3D) bioprinting has been introduced [8]. Bioprinting or also called biofabrication is one facet of tissue engineering where the aim is to build a 3D construct containing biological cells [9]. 3D bioprinting is made by the advancement of 3D printing technology, cell biology and materials science [10].

In general, bioprinting is a process that builds a 3D construct using computer-controlled 3D printing devices by depositing the cells and biomaterials precisely into geometries that create anatomically correct biological structures. It prints by outputting layer upon layer of living cells in hydrogels or viscous fluid, or cell-seeded microcarriers, which is termed bioink [9]. Different from conventional 3D printing techniques, which print temporary cell-free scaffolds, bioprinting requires a different technical approach that is compatible with depositing living cells [11, 12]. Compared to 3D printing, bioprinting involves extra complexities, such as the choice of materials, cell types, growth and differentiation factors, and technical challenges related to the sensitivities of living cells and the construction of tissues. Thus, bioprinting requires the incorporation of technologies from engineering, biomaterials science, cell biology, physics and medicine fields [10].

The bioprinting process can be divided into three sequential technological steps, which are pre-processing, processing (actual printing), and post-processing [12]. Before these three processes, the desired cell that will be used in printing must first be isolated and expanded or proliferated [13]. Pre-processing is a process of designing a scaffold, tissue or organ using imaging and computer-aided design techniques. Then the design is printed through a bioprinter. The post-processing of 3D bioprinted constructs involves the process of tissue remodelling and maturation in a specially designed chamber bioreactor, which accelerates tissue maturation [14].

Bioprinting is present in various biologically applied printing systems, such as inkjet, extrusion and laser-assisted bioprinting. These printing systems vary in their method of deposition. Extrusion-based bioprinting is the most common type of bioprinter used. It used compressed air to dispense and control the amount of liquid extruded from the syringe or the nozzle [15]. The most common methods to extrude the biological material from the nozzle are pneumatic [16, 17] and mechanical which use either piston or screw dispensing system [18, 19]. Inkjet bioprinter used thermal or acoustic force system to eject the drops of biological material liquid in a controllable size [20, 21]. The thermal inkjet printer electrically heats the printer head, which generates a pulse of pressure and subsequently forces the droplets from the nozzle [10]. Whereas acoustic printers use pulses formed by piezoelectric or ultrasound pressure to eject the droplets of the materials. Laser-assisted bioprinter is less commonly used than inkjet and micro extrusion bioprinter, which may be because of the high cost, the cumbersome and the complexities of the laser-assisted bioprinter. It uses lasers focused on an absorbing substrate to generate pressures that propel cell-containing materials onto a collector substrate.

2. THREE DIMENSIONAL BIOPRINTING FOR BONE TISSUE ENGINEERING APPLICATION

In bone tissue engineering, the strategies to engineer bone are primarily focused on the development of scaffolds using natural or synthetic materials for cell transplantation or as conduits to guide new bone growth [3]. The success of this strategy is dependent on the properties of the material, where the material is supposed to be biocompatible, osteoconductive and can degrade to a non-toxic product that can be metabolized or excreted [3]. Bose et al have provided certain requirements to become an ideal scaffold for bone tissue engineering applications to suit with the complexity of the biomechanical system of bone (Table 1) [22]. Before, scaffolds were engineered to be bioactive or bioresorbable to enhance tissue growth, but now scaffolds are often designed to induce bone formation and vascularization. These scaffolds can be incorporate with bioactive molecules, hormones or growth factor such as transforming growth factor beta (TGF-β), bone morphogenetic proteins (BMP), insulin-like growth factor (IGF), fibroblast growth factors (FGF), and vascular endothelial growth factor (VEGF) control osteogenesis, bone tissue regeneration, ECM formation via recruiting and differentiating osteoprogenitor cells to specific lineages and vascularization [23, 24].

2.1. Choices of Cells for 3D Bioprinting of Bone Scaffold

The main regulators of bone deposition, modelling, and remodelling are osteoblasts and osteocytes. Hence, osteoblasts and their precursors are a great source for a successful cell-based bone therapy. However, the use of mesenchymal stromal cells (MSC) in bone tissue engineering has been a significant step forward. The most commonly used cells are bone marrow stromal cells (BM-MSC), which are relatively simple to obtain, recruit in situ, and are well-characterized [28, 29]. Apart from that, adipose tissue-derived stem cells (ASC), oral cavity MSC and skin basal layer have also been used in bone regeneration. ASC have osteogenic potential in vitro, are abundant and easy to obtain, and can live in low oxygen and glucose conditions [30, 31]. Oral cavity MSC have also been proposed for biofabrication of bone [32, 33]. Whereas skin basal layer multipotent stem cells have ability to differentiate to become adipocytes, osteoblasts, chondrocytes, which make it suitable to be used in bone therapy [34].

Table 1. Requirements to become an ideal scaffold for bone tissue engineering application

Requirements	Notes
Biocompatibility	Biocompatibility is defined as the ability to support normal cellular activity without any local and systematic toxic effects to the host tissue [25]. The scaffold also must be osteoconductive which permit the adhesion and proliferation of the bone cells and also formation of the extracellular matrix on the surface and pores of the scaffolds
Mechanical properties	The mechanical properties of the scaffold should match host bone properties where it varies widely from cancellous to cortical bone. Proper load transfer is important as well [22].
Pore size	Rouwkema et al., suggest the pore size of the scaffolds should be at least 100 μm in diameter [26]. However, pore sizes in the range of 200 to 350 μm are found to be optimum for bone tissue in-growth [27]. The porosity percentage also play a role where high porosity scaffolds can provide a maximal cell loading and support cell growth and differentiated function [3]
Bioresorbability	Bioresorbability is the ability of the scaffold to degrade with time in vivo at a controlled rate which will create a space for the growth of new bone tissue [22].

3. THREE DIMENSIONAL BIOPRINTING OF POLYCAPROLACTONE FOR BONE TISSUE ENGINEERING APPLICATION FOR BONE TISSUE ENGINEERING APPLICATION

Polycaprolactone (PCL) is a synthetic, hydrophobic, semicrystalline polymer which was among the earliest polymers produced in the early 1930s by the Carothers group [35]. PCL is elastic and consists of nonpolar methylene groups and one semi-polar ester group (Figure 1). The low melting point (59-64 °C), good solubility, and exceptional blend-compatibility have stimulated extensive research on PCL into its potential application in the biomedical field.

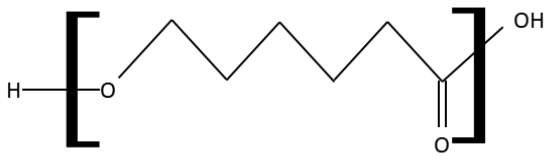

Fig. 1. Chemical structure of PCL

Recently, PCL is commonly used polymer in biomedical and tissue engineering applications for hard tissue regeneration due to its non-toxic, biodegradable and biocompatible properties [36]. PCL has a significantly slower biodegradation rate than other biodegradable polymer materials, which makes it suitable to serve as long-term implantable systems and in applications where a more resilient material is required, such as in bone substitution therapy [37]. PCL possesses a unique advantage due to its ability to form compatible blends with a diverse range of other polymers. In addition to this versatility, PCL exhibits superior mechanical properties when compared to many other biodegradable polymers. Its strength and elasticity are particularly notable, varying according to its molecular weight [38]. Therefore, PCL is suitable for use in sutures, tendons, cartilage, bone, and other biomedical applications in which mechanical strength is required [39]. Williams et al. were able to fabricate a 3D scaffold of PCL via selective laser sintering, which has mechanical properties within the lower range of trabecular bone, suggesting they may have the ability to withstand early functional loading [40]. PCL scaffolds resemble native bone in mechanical properties and exhibit good biocompatibility, as demonstrated by Shor et al. They fabricated PCL scaffolds using extrusion 3D printing and conducted an in vivo study by implanting osteoblast-seeded scaffolds in nude mice. Microscopy and radiological examinations showed that these scaffolds promoted osteogenesis. [41].

However, it is impossible to prepare scaffolds made of a single material that can satisfy numerous requirements. Therefore, fabrication of scaffolds using composite materials is a promising technique to meet all the requirements. For bone tissue engineering applications, PCL scaffolds have been incorporated with ceramics, natural and synthetic polymer to improve their mechanical properties, controllable degradation rates, and bioactivity [42, 43]. This can be supported by Wu et al., 2012; Diba et al., 2012; and Yao et al., 2017. Wu et al. found that PCL scaffolds incorporated with magnesium phosphate improve the hydrophilicity of the scaffolds [44]. Whereas Diba et al. prove incorporation of forsterite nanocomposite into the PCL scaffolds significantly improved the mechanical properties [45]. Yao et al., also shows the same improvement on mechanical properties when blending the PCL with polylactic acid (PLA). Not only that, the blending of PCL and PLA also enhanced the cell viability of mesenchymal cells and promoted the osteogenic differentiation [46]. In placing more emphasise, Wang et al. and Bao et al., claimed that the material densification characteristics, compressive strength, and value of MTT assay were better than used PCL alone but only when the content of PCL is higher than the ceramic content in hybrid composite [47, 48]. PCL has also have been incorporated with natural polymers such as alginate, gelatin and chitosan to produce hybrid composite scaffolds which have improved their bioactive properties [49-51]. They all claimed that the PCL scaffolds incorporated with natural polymer have improved the cell adhesion and proliferation of the cells. All these findings prove that PCL is suitable to be used in bone tissue engineering applications when incorporated with other polymers or ceramics.

3.1 Previous Study on 3D Bioprinted Polycaprolactone Bone ScaffolD

Since 3D bioprinting using only hydrogel is insufficient to preserve the 3D structure throughout the regenerative period, many researchers consider using solid-state synthetic biomaterials like polycaprolactone (PCL), a thermoplastic material, together with cell-laden hydrogel in the printing process to improve mechanical stability. This combination of thermoplastic material and hydrogel constructs or scaffolds is often termed as hybrid tissue construct. The hybrid tissue construct mimics the native bone, where it comprises of an acellular structural component characterized by a high degree of stiffness and surrounded by softer matrix with a varying amount of cells. The thermoplastic polymer component will provide mechanical support, structural stability, and 3D space for tissue development, while the hydrogel component will serve as a diffusible component as a vascular conduit or to deliver bioagents such as cells and growth factors to improve the construct's biological functionality [52]. The printing process of hybrid construct usually consist of alternately printed of thermoplastic polymer and hydrogels or hydrogels were deposited between the thermoplastic polymer struts (Figure 2).

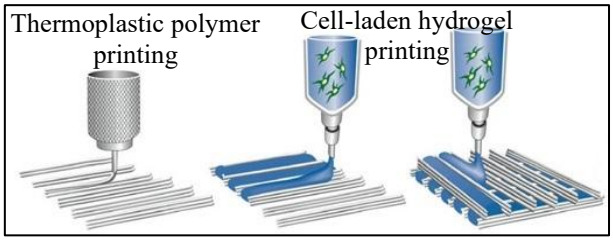

Fig. 2. Printing process the hybrid construct

Schuurman et al., 2011 were successfully printed a hybrid construct of PCL and chondrocyte cells-laden sodium alginate. The results showed improved in mechanical stability and high cell viability in the generated hybrid constructs immediately after printing, as well as after 1 and 3 days of in vitro culture [53]. In other studies, the hybrid construct was printed by dispensing the PCL struts first, followed by dispensing the osteoblast and chondrocyte cell-laden alginate between the pores separately [54]. Separately dispensed osteoblasts and chondrocyte cell-laden alginate between the PCL struts not only retained their initial position and viability, but also proliferated up to 7 days after being dispensed. Yu et al. developed a PCL/fetal cartilage-derived progenitor cells-laden alginate bipartite hybrid scaffold where they printed the PCL/cell-laden alginate hybrid scaffold onto a PCL membrane in a vertical direction [55]. They also designed and developed a polydimethylsiloxane coculture system for osteochondral tissue (PCSOT). The PCL/alginate bipartite scaffold was successfully fabricated, importantly, the PCSOT system may be applicable as an in vitro platform for osteochondral tissue engineering as it allows fetal cartilage-derived progenitor cells in the hybrid construct to undergo differentiation into osteochondral tissues.

To mimic the complexity of natural ECM and enhance cellular efficiency, Pati et al., created a novel cell-laden

bio-ink made of decellularized extracellular matrix pre-gel and PCL [56]. According to their findings, the printed decellularized ECM constructs improved the viability of functional cells and 3D tissue growth. In other study, Kang et al. developed a specialized 3D bioprinter called the integrated tissue-organ printer [57]. Using this printer, they were able to print mandible bone, calvaria bone, cartilage and skeletal muscle using PCL, pluronic-F127 as sacrificial ink and gelatin, fibrinogen, hyaluronic acid (HA) and glycerol as cell-laden bioink. Daly et al. created a 3D bioprinted model of hypertrophic cartilage using gamma-irradiated alginate bioink incorporating Arg-Gly-Asp adhesion peptides, mechanically reinforced with PCL fibres, to mimick the anatomy of the vertebral body [58]. The compressive modulus were increased by ~350 fold which providing the stiffness needed to implant these immature cartilaginous rudiments into load-bearing areas.

Murphy et al. developed a novel solvent based extrusion 3D bioprinting process [59]. PCL was mixed with borate glass to make an extrudable paste, and human adipose stem cells (ASCs) were incorporated into matrigel and extruded through a different nozzle to make cell-laden scaffolds with pore sizes varying from 100 to 300 μm. The findings revealed a high potential for creating a bioactive and highly angiogenic three-dimensional matrix while increasing angiogenic ability with borate glass enhancement. In other study, hybrid construct of PCL/hydroxyapatite composite with stromal vascular fraction from adipose tissues derived cells-laden hydrogel bioink (methacrylated hyaluronic acid (HaMa) , gelatin methacrylamide (GelMa) and hyaluronic acid) were fabricated [60]. The hybrid construct then was maintained in normoxic and hypoxic conditions. The results showed hybrid construct maintained at short-term hypoxic treatment promotes the prevascularization of bioprinted bone constructs in vitro to achieve rapid anastomosis in vivo and enhances bone repair.

Antibacterial drug, doxycycline, have been loaded into mesoporous bioactive glass and combine with PCL to form composite material [61]. This composite then were used together with cell-laden (bone morphogenetic protein-2 (BMP2) transfected mouse mesenchymal stem cells (C3H10T1/2)) GelMA, HAMA, and the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) to form hybrid construct by extrusion printing. In vivo and in vitro experiments confirmed that the scaffold could actively secrete BMP2 to significantly promote osteoblast differentiation and induce ectopic bone formation while inhibiting bacterial infection. Shanjani et al. successfully developed a novel hybrid printing system which combining digital light processing-based stereolithography (DLP-SLA) and extrusion [62]. The printing process consist of melting and depositing PCL layer-by-layer using extrusion module then human umbilical vein endothelial cells-laden poly-ethylene glycol diacrylate was printed using DLP-SLA module. The printed hybrid construct has high compressive mechanical stiffness and 90% cells viability. Table 2 summarizes the previous study on 3D bioprinted polycaprolactone bone scaffold.

4. CONCLUSION

3D bioprinting has gained more attention and interest in recent years to fabricate human cell based tissue models and biological products, especially in bone tissue engineering applications. Combination of thermoplastic polymer and hydrogels are usually used to fabricate 3D hybrid construct for bone scaffolds. Despite all the benefits of 3D bioprinting, creating a tissue construct or artificial organ with complex microarchitecture and completely biological functions remains a challenge. As a result, further attention should be devoted to the development of high-performance bioinks and high-resolution bioprinters.

Table 2. Previous study on 3D bioprinted polycaprolactone bone scaffolds.

Method	Materials/Hydrogels	Type of Cells	Ref.
Extrusion - dual extruder	- Sodium Alginate	- Chondrocyte cells (C20A4 cells) - Human osteoblast cell line (MG63)	[53]
Extrusion - dual extruder	- Alginate	- Chondrocytes cells (isolated from human nasal septum cartilage) - Human adipose stem cells	[54]
Extrusion - dual extruder	- Decellularized extracellular matrix pre-gel (dECM)	- Human turbinate mesenchymal stromal cells	[56]
Hybrid printing system - combining digital light processing-based stereolithography (DLP-SLA) and extrusion	- Poly-ethylene glycol diacrylate (PEGDA)	- Human umbilical vein endothelial cells	[62]
Extrusion - triple extruder	- Composite hydrogel from gelatin, fibrinogen, hyaluronic acid (HA) and glycerol - Pluronic F-127 as sacrificial ink	- Human amniotic fluid-derived stem cells	[57]
Extrusion - dual extruder	- Gamma irradiated alginate incorporating Arg-Gly-Asp (RGD) specific adhesion peptides (RGD-γ alginate) - Poly (ethylene glycol) methacrylate (PEGMA) - gelatin methacrylamide (GelMA)	- Bone marrow derived mesenchymal stem cells	[58]
Extrusion - dual extruder	- PCL)/bioactive borate glass composite - Matrigel	- Human adipose stem cells	[59]
Extrusion - dual extruder	- PCL/hydroxyapatite composite - Methacrylated HA (HaMa) GelMa and HA	- Stromal vascular fraction from adipose tissues derived cells	[60]
Extrusion - dual extruder	- RGD-γ-irradiated alginate and nano-hydroxyapatite- plasmid DNA complexed	- Bone marrow derived mesenchymal stem cells	[63]
Extrusion - dual extruder	- Alginate	- Fetal cartilage-derived progenitor cells	[55]
Extrusion - dual extruder	- PCL/doxycycline loaded mesoporous bioactive glass composite - GelMA, HAMA, and the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP)	- Bone morphogenetic protein-2 (BMP2) transfected mouse mesenchymal stem cells (C3H10T1/2)	[61]
Extrusion - dual extruder	- GelMa	- Dental pulp stem cells	[64]
Extrusion - dual extruder	- Alginate and gelatin	- Immortalized mesenchymal stem cells (iMSCs)	[65]
Extrusion - dual extruder	- Composite hydrogel - fibrinogen, type A gelatin, hyaluronic acid and glycerol	- Human mesenchymal stem/stromal cells (hMSCs)	[66]
Extrusion - dual extruder	- Gelatin and alginate	- Autologous bone and bone marrow-derived mesenchymal stem cell (BMSC)	[67]

ACKNOWLEDGEMENT
This publication is supported by Universiti Teknologi Malaysia and the Ministry of Higher Education, Malaysia, under the Fundamental Research Grant Scheme (Grant no.: FRGS/1/2023/STG05/UTM/02/8).

REFERENCES

[1] Busch, A., Wegner, A., Haversath, M., & Jäger, M. (2021). Bone substitutes in orthopaedic surgery: current status and future perspectives. *Zeitschrift für Orthopädie und Unfallchirurgie*, *159*(03), 304-313.

[2] Vacanti, J. P., & Langer, R. (1999). Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. *The lancet*, *354*, S32-S34.

[3] Crane, G. M., Ishaug, S. L., & Mikos, A. G. (1995). Bone tissue engineering. *Nature Medicine*, *1*(12), 1322-1324.

[4] Varghese, M. G., Thomas, A., Rupesh, S., Sameer, K. M., Joseph, D., & Thomas, N. G. (2024). Fabrication Techniques for Scaffolds Applied in Regenerative Medicine. In *Novel Biomaterials for Tissue Engineering*. IntechOpen.

[5] Choi, S. W., Zhang, Y., & Xia, Y. (2010). Three-dimensional scaffolds for tissue engineering: the importance of uniformity in pore size and structure. *Langmuir*, *26*(24), 19001-19006.

[6] Bobbert, F. S. L., & Zadpoor, A. A. (2017). Effects of bone substitute architecture and surface properties on cell response, angiogenesis, and structure of new bone. *Journal of Materials Chemistry B*, *5*(31), 6175-6192.

[7] Blaudez, F., Vaquette, C., & Ivanovski, S. (2022). Cell Seeding on 3D Scaffolds for Tissue Engineering and Disease Modeling Applications. In *Oral Biology: Molecular Techniques and Applications* (pp. 473-483). New York, NY: Springer US.

[8] Mironov, V., Visconti, R. P., Kasyanov, V., Forgacs, G., Drake, C. J., & Markwald, R. R. (2009). Organ printing: tissue spheroids as building blocks. *Biomaterials*, *30*(12), 2164-2174.

[9] Atala, A., & Yoo, J. J. (2015). *Essentials of 3D biofabrication and translation*. Academic Press, 1.

[10] Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs.*Nature biotechnology*, *32*(8), 773-785.

[11] Bracci, R., Maccaroni, E., & Cascinu, S. (2013). Transient sunitinib resistance in gastrointestinal stromal tumors. *New England Journal of Medicine*, *368*(21), 2042-2043.

[12] Mandrycky, C., Wang, Z., Kim, K., & Kim, D. H. (2016). 3D bioprinting for engineering complex tissues. *Biotechnology advances*, *34*(4), 422-434.

[13] Mironov, V., Reis, N., & Derby, B. (2006). Bioprinting: a beginning. *Tissue engineering*, *12*(4), 631-634.

[14] Dababneh, A. B., & Ozbolat, I. T. (2014). Bioprinting technology: a current state-of-the-art review. *Journal of Manufacturing Science and Engineering*,*136*(6), 061016.

[15] Khatiwala, C., Law, R., Shepherd, B., Dorfman, S., & Csete, M. (2012). 3D cell bioprinting for regenerative medicine research and therapies. *Gene Therapy and Regulation*, *7*(01), 1230004.

[16] Khalil, S., & Sun, W. (2007). Biopolymer deposition for freeform fabrication of hydrogel tissue constructs. *Materials Science and Engineering: C*, *27*(3),469-478.

[17] Chang, C. C., Boland, E. D., Williams, S. K., & Hoying, J. B. (2011). Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *98*(1), 160-170.

[18] Cohen, D. L., Malone, E., Lipson, H. O. D., & Bonassar, L. J. (2006). Direct freeform fabrication of seeded hydrogels in arbitrary geometries. *Tissue engineering*, *12*(5), 1325-1335.

[19] Visser, J., Peters, B., Burger, T. J., Boomstra, J., Dhert, W. J., Melchels, F. P., & Malda, J. (2013). Biofabrication of multi-material anatomically shaped tissue constructs. *Biofabrication*, *5*(3), 035007.

[20] Cui, X., Boland, T., D D'Lima, D., & K Lotz, M. (2012). Thermal inkjet printing in tissue engineering and regenerative medicine. *Recent patents on drug delivery & formulation*, *6*(2), 149-155.

[21] Xu, T., Kincaid, H., Atala, A., & Yoo, J. J. (2008). High-throughput production of single-cell microparticles using an inkjet printing technology. *Journal of Manufacturing Science and Engineering*, *130*(2), 021017.

[22] Bose, S., Roy, M., & Bandyopadhyay, A. (2012). Recent advances in bone tissue engineering scaffolds. *Trends in biotechnology*, *30*(10), 546-554.

[23] Percival, K. M., Paul, V., & Husseini, G. A. (2024). Recent advancements in bone tissue engineering: integrating smart scaffold technologies and bio-responsive systems for enhanced regeneration. *International Journal of Molecular Sciences*, *25*(11), 6012.

[24] Todd, E. A., Mirsky, N. A., Silva, B. L. G., Shinde, A. R., Arakelians, A. R., Nayak, V. V., arcantonio, R.A.C., Gupta, N., Witek, L. & Coelho, P. G. (2024). Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions. *Journal of Functional Biomaterials*, *15*(10), 280.

[25] Williams, D. F. (2008). On the mechanisms of biocompatibility. *Biomaterials*, *29*(20), 2941-2953.

[26] Rouwkema, J., Rivron, N. C., & van Blitterswijk, C. A. (2008). Vascularization in tissue engineering. *Trends in biotechnology*, *26*(8), 434-441.

[27] Murphy, C. M., Haugh, M. G., & O'Brien, F. J. (2010). The effect of mean pore size on cell attachment, proliferation and migration in collagen– glycosaminoglycan scaffolds for bone tissue engineering. *Biomaterials*, *31*(3), 461-466.

[28] Tang, D., Tare, R. S., Yang, L. Y., Williams, D. F., Ou, K. L., & Oreffo, R. O. (2016). Biofabrication of bone tissue: approaches, challenges and translation for bone regeneration. *Biomaterials*, *83*, 363-382.

[29] Orciani, M., Fini, M., Di Primio, R., & Mattioli-Belmonte, M. (2017). Biofabrication and bone tissue regeneration: cell source, approaches, and challenges. *Frontiers in Bioengineering and Biotechnology*, *5*, 17.

[30] El Tamer, M. K., & Reis, R. L. (2009). Progenitor and stem cells for bone and cartilage regeneration. *Journal of tissue engineering and regenerative medicine*, *3*(5), 327-337.

[31] Szpalski, C., Barbaro, M., Sagebin, F., & Warren, S. M. (2012). Bone tissue engineering: current

strategies and techniques—part II: cell types. *Tissue Engineering Part B: Reviews*, *18*(4), 258-269.

[32] Wang, F., Yu, M., Yan, X., Wen, Y., Zeng, Q., Yue, W., Yang, P. and Pei, X., (2011). Gingiva-derived mesenchymal stem cell-mediated therapeutic approach for bone tissue regeneration. *Stem cells and development*, *20*(12), 2093-2102.

[33] Zhu, W., & Liang, M. (2015). Periodontal ligament stem cells: current status, concerns, and future prospects. *Stem cells international*, *2015*.

[34] Orciani, M., Mariggiò, M. A., Morabito, C., Di Benedetto, G., & Di Primio, R. (2010). Functional characterization of calcium-signaling pathways of human skin-derived mesenchymal stem cells. *Skin pharmacology and physiology*, *23*(3), 124-132.

[35] Natta, F. J. V., Hill, J. W., & Carothers, W. H. (1934). Studies of polymerization and ring formation. XXIII. 1 ε-Caprolactone and its polymers. *Journal of the American Chemical Society*, *56*(2), 455-457.

[36] Ramírez-Ruiz, F., Núñez-Tapia, I., Piña-Barba, M. C., Alvarez-Pérez, M. A., Guarino, V., & Serrano-Bello, J. (2025). Polycaprolactone for Hard Tissue Regeneration: Scaffold Design and In Vivo Implications. *Bioengineering*, *12*(1), 46.

[37] Pitt, C. G. (1990). Poly-ε-caprolactone and its copolymers. *Drugs and the pharmaceutical sciences*, *45*, 71-120.

[38] Darwis, D., Mitomo, H., & Yoshii, F. (1999). Degradability of radiation crosslinked PCL in the supercooled state under various environments. *Polymer degradation and stability*, *65*(2), 279-285.

[39] S. A. Zulkefli, N. S. Ismail, M. I. Abd Samad, et al., Development and analysis of 3D-printed knee orthosis for post-anterior cruciate ligament injuries rehabilitation. *Proc. Int. Exch. Innov. Conf. Eng. Sci. (IEICES)*, vol. 10, pp. 583 – 589, 2024.

[40] Williams, J.M., Adewunmi, A., Schek, R.M., Flanagan, C.L., Krebsbach, P.H., Feinberg, S.E., Hollister, S.J. and Das, S., (2005). Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials*, *26*(23), 4817-4827.

[41] Shor, L., Güçeri, S., Chang, R., Gordon, J., Kang, Q., Hartsock, L., An, Y. and Sun, W., (2009). Precision extruding deposition (PED) fabrication of polycaprolactone (PCL) scaffolds for bone tissue engineering. *Biofabrication*, *1*(1), 015003.

[42] Stratton, S., Shelke, N. B., Hoshino, K., Rudraiah, S., & Kumbar, S. G. (2016). Bioactive polymeric scaffolds for tissue engineering. *Bioactive materials*, *1*(2), 93-108.

[43] Hajiali, F., Tajbakhsh, S., & Shojaei, A. (2018). Fabrication and properties of polycaprolactone composites containing calcium phosphate-based ceramics and bioactive glasses in bone tissue engineering: a review. *Polymer reviews*, *58*(1), 164-207.

[44] Wu, F., Liu, C., O'Neill, B., Wei, J., & Ngothai, Y. (2012). Fabrication and properties of porous scaffold of magnesium phosphate/polycaprolactone biocomposite for bone tissue engineering. *Applied Surface Science*, *258*(19), 7589-7595.

[45] Diba, M., Kharaziha, M., Fathi, M. H., Gholipourmalekabadi, M., & Samadikuchaksaraei, A. (2012). Preparation and characterization of polycaprolactone/forsterite nanocomposite porous scaffolds designed for bone tissue regeneration. *Composites Science and Technology*, *72*(6), 716-723.

[46] Yao, Q., Cosme, J. G., Xu, T., Miszuk, J. M., Picciani, P. H., Fong, H., & Sun, H. (2017). Three dimensional electrospun PCL/PLA blend nanofibrous scaffolds with significantly improved stem cells osteogenic differentiation and cranial bone formation. *Biomaterials*, *115*, 115-127.

[47] Wang, Y., Liu, L., & Guo, S. (2010). Characterization of biodegradable and cytocompatible nano-hydroxyapatite/polycaprolactone porous scaffolds in degradation in vitro. *Polymer Degradation and Stability*, *95*(2), 207-213.

[48] Bao, T. Q., Franco, R. A., & Lee, B. T. (2012). Preparation and characterization of a novel 3D scaffold from poly (Ɛ-caprolactone)/biphasic calcium phosphate hybrid composite microspheres adhesion. *Biochemical engineering journal*, *64*, 76-83.

[49] Gerçek, I. Ş. I. L., Tığlı, R. S., & Gümüşderelioğlu, M. (2008). A novel scaffold based on formation and agglomeration of PCL microbeads by freeze-drying. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, *86*(4), 1012-1022.

[50] Thuaksuban, N., Nuntanaranont, T., Pattanachot, W., Suttapreyasri, S., & Cheung, L. K. (2011). Biodegradable polycaprolactone-chitosan three-dimensional scaffolds fabricated by melt stretching and multilayer deposition for bone tissue engineering: assessment of the physical properties and cellular response. *Biomedical Materials*, *6*(1), 015009.

[51] Grandi, C., Di Liddo, R., Paganin, P., Lora, S., Dalzoppo, D., Feltrin, G., Giraudo, C., Tommasini, M., Conconi, M.T. and Parnigotto, P.P., (2011). Porous alginate/poly (ε-caprolactone) scaffolds: preparation, characterization and in vitro biological activity. *International journal of molecular medicine*, *27*(3), 455-467.

[52] A. M. Ab Rashid, A. H. D. Mukhainizam, H. Rashid, et al., Enhancing 3-D printed bed-resting ankle-foot orthosis design through topology optimization. *Proc. Int. Exch. Innov. Conf. Eng. Sci. (IEICES)*, vol. 10, pp. 142 – 147, 2024.

[53] Schuurman, W., Khristov, V., Pot, M. W., van Weeren, P. R., Dhert, W. J., & Malda, J. (2011). Bioprinting of hybrid tissue constructs with tailorable mechanical properties. *Biofabrication*, *3*(2), 021001.

[54] Shim, J. H., Lee, J. S., Kim, J. Y., & Cho, D. W. (2012). Bioprinting of a mechanically enhanced three-dimensional dual cell-laden construct for osteochondral tissue engineering using a multi-head tissue/organ building system. *Journal of Micromechanics and Microengineering*, *22*(8), 085014.

[55] Yu, J., Lee, S., Choi, S., Kim, K.K., Ryu, B., Kim, C., Jung, C.R., Min, B.H., Xin, Y.Z., Park, S.A. and

Kim, W., (2020). Fabrication of a polycaprolactone/alginate bipartite hybrid scaffold for osteochondral tissue using a three-dimensional bioprinting system. *Polymers*, *12*(10), 2203.

[56] Pati, F., Jang, J., Ha, D.H., Kim, S.W., Rhie, J.W., Shim, J.H., Kim, D.H. and Cho, D.W., (2014). Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature communications*, *5*(1), 1-11.

[57] Kang, H. W., Lee, S. J., Ko, I. K., Kengla, C., Yoo, J. J., & Atala, A. (2016). A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature biotechnology*, *34*(3), 312-319.

[58] Daly, A. C., Cunniffe, G. M., Sathy, B. N., Jeon, O., Alsberg, E., & Kelly, D. J. (2016). 3D bioprinting of developmentally inspired templates for whole bone organ engineering. *Advanced healthcare materials*, *5*(18), 2353-2362.

[59] Murphy, C., Kolan, K., Li, W., Semon, J., Day, D., & Leu, M. (2017). 3D bioprinting of stem cells and polymer/bioactive glass composite scaffolds for bone tissue engineering. *International Journal of Bioprinting*, *3*(1).

[60] Kuss, M. A., Harms, R., Wu, S., Wang, Y., Untrauer, J. B., Carlson, M. A., & Duan, B. (2017). Short-term hypoxic preconditioning promotes prevascularization in 3D bioprinted bone constructs with stromal vascular fraction derived cells. *RSC advances*, *7*(47), 29312-29320.

[61] Wang, M., Li, H., Yang, Y., Yuan, K., Zhou, F., Liu, H., Zhou, Q., Yang, S. and Tang, T., (2021). A 3D-bioprinted scaffold with doxycycline-controlled BMP2-expressing cells for inducing bone regeneration and inhibiting bacterial infection. *Bioactive materials*, *6*(5), 1318-1329.

[62] Shanjani, Y., Pan, C. C., Elomaa, L., & Yang, Y. (2015). A novel bioprinting method and system for forming hybrid tissue engineering constructs. *Biofabrication*, *7*(4), 045008.

[63] Cunniffe, G. M., Gonzalez-Fernandez, T., Daly, A., Sathy, B. N., Jeon, O., Alsberg, E., & Kelly, D. J. (2017). Three-dimensional bioprinting of polycaprolactone reinforced gene activated bioinks for bone tissue engineering. *Tissue Engineering Part A*, *23*(17-18), 891-90.

[64] Buyuksungur, S., Hasirci, V., & Hasirci, N. (2021). 3D printed hybrid bone constructs of PCL and dental pulp stem cells loaded GelMA. *Journal of Biomedical Materials Research Part A*, *109*(12), 2425-2437.

[65] Koch, F., Thaden, O., Conrad, S., Tröndle, K., Finkenzeller, G., Zengerle, R., Kartmann, S., Zimmermann, S. & Koltay, P. (2022). Mechanical properties of polycaprolactone (PCL) scaffolds for hybrid 3D-bioprinting with alginate-gelatin hydrogel. *journal of the mechanical behavior of biomedical materials*, *130*, 105219.

[66] Pitacco, P., Sadowska, J. M., O'Brien, F. J., & Kelly, D. J. (2023). 3D bioprinting of cartilaginous templates for large bone defect healing. *Acta Biomaterialia*, *156*, 61-74.

[67] Huan, Y., Zhou, D., Wu, X., He, X., Chen, H., Li, S., Jia, B., Dou, Y., Fei, X., Wu, S. & wei J. (2023). 3D bioprinted autologous bone particle scaffolds for cranioplasty promote bone regeneration with both implanted and native BMSCs. *Biofabrication*, *15*(2), 025016.