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# 3D Bioprinting Strategies for Melatonin-Loaded Polymers in Bone Tissue Engineering

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**Bone pathologies are still among the most challenging issues for orthopedics. Over the past decade, different methods are developed for bone repair. In addition to advanced surgical and graft techniques, polymer-based biomaterials, bioactive glass, chitosan, hydrogels, nanoparticles, and cell-derived exosomes are used for bone healing strategies. Owing to their variation and promising advantages, most of these methods are not translated into clinical practice. Three dimensonal (3D) bioprinting is an additive manufacturing technique that has become a next-generation biomaterial technique adapted for anatomic modeling, artificial tissue or organs, grafting, and bridging tissues. Polymer-based biomaterials are mostly used for the controlled release of various drugs, therapeutic agents, mesenchymal stem cells, ions, and growth factors. Polymers are now among the most preferable materials for 3D bioprinting. Melatonin is a well-known antioxidant with many osteoinductive properties and is one of the key hormones in the brain–bone axis. 3D bioprinted melatonin-loaded polymers with unique lipophilic, anti-inflammatory, antioxidant, and osteoinductive properties for filling large bone gaps following fractures or congenital bone deformities may be developed in the future. This study summarized the benefits of 3D bioprinted and polymeric materials integrated with melatonin for sustained release in bone regeneration approaches.**

## 1. Introduction

Melatonin (Mel) has the chemical formula "N-acetyl-5-hydroxytryptamine", which is released from the pineal gland during the dark phase. Tryptophan- and serotonin-mediated enzymatic activities facilitate the synthesis of Mel. Owing to its lipophilicity, Mel can cross all cell membranes via the MT1 and MT2 receptors and plays many beneficial roles in pathophysiological processes, including cardiovascular, metabolic, musculoskeletal, and visceral mechanisms related to healing. It is also known as an immunomodulatory, anti-inflammatory, antioxidant, and free-radical scavenger. Therefore, Mel has been suggested in numerous studies and has repeatedly been proven to be one of the essential molecules for humans.

Mel is proposed as a regulator component of the "brain–bone axis" theory, which suggests that the central nervous system has regulatory effects on bone remodeling, as represented in **Figure 1**.[1] Mel has direct positive effects on osteoblast differentiation and proliferation. It increases bone mass by increasing alkaline phosphatase (ALP) and collagen type 1 (COL I) levels and facilitates mineralization. As a result, Mel has osteoinductive effects on bone, contributing to decreased ROS and RANKL levels, preventing bone resorption, and inhibiting osteoclastogenesis.

In addition, the migration and proliferation of mesenchymal stem cells (MSCs) are essential for bone regeneration.[2] Mel stimulates the chondrogenic and osteogenic differentiation of MSCs and promotes new bone formation. It is also a well-known vascular endothelial growth factor (VEGF) activator. Following an injury, enhanced blood flow to the fracture site is needed. Previous studies have revealed that Mel promotes angiogenesis during bone injury repair through increasing VEGF levels and provides antioxidant benefits against ischemic damage.[3] Recent in vitro studies have demonstrated that Mel stimulates the osteogenic gene expression of runt-related transcription factor 2 (Runx2), osteocalcin (OCN), and bone morphogenic protein (BMP) by providing MSCs and stimulating bone mineralization through the BMP, ERK, Wnt, and PKA/PKC signaling pathways.[4]

Biodegradable polymers incorporated with Mel have been shown to have various effects on bone regeneration. The sustained and controlled release of these materials continues to impact the fracture site to promote robust regeneration. Mel has

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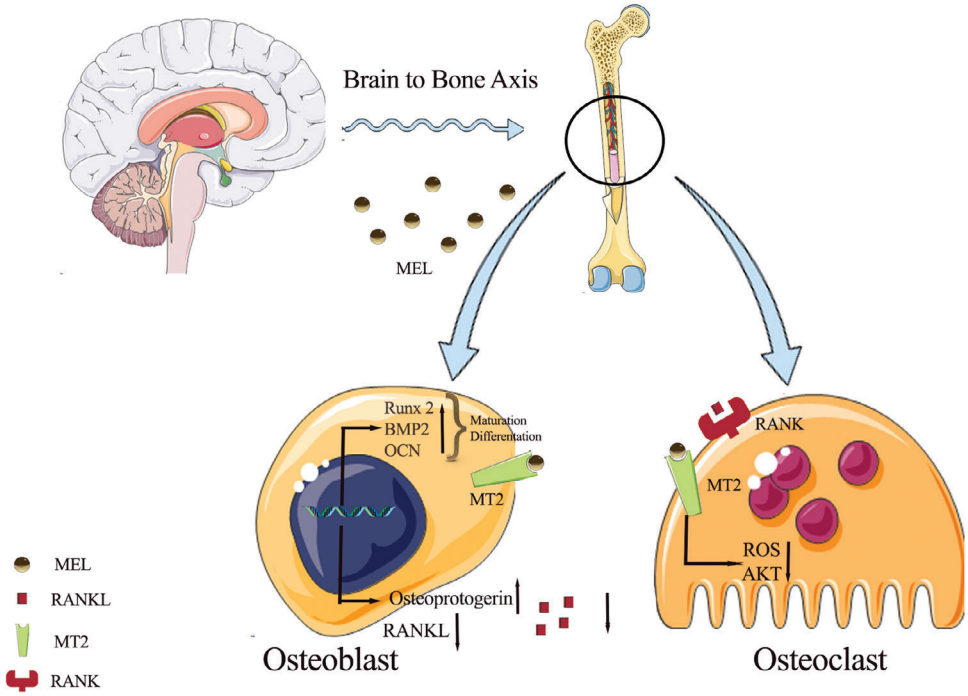


**Figure 1.** Contribution of Mel to the brain-to-bone axis.

been used along with various polymers in the field of bone tissue engineering experimental studies, and it has been shown to enhance bone healing.[5] However, large gaps in bone loss still require effective methods to accelerate healing. At this point, 3D bioprinting seems to be a viable alternative to the present gold standard methods, which are still in use in clinical practice. 3D bioprinting offers personalized treatment for such pathologies in bone tissue.[6]

### 1.1. Biodegradable Polymers

Over the past thirty years, notable developments have been achieved in biodegradable polymers for medical applications. The first polymer devices were surgical sutures, which emerged in the 1960s. Since then, applicable biomaterials have led to tremendous fluctuations in various medical fields, such as the sustained delivery of drugs, agents, MSCs, and regenerative medicines. Extensive research has yielded promising biodegradable, biocompatible, bioresorbable polymer-based products, which have been integrated into clinical trials in an accelerated manner. The simple, flexible design capacity of polymers for the required treatment and pathology has gained attention because of their easily applicable properties. Polymer membranes facilitate the carriage and controlled delivery of the loaded molecules not systemically but primarily to the origin of the disease.

A better choice for Mel entrapment with polymeric materials can improve its half-life and antioxidant, anti-inflammatory, and regenerative properties. This mostly depends on enhancing bioavailability with lower degradation and providing long-term effects. In bone tissue engineering, bone healing requires a long process and robust mechanical bearing. Thus, the preferred polymeric material should provide lower biodegradation and protect the molecular structure of Mel to ensure a long half-life. Furthermore, to design a better polymeric tissue structure, multiple biocompatible and biodegradable synthetic and natural polymers have been used to create an in vivo mimicking microenvironment.[7]

Polymers utilized as medical biodegradable carriers are generally classified into two subheadings—i.e., natural and synthetic polymers. As illustrated in **Figure 2**, polymer bends are usually prepared via various techniques and are applied to bone-related pathologies to stimulate bone regeneration. Naturally, derived polymers provide excellent biocompatibility with a similar structure as the extracellular matrix (ECM) and facilitate cell adhesion and functionality. These polymers contain high protein and polysaccharide contents and attract tissue engineers because of their chemical versatility and biomechanical properties. The most common types of natural polymers used in bone tissue engineering are hydrogels, collagen, silk fibroin, chitosan, gelatin, and alginate substances, which exhibit improved cell proliferation and differentiation.[8] Natural polymers are mostly preferred in ocular, skin, intestinal, and brain tissues because of their rapid biodegradability, low cost, nontoxicity, bioadhesive, and eco-friendly properties. Natural polymers absorb water, swell, and retain water up to their dry weight. Hence, insoluble molecules can be easily entrapped and released from natural polymeric blends. Owing to the mentioned properties of natural polymers, their mechanical strength, stability, and biodegradation are low; they have uncontrollable degradation rates and exhibit antigenicity.

Additionally, there are multiple concerns in the development of natural polymeric blends in terms of pore size, surface activity, low versatility, elevated contamination risk, instability, and

 

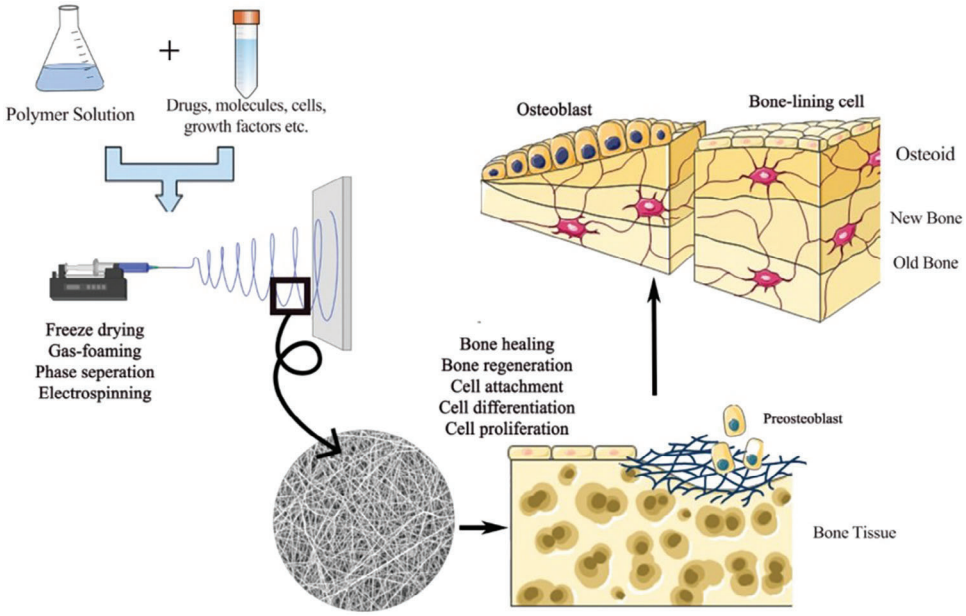


**Figure 2.** Preparation of polymer blends.

structural design difficulties.[9] To promote mechanical properties, an optimum porous structure for osteoconductivity and surface reactions, and the desired designation, natural polymeric matrices should be blended with synthetic polymeric composites. Among them, synthetic polymeric blends incorporating collagen chitosan and alginate hydrogels have been used as alternative options in bone tissue engineering.[10]

Compared with natural polymers, synthetic polymers represent more controllable conditions and can be designed with various properties, such as biodegradation period and rate, biomechanical strength levels, and convenience to the microstructure of the related tissue. Additional advantages of synthetic polymeric materials include their low cost, long-term storage, and easy serial manufacturing, as represented in **Figure 3**.[11] In the field of bone tissue engineering, the synthetic polymers PLA (poly-lactic acid), PCL (polycaprolactone), and PLGA (lactic-co-glycolic acid) are the most widely fabricated and utilized polymer structures for bone healing. The biodegradability of these synthetic polymers results in nontoxic performance and metabolized leftovers. Owing to their biomechanical benefits, the osteoconductivity and osteoinductivity of synthetic polymers are limited. Therefore, to enhance the performance of synthetic polymers, they must be improved by seeding cells; coating them with peptides, proteins, and growth factors; or blending them with hydroxyapatite crystals. The use of polymers in bone tissue engineering for bone healing is relevant, but as suggested, improvements in osteoinductivity and osteoconductivity are essential. Thus, studies in this field focus on carriers loaded with therapeutic agents or molecules.[12] Mel-entrapped synthetic polymeric composites have been widely used in bone tissue engineering studies. The results revealed that the encapsulation efficiency of Mel was 70–80% greater than that of natural polymers.[13] However, aspects such as the choice of optimal synthetic polymeric material, Mel dosage, drug release timing, and interaction with additive molecules and tissue must be further evaluated.

Taken together, in bone tissue engineering, the fabricated scaffold should exhibit better encapsulation efficiency, porosity, and degradation rate for the required healing process, biocompatibility, and mechanical strength to gain human load-bearing capacity. The information above suggests that synthetic polymeric blends might have a better effect on bone diseases. The choice of polymeric matrix significantly influences the activity of Mel in bone regeneration.

### 1.2. Bone Tissue Engineering and 3D Bioprinting

Bone tissue is a tough tissue, and large gaps in bone fractures require fixation with bone tissue-like objects or biomaterials. Autografts have long been known as the gold standard method for filling large gaps. However, autografting has many drawbacks, such as unexpected donor site reactions, inadequate graft material, inflammation, and long-term pain, which limit the use of autograft techniques in clinical applications.[14] Other graft materials, such as allografts and xenografts, are not always available or feasible because of immune reactions.[15] Biomaterial technology in the field of bone tissue engineering offers promising outcomes in terms of osteoinductivity, osteoconductivity, tunable biodegradability, and personalized architecture. Unlike the limitations of bone graft applications with autografts, allografts, or xenografts, artificial biomaterials enriched with multiple factors, such as seeded cells, growth factors, drugs, molecules, and hormones, result in enhanced regeneration and feasibility for any type of bone defect or loss. With the increasing trend of additive manufacturing, 3D-printed biomaterials have recently attracted substantial attention in bone tissue engineering. 3D bioprinting offers the best match for bone loss and large gaps to regenerate. This technology allows for the designation of the required architecture and the incorporation of essential additives according to tissue requirements. One of the main advantages of 3D

 

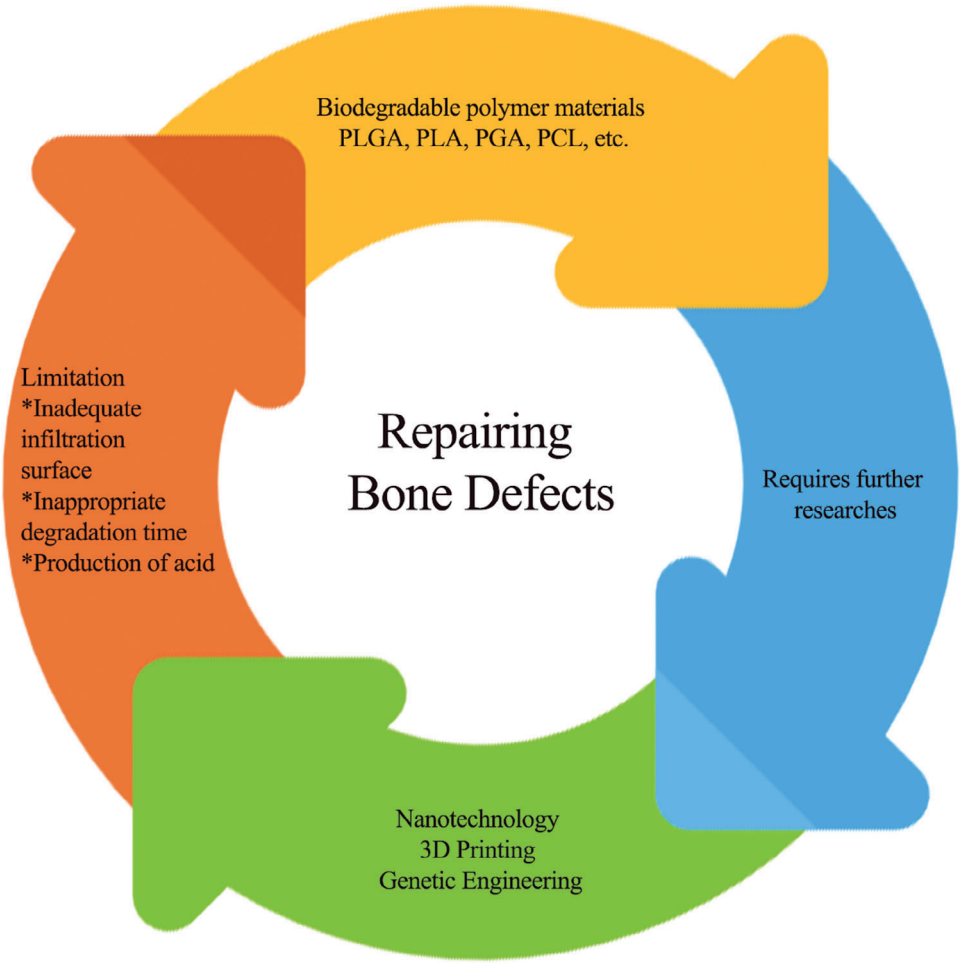


**Figure 3.** Approaches in synthetic polymer matrices for bone regeneration.

bioprinting in bone tissue engineering is the ability to fabricate interconnected porous artificial devices. Porosity is the main feature of a graft material that must have a proper diameter. The porous structure allows cells (osteoblasts or BMSCs) to adhere to and infiltrate the material surface. Studies have revealed that the 10–50 μm pore size of scaffolds facilitates osteoblast migration and proliferation.[16] In addition, owing to the lack of load bearing in tough tissues, 3D-bioprinted biomaterials provide the mechanical stability of the grafts developed for large bone gaps. The 3D bioprinting approach also incorporates cells, molecules, drugs, and various agents with natural or synthetic polymers to mimic the bone structure and microenvironment for bone regeneration.[17]

Bioinks for 3D bioprinting allow for the fabrication of composite scaffolds, while various additives are released from a scaffold and contribute to regeneration.[18] These bioinks should be concise, biocompatible, biodegradable, have osteointegration properties, and robust in terms of the mechanical strength of the scaffold to provide adequate load-bearing capacity. Additionally, natural bioinks, such as polymer solutions for bioprinting, have contributed to the development of the newest smart devices for bone regeneration. These types of scaffolds have also been tested for their cytotoxicity and thermal and mechanical strength.[19]

Furthermore, in vivo studies are needed to clarify the effects on bone regeneration in critical-sized defect models. To date, few studies have used Mel for 3D bioprinted biomaterials in different models. In this review, we summarize the scope of this incorporation.

In general, there are several challenges in translating 3D bioprinted composites to clinical applications that should be avoided and cannot be ignored. Tissue regeneration requires a variety of signaling pathways to be stimulated and genes to be expressed. The fabricated scaffold must improve regeneration through involvement and increased porosity for cell interactions.[20] From this point of view, loading multiple agents into the scaffold decreases the porosity, which may lower the mechanical strength of the composite material. Indeed, Mel entrapment may meet all tissue healing process requirements, as mentioned above, regarding the osteoinductivity of Mel itself. Indeed, a proper method for fabricating Mel-encapsulated scaffolds may enhance both the mechanical strength and regeneration efficiency of 3D bioprinted scaffolds. Moreover, Tabriz et al. (2023) reported that 3D-printed scaffolds may possess the ability for internal vascularization due

 

to the peripheral migration of new bone.[21] Mel can be proposed as a good angiogenesis stimulant and may be cell seeded, and Mel-loaded 3D bioprinted scaffolds may be an option for such a challenge.

Overall, 3D bioprinting appears to be a promising technology for bone tissue engineering with Mel-loaded blends, and clinical translation is attainable in the near future.

### 1.3. Mel-Loaded Biodegradable Polymers

Many different methods have been developed for bone regeneration for bone fractures and large bone loss. However, the post-surgery healing process takes too long, immobilizes the patient, and requires the use of numerous medications.[22] Generally, treatment methods are limited by systemic effects and dosage constraints of therapeutic agents. Thus, novel treatment methods have directed the controlled sustainable delivery of these agents to the primary target area. At that point, bone tissue engineering provides promising treatment options for the sustained release of various therapeutic agents, cells, ions, antibiotics, and growth factors for bone fracture healing. As a therapeutic agent, Mel is commonly used to relieve pain and improve postoperative sleep quality.[23] The combined application of Mel and bone tissue engineering biomaterials also has a certain value in bone tissue engineering. Since Mel is influenced by light exposure and rapidly degrades, encapsulation methods have gained importance because of their benefits. In addition, Mel has low solubility in water. The therapeutic potential of Mel depends on its long-term stability; therefore, studies have claimed that chitosan-based Mel-loaded biomaterials might be effective in uncovering its effects.

Mel-loaded chitosan microparticles enhanced the osteogenic differentiation and calcium deposition of preosteoblast cells and, in vitro, increased the expression of OCN, BMP, and COL1, which were also significantly upregulated.[24] A study revealed that a Mel-loaded alginate-chitosan/beta-tricalcium phosphate composite hydrogel facilitated periodontal healing and bone regeneration in a critical-sized furcation model. In this particular study, alginate and chitosan were blended with hydrogels to increase their biocompatibility and mechanical strength and increase their cell activities.[25] Mel and BMP-2 were embedded into chitosan/hydroxyapatite scaffolds, which inhibited the differentiation of RAW 264.7 cells into osteoclasts and decreased cathepsin K gene expression.[26] The scaffold showed slow biodegradability and biocompatibility. Chitosan-based scaffolds are appropriate for slow degradation, and they have relatively high levels of encapsulating efficiency for Mel. However, the use of chitosan-based scaffolds is limited because of their lower bioavailability and toxicity. The concentration-dependent cytotoxicity makes its common use difficult. Even though incorporating systems decreases the cytotoxicity of pure chitosan, it has become essential to look for safer materials.

The development and application of polymeric composites is a very recent method for bone tissue engineering, and it offers promising bone regeneration potential. Polymer-based scaffolds provide a proper microenvironment for robust mechanical strength, cell interactions, and long-term stability for degradation.[27] The preference for polymeric materials depends on porosity, surface charge, biodegradability, cytotoxicity, drug loading, and biocompatibility properties. Thus, polymeric materials offer promising application opportunities for tissue engineering. Among them, as mentioned above, PCL is an inexpensive and stable polymeric material.[28]

Moreover, PCL seems to be more suitable for application in tough tissues. In a recent study, a Mel-encapsulated PCL composite markedly increased bone volume and promoted remodeling in vitro and in vivo. In this study, the PCL microstructure, surface energy, and electrospray method were used, and the encapsulation efficiency of Mel was 73%. These findings suggest that PCL-based Mel-encapsulated graft materials have increased potential for bone regeneration.[29]

In a tendon-to-bone healing animal model study, Mel-loaded aligned PCL scaffolds were fabricated and implanted to utilize the prochondrogenic and immunomodulatory effects of sustained Mel release. Electrospun fibrous PCL membranes incorporating Mel promoted chondrogenic differentiation in vitro. In vivo, the sustained release of Mel from PCL scaffolds inhibited macrophage infiltration, which is the main challenge of the tendon-to-bone healing process. Furthermore, it was reported that the scaffold enhanced collagen maturation and improved biochemical strength.[30] In summary, owing to their exceptional availability and easy application, PCL polymers have low bioactivity and surface energy with high hydrophobicity, negatively affect cell affinity, and exhibit inadequate tissue regeneration.[31] Taken together, Mel has low solubility in water, and this composite has improved with further techniques or the incorporation of regenerative molecules.

PLGA is the most preferable polymeric material for tissue engineering applications because of its enhanced biocompatibility, biodegradability, and ability to mimic the ECM of bone tissue appropriately. In addition to their osteoinductive and antioxidant and anti-inflammatory properties, Mel-incorporated PLGA biomaterials appear to be better blends for bone regeneration. A study was performed on the basis of the in vitro antioxidant interactions and cytotoxicity of Mel-based PLGA NPs and pure Mel over erythrocytes. The scaffold was fabricated via the emulsion–solvent evaporation method, which is low-cost and versatile and is a widely used technique to improve the water solubility of insoluble drugs. The encapsulating efficiency of Mel was higher at a rate of 41% with PLGA. Compared with pure Mel, Mel-loaded PLGA NPs showed no hemolysis and enhanced scavenging activity. This study indicated that PLGA might be a promising carrier for Mel in a range of pathological conditions.[32]

Similarly, in a cerebral ischemia–reperfusion model, Mel-loaded PLGA NPs were administered orally to rats, and their antioxidant activity was assessed. Prophylactic-administered Mel-loaded PLGA NPs exhibited better antioxidant activity than did Mel alone. This may depend on the slow and long-term degradation of Mel from PLGA NPs[33]. By utilizing a tibial bone defect model in rats, Wang et al. (2022) fabricated a Mel-loaded PLGA blend for type 1 diabetes mellitus (T1DM) mice. Mel/PLGA scaffolds were fabricated via the electrospinning method with 30 days of sustained release. Mel-loaded composite biomaterials exert dual effects and accelerate bone regeneration via the inhibition of ROS overproduction and promote cell proliferation by activating the BMP-4/WNT pathway[34]. Notably, Mel has been suggested

**Table 1.** Overview of Mel-loaded polymer composites.

| Mel Composites | Application | Structural Properties | Major Outcomes | References |
|---|---|---|---|---|
| Chitosan microparticles | • In vitro Preosteoblastic cells | • Slow degradability<br>• Encapsulation efficiency with a rate of 85–90% | • Enhanced osteogenic differentiation and calcium deposit of preosteoblast cells.<br>• Significantly upregulated the expression of OCN, BMP, and COL1. | [69] |
| Alginate-chitosan/beta-tricalcium phosphate composite hydrogel | • In vitro cytotoxicity<br>• In vivo critical-sized bilateral class II furcation defects | • Proliferative influence on BMMSCs at low concentrations up to 3 mg/ml<br>• Porosity value of ≈ 83.64 ± 1.2%<br>• Higher viscous elastic modulus | • Accelerated bone formation and advanced maturity.<br>• After 4 and 8 weeks, the scaffold showed a significant increase in cementum length concurrent weeks.<br>• The scaffold provided is similar to normal compact bone, with potent periodontal ligament attachment | [25] |
| Chitosan/hydroxyapatite scaffolds | In vitro RAW 264.7 cell line | • Slow biodegradability<br>• Good biocompatibility | • Inhibition of the differentiation of RAW 264.7 cells to osteoclasts and decreased cathepsin K gene expressions | [26] |
| PCL composite | • In vitro Primary human osteoblasts<br>• In vivo female Sprague Dawley rats | • Encapsulation efficiency of Mel was 73%<br>• Biocompatible, biodegradable | • Mel in PCL composite markedly increased bone volume and remodeling both in vitro and in vivo | [29] |
| PCL nanofibers | • In vitro human bone marrow-derived mesenchymal stem cells (hBMSCs)<br>• In vivo rat acute rotator cuff tear model | • Enhanced hydrophilicity<br>• Appropriate diameter size with 1.2- 0.1 μm | • Mel provided chondrogenic differentiation in vitro<br>• Scaffolds inhibit macrophage infiltration, which is the main challenge of the tendon-to-bone healing process. Furthermore, it was claimed that the scaffold enhanced collagen maturation and improved biochemical strength | [30] |
| PLGA NPs | • In vitro antioxidant interactions and cytotoxicity over erythrocytes | • Encapsulating efficiency of Mel was higher at a rate of 41% with PLGA | • No hemolysis and enhanced scavenging activity | [32] |
| PLGA NPs | In vivo cerebral ischemia–reperfusion | • Encapsulation efficiency was 69–74%<br>• Nanofibers were semispherical with a size range of 83 ± 7.7 nm<br>• Narrow size distribution and smooth surface morphology | • Better antioxidant activity | [33] |
| PLGA | • In vitro<br>• In vivo tibial bone defect in DM mice | • MT was fully encapsulated in the PLGA and released for 30 days | • Inhibition of ROS overproduction<br>• Promoted cell proliferation by activating the BMP-4/WNT pathway | [34] |

as a dose-reliable agent so that Mel can be used at long dose intervals with any toxicity[35]. The abovementioned applications are summarized in **Table 1**.

Most tissue engineering studies agree that Mel-incorporated polymeric materials exhibit enhanced effects on bone regeneration from various perspectives, as represented in **Figure 4**. Compared with other scaffolds, polymeric material-based Mel composites have functional significance for experimental interventions. Personalized decoration with Mel-loaded polymers might be used in the future for clinical interventions. Thus, additive manufacturing with 3D applications offers promising applications for bone tissue engineering in the field of polymeric-based Mel scaffolds.

### 1.4. PLGA Scaffolds

Poly(lactic-co-glycolic acid) (PLGA) is a synthetic bioresorbable and biodegradable polymer structure that mimics smart drug delivery systems in the field of bone tissue engineering. PLGA offers various advantages, including designation according to any request for the tissue and loading of any molecules, cells, agents, or growth factors. In addition, PLGA allows osteoblastic cell adhesion, proliferation, and differentiation and provides the required time profile for the degradation of loaded agents.[12] One of the widespread reasons for the use of PLGA in bone tissue engineering is that it mimics the extracellular matrix of bone tissue and supplies a microenvironment for osteoblast proliferation and

 

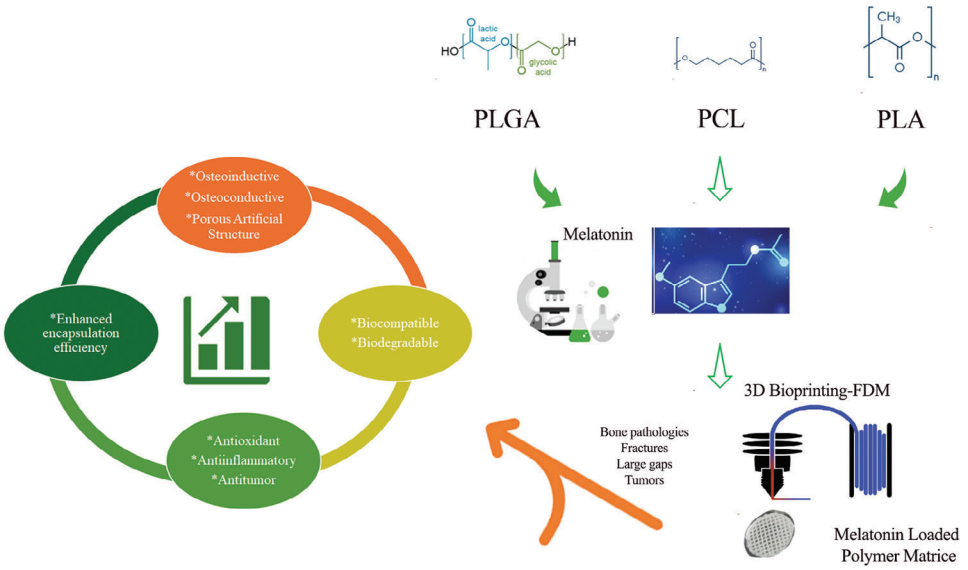


**Figure 4.** 3D bioprinting of Mel-encapsulated synthetic polymer matrices and the contribution of the scaffolds to bone regeneration.

differentiation. However, the mechanical strength of bone-like tough tissues is limited. In bone tissue engineering, the mechanical stress and load-bearing efficiency of the scaffolds are important for robust bone regeneration. In line with this purpose, previous studies have reported that PLGA contains two main components, lactic acid and glycolic acid monomers (LA:GA) and that the LA:GA ratio affects the mechanical properties of PLGA-based scaffolds. It has been reported that if the LA:GA ratio is 50/50, the biodegradability of the scaffolds might be rapid. The optimal ratios have been revealed to be 85/15 and 75/25 for bone tissue engineering applications with ≈ 8- or 16-week degradation periods, respectively, which is approximately proper for bone regeneration.[36]

Furthermore, these substances, due to their acidic nature, change the local pH and may lead to inflammation. Additionally, because PLGA alone is unable to provide mechanical strength, improved techniques should be applied to PLGA-based scaffolds. Mel has low solubility in aqueous solutions; therefore, PLGA may increase the hydrophilicity of the composite and enhance cell adhesion, proliferation differentiation, and all contributing effects of Mel. Thus, Mel has pH modulator effects and may reduce unexpected changes in pH after implantation.[37]

Recently, 3D bioprinted PLGA scaffolds have become a proper option for bone regeneration. Even so, 3D bioprinted PLGA scaffolds alone may not be able to meet all osteoinductive requirements. Large bone fractures mostly require grafting with biomaterials incorporated with cell seeding procedures, osteoinductive agents, facilitated osteoblastic activity, and inhibition of osteoclastogenesis, which may efficiently compensate for the lack of biocompatibility of fabricated scaffolds and can effectively address the lack of biocompatibility of scaffolds.[37] Recently, 3D bioprinting of PLGA scaffolds has been confirmed to have great potential for the development of artificial bone grafts with excellent mechanical properties. Multiple in vivo and in vitro studies have shown that PLGA scaffolds have promising effects on bone regeneration via angiogenesis, osteogenesis, and loading.

However, further research is still needed before its use in the clinic. While creating artificial structures and fabricating ideal porosities are relatively simple and have been shown to be effective, the printing process has several challenges. The printability of the preferred molecules or drugs and compounds, preparation for mass production, and speed and improvement of additive biological materials still suffer from low applicability.[38] Nevertheless, promising methods for bioprinting technologies and widely used techniques, such as fused deposition modeling (FDM), exist. FDM allows the creation of layer-by-layer materials by melting polymers and uses flexible dosages of molecules to form the final object. Through the FDM bioprinting technique, PLGA-based scaffolds can be fabricated with complex artificial structures and multiple geometries, offering customized treatment options. Owing to the thermal processing involved in FDM bioprinting, thermosensitive drugs may be negatively affected by printing. The risk of deterioration of drug stability may not be ignored. At this point, Mel-loaded scaffolds may have some drawbacks. A previous study indicated various thermal exposures to Mel at relatively high temperatures (60–90 °C) and reported that increased temperature accelerated the degradability of Mel. This may be avoided by PLGA encapsulation and should be further evaluated in vitro and in vivo.[39]

Chen et al. (2024) fabricated a multitasked biomaterial for treating diabetic bone disease with 3D bioprinted Mel-loaded PLGA nanoparticles and incorporated it with alginate and PCL/$\beta$-tricalcium phosphate via FDM. The in vitro (MC3T3-E and bEnd.3 cell lines) and in vivo effects of the scaffold were assessed. The Mel released from the composites was more sustainable. Additionally, the scaffold exhibited osteoinductivity, facilitated bone regeneration, and stimulated angiogenesis, promoting VEGF production via Nrf2/HO-1 signaling.[40] In this study, the sustained release of Mel from the scaffolds increased antioxidant activity by increasing SOD and CAT and inhibited ROS release at high plasma glucose levels. Mel also affects the expression of musculoaponeurotic fibrosarcoma oncogene ho-

 

molog (MAF) genes and activates the release of HO-1 to stimulate VEGF activation to increase angiogenesis following osteogenesis.

In a dual delivery system with BMP2 and Mel-loaded PLGA nanoparticles achieved by Jarrar et al. (2021), dual delivery enhanced ALP and Runx2 expression and mineralization in vitro, suggesting that this smart device might be a promising option for bone repair in clinical applications. Mel was used to prevent the drawbacks of BMP2 use, which may stimulate activation. Owing to the improved effects of the BMP2-loaded PLGA nanoparticles in that study, the addition of Mel minimized the side effects of BMP2 and enhanced the effects of low-dose BMP-2. Runx2 and ALP expression levels were found to be increased in the Mel-loaded BMP2/PLGA groups.[41] As a sustained release agent to the local area, Mel facilitates healing due to its excellent antioxidant and anti-inflammatory properties. In a recent study, a Mel-based PLGA-COLBP scaffold was applied to an osteoarthritis model. Scientists reported that Mel inhibited the TLR2/4-MyD88-NF$\kappa$B pathway and reduced ROS.

Furthermore, cartilage matrix metabolism was improved, and the progressive development of osteoarthritis was prevented.[42] One osteosarcoma study in the field of bone tissue engineering was performed to clarify the composite effect of Mel-loaded PLGA microparticles (PLGA-MPs). In their study, Altındal et al. (2019) aimed to investigate the anticarcinogenic effects of Mel through a sustained release method. Mel-loaded PLGA-MPs inhibited the growth of MG-63 cells and were noted to be a promising system for osteosarcoma chemotherapy via sustained Mel delivery. This study revealed that the incorporation of Mel with PLGA MPs resulted in a slower release than the incorporation of Mel blended with chitosan. Thus, it is clear that PLGA carriers offer long-term release of loaded agents.[43] An in vitro study on human mesenchymal stem cells (hSMCs) was conducted to evaluate the osteogenic effects of Mel-encapsulated PLGA microspheres, which exhibited good osteoinductivity and osteoconductivity.

Moreover, the composite increased the expression of ALP, osteopontin, osteocalcin, and Runx2. In this application, Mel-induced osteogenic differentiation and the PLGA scaffold generated a proper microenvironment for osteoblastic cells, suggesting that this incorporation could be used as a simulator of bone healing in vivo.[44] Another Mel-based composite scaffold area is generally studied in consideration of osteoporotic fracture healing. PLGA polymer nanocarriers offer large-interval loading molecules with unique structures. In osteoporotic fractures, improving osteoclastogenesis and osteoinductivity is the main objective. Therefore, a previous study by Liu et al. (2022) focused on improving bone strength and inhibiting osteoclastic activity in an osteoporotic fracture model. In this study, an ion and drug dual delivery system was developed and applied. For that purpose, a magnesium (Mg) alloy-based Mel-loaded PLGA polymer was fabricated. The results showed that while the Mg alloys restored the bone structure to be denser and stronger, Mel increased the expression levels of ALP, OCN, and OPG. It has been suggested that an increase in OPG by Mel inhibits osteoclastogenesis and prevents the fragility of bone.[45] In general, PLGA blends result in slow release but are safe and stable for the sustained release of agents. Mel-based PLGA delivery systems generate simple sustained release, and this type of controlled release provides a proper microenvironment to accelerate osteogenic effects. On the other hand, there is still a lack of 3D bioprinted Mel-loaded PLGA scaffolds for bone regeneration, and further studies are needed.

Although Mel-loaded PLGA scaffolds have been widely used in the repair of bone defects, mechanical load bearing is among the main challenges in the bone regeneration process. Even though Mel has excellent bone regeneration properties, the mechanical properties of PLGA should be maintained. Furthermore, 3D bioprinting methods, multilayered structures, and composite blends may yield promising results when Mel is incorporated.

### 1.5. PLA Scaffolds

PLA is a biodegradable polymer made of lactic acid chains and is used to synthesize barrier membranes for biomedical applications, in particular. It is a synthetic polymer made from repeating units of lactic acid and a member of the polyester family. PLA polymers are applicable devices manufactured in different forms, such as films, fibers, and patches, and they have been approved by the Food and Drug Administration (FDA) for biomedical applications.[46] Additionally, the structure of PLA allows the combination of osteoconductive and osteoinductive materials, such as hydroxyapatite, growth factors, and therapeutic agents, for robust bone repair. These polymer membranes facilitate cell ingrowth and adhesion for osteogenesis.[47] PLA carrier properties can be designed according to the required molecular weight and solubility to enhance stability, applicability, and handling. Despite various advantages, such as a high Young's modulus and tensile strength, enhanced sintering activity, and good biodegradability, PLA still has multiple drawbacks. During the preparation of a PLA solution, toxic solvents such as dichloromethane, which are risky for health, are used, and the hydrophobicity of PLA decreases cell migration and adhesion for osteogenesis. Furthermore, pH changes and long-term inflammation in the local area limit the wide use of PLA in bone tissue engineering applications. To overcome these drawbacks, incorporation of natural polymers or proper coatings for cell survival, modulation of pH, and avoidance of inflammatory responses may improve scaffold fabrication.[48]

To overcome these detrimental outcomes, scientists have focused on different blends and composites incorporated with PLA polymer carriers to improve biodegradability and biocompatibility. The 3D printing of PLA scaffolds has limited potential because of the high process temperature, which is inappropriate for cell survival or for most additive molecules. To achieve an optional biomaterial with 3D printing, there are multiple available printing methods for utilizing PLA-based 3D bioprinting materials, such as FDM, selective laser sintering, solvent evaporation, stereolithographic approaches, and selective electron beam melting. Each technique uses special principles to fabricate the most beneficial material for cell adhesion.[49] Even the FDM technique provides many advantages for 3D bioprinting of PLA-based scaffolds due to its controlled thermal opportunities, simplicity, fast manufacturing, and highly porous properties; other methods have also been used in a wide range of 3D approaches for PLA application.[50] To determine the chemical and morphological efficacy of FDM bioprinted PLA scaffolds, three differ-

 

ent pore sizes (150, 200, and 250 μm) of structured scaffolds were fabricated. The results of the cell viability assay indicated that all three structures are biocompatible and have no cytotoxicity. The FDM technique did not lower the porosity or modulate the thermostability of the polymeric matrices.[37] 3D bioprinted approaches for PLA-based scaffolds mostly include incorporating coatings with robust osteoinductivity and osteoconductivity. Polydopamine-coated PLA composites were fabricated to improve cell adhesion onto the material surface and increase COL1 accumulation. The characterization of the scaffold revealed a porous structure of 60%, where the cancellous bone porosity was between 30% and 90%. In vitro, incubation of MSCs with the composite indicated that polydopamine coating of PLA enhanced the cell response, ECM deposition, and ALP activity at 7, 14, and 21 days.[51] In a study, PLA/HA scaffolds enriched with bone marrow and growth factors were fabricated via an extrusion-based 3D bioprinting method in a layer-by-layer manner. The characterization of the scaffolds indicated that high porosity and pore diameters were appropriate for cell attachment (mean 500 μm). In this study, HA was incorporated with PLA to provide an appropriate microenvironment for cells. In addition, HA may enhance the mechanical stability of the scaffold; however, the mechanical strength was not evaluated. The results of this study suggested that osteoinductive additives to PLA improved cell proliferation in vitro in combination with MSCs and promoted bone regeneration in vivo in rabbit radius defects.[52] Owing to the low mechanical stability of PLA itself, incorporating $\beta$-tricalcium phosphate may better enhance the bioactivity of the final scaffold. Backes et al. (2021) developed a novel scaffold with PLA and $\beta$-tricalcium phosphate at different concentrations. All formulations (5%, 10%, and 25% wt.) improved the rheological behavior. The scaffold was incubated with simulated body fluid to observe HA formation on the scaffold surface. In the early days (day 7), the addition of 10% tricalcium phosphate containing 3D bioprinted PLA enhanced cell interactions. $\beta$-Tricalcium phosphate might be a good candidate for the development of PLA composite materials for bone tissue.[53] One of the other matrices incorporated with PLA is poly(glycolic acid) (PGA), which is a biocompatible thermoplastic aliphatic polyester. Scientists have developed a craniomaxillofacial mold with 3D bioprinting based on PLA/PGA. First, the main aim of the study was to compare the artificial and original morphologies of the dog mandible. The computed tomography results confirmed the feasibility of this method. In vitro cell culture results also revealed that the PLA/PGA composite improved cell adhesion and ECM deposition on the material surface.[54]

There are no in vitro or in vivo studies on bone healing with Mel, but PLA has been widely used in bone regeneration studies, and Mel-loaded PLA, not more than PLGA, seems beneficial in bone tissue engineering.

Owing to the inflammatory conditions and changes in the microenvironment of the injured tissues and bone, the healing process initially affects the local area via inflammatory cytokines and concludes with hematoma. Therefore, the applied agents should reduce inflammatory activity and accelerate healing. Mel is a well-known anti-inflammatory agent for a range of pathological disorders. The Mel-based PLA membrane seems to be a good stimulator of bone regeneration and an inhibitor of inflammatory processes. In an osteoporosis model, Mel-based PLA nanoparticles were synthesized and characterized after being applied to rats. Mel-loaded PLA was administered to the rats for seven days. The results showed that the composite scaffold had no toxicity in rats. In this study, the scientists claim that the biomaterial did not significantly affect the serum ALP levels of rats but reduced the serum IL-6, IL-1$\beta$, AST, and ALT levels.[55] Considering the pH levels of the microenvironment of synthetic polymers, it was previously reported that polymers reduce the pH. Owing to the applicable properties of PLA, it reduces the pH of the applied area and can cause detrimental changes in cell metabolism. Therefore, incorporating Mel with PLA may contribute to regulating the pH. In a previous study, Mel treatment alleviated acidosis-related glycogen synthase kinase-3$\beta$ activity, NF-$\kappa$B signaling, endoplasmic reticulum stress, Golgi stress, and abnormal autophagy–lysosome signals. These data indicate that Mel is beneficial for acidosis-induced injuries. Thus, Mel may be a regulator agent of the PLA-implanted area.[56] One of the main properties of Mel is its antioxidative efficiency. Accumulating evidence has shown that Mel is a ROS scavenger. In general, most bone injuries are treated with various types of implants. After surgery, the implants trigger inflammatory and oxidative processes around the implanted site. At this point, bone tissue engineering offers promising facilitated healing via applicable biomaterials. A study by Pandey et al. (2015) reported that Mel encapsulated with PLA significantly reduced ROS levels in vitro and suggested that Mel-based PLA nanoparticles possess high clinical value for the improvement of different pathologies.[57] It is clear from these studies that a single PLA polymer needs to meet tissue requirements, such as biocompatibility, biodegradability, and antioxidative or anti-inflammatory effects at the same time.

### 1.6. PCL Scaffolds

As there is a consensus about the beneficial effects of polymeric biomaterials in bone healing, which the FDA approves, polymeric scaffolds provide the optimal microenvironment for mechanical robustness, cell ingrowth, and sustained release. PCL is among the most widely used polymeric blends in bone tissue engineering owing to its good physical properties. PCL offers an exceptionally low-cost polymeric scaffold, an optimal environment for supporting mechanically robust cell growth and controlling biodegradation. PCL is exceptionally inexpensive and has desirable mechanical properties compared with other polymeric carriers, which makes this polymer preferable. Owing to its slow degradation rate, complexity, and relevance to various processes, PCL is very suitable for entrapping drugs in micro- and nanoparticles, facilitating the permeability of small drug molecules. PCL presents a less acidic environment than does PLA.[29]

Rapid prototyping is a production method for 3D-printed PCL scaffolds. In one study, different 3D-printed PCL structures were fabricated and evaluated for their chemical and morphological properties. The rapid prototyping method allowed the formation of higher porosities with 50–60% porosity for all the structures. Both scaffolds improved cell attachment, ALP release, and cell proliferation.[28] Owing to the hydrophilic surface of PCL, cell attachment mostly occurs on scaffold surfaces. Therefore,

facilitating cell adhesive strategies must be applied. Therefore, PLGA-incorporated PCL scaffolds were blended because of the ideal properties of PLGA, with the exception of acidic substitutes. Slowering the degradation of PLGA with PCL incorporation may avoid pH changes in the local area. PCL also enhances the mechanical stability of PLGA for use in tough tissues. The mechanical and morphological properties of the final composite with PCL/PLGA/HA were evaluated. The scaffold porosity was high, and the mean pore diameter was 500 µm, which is ideal for cell activity and angiogenesis. In addition to improved mechanical properties, the scaffold enhanced the attachment, proliferation, and adhesion of cells and seems to be a good candidate for bone tissue engineering.[58]

In a concentration-dependent study, PCL was fabricated at different concentrations (15%, 12%, and 18% wt.) via 3D printing, and the regenerative effects of the scaffold were evaluated. Interestingly, the porosity of the scaffolds was between 73.06% and 55.07%, and the mechanical strength increased in a PCL dose-dependent manner. The 3D bioprinted PCL scaffolds also showed significant accumulation of newly formed apatite on the scaffold surface. The drug release of both scaffolds was also impressive. BMP2 was loaded into all the developed scaffolds, and drug release was profiled. Owing to the low biodegradability of PCL, the sustained release of drugs provides time for the enhancement of osteogenesis and cell activities.[59]

PCL incorporation with 3D bioprinting provides promising applications for treating bone diseases. 3D-printed PCL scaffolds have been used in numerous clinical trials and approved by the FDA. 3D bioprinted PCL scaffolds are low-cost, nontoxic, have low immunoreactivity, have a low degradation profile, and do not change the pH of their products. Compared with other polymeric materials, PCL provides sufficient load-bearing potential, especially for tough tissues, e.g., bones. In vivo studies have indicated that 3D-printed PCL scaffolds possess effective and safe properties. This incorporation maintains cell proliferation, adhesion, angiogenesis, and osteoinductive effects. 3D-printed PCL scaffolds seem to be better at mimicking the microenvironment of bone tissue and offer a future treatment option for bone healing.[60] 3D bioprinted Mel-loaded PCL scaffolds have been used in several studies for nerve regeneration and repair. Studies suggest that the scaffold has increased regenerative effects and that Mel prevents oxidative stress and inflammation. The structure of the scaffolds had mechanical stability, and drug release was adequate. The degradation rates of the abovementioned scaffolds were appropriate during nerve repair.[61]

In a recent study, 3D bioprinted Mel/PCL scaffolds improved nerve regeneration and reduced ROS and inflammation both in vitro and in vivo after 6- and 12-week applications. The Young's modulus of the scaffold was 47.06 MPa, which adequately supports the loading of tough tissues. Moreover, Mel-loaded PCL scaffolds improved cell viability.[62]

In addition to Mel's osteoinductivity, 3D bioprinting technology integrated with PCL scaffolds can be a good candidate for treating bone diseases or fractures. However, there are only a few studies in this field. Zhang et al. (2021) fabricated 3D-printed Mel-loaded Mg-PCL scaffolds to observe the effects in vivo and in vitro osteosarcoma models. In this study, Mel-loaded Mg-PCL scaffolds aimed to inhibit the key cell-in-cell (CIC) pathway, which involves an increased number of living cells in adjacent living cells and promotes tumor metastasis through Rho/ROCK signaling. During this process, Mel first interacts with the cAMP/PKA pathway via the MT1 receptor, which may be the key to preventing tumor invasion and metastasis in osteosarcoma cells. The results produced promising outcomes. The scaffold has good biodegradability, biocompatibility, and nontoxicity. The in vivo results showed that the scaffold inhibited osteosarcoma cell proliferation and metastasis.[63]

Mel-loaded PCL membranes have already been shown to have positive effects on various bone regeneration models. Studies have reported that the physiochemical properties of fabricated membranes include excellent biocompatibility and biodegradation. Mel-loaded PLCL biofilms promoted the osteogenic differentiation potential of MC3T3-E1 cells and increased the mRNA expression levels of ALP, OCN, OPN, and Runx2.[64] 3D fibrous scaffolds based on a polymer blend solution containing PCL-loaded Mel diatom frustules were fabricated via the wet electrospinning method. Diatom frustules are made of nanopores that provide excellent drug-loading properties and are used to enhance osteoinductivity. However, some drawbacks of diatom frustules exist. Diatoms rapidly degrade and release the involved drug in the early phases. Hence, researchers have used PCL to prevent rapid degradation. The final composite bearing Mel-loaded frustules significantly improved the ALP activity of the human primary sarcoma cell line (Saos-2 cells). The developed scaffold system was fabricated with increased porosity and successfully induced osteogenic activity via controlled Mel delivery, and the final biomaterial has potential for use in bone tissue engineering.[65]

In addition to fracture healing, tendon-to-bone healing is still a challenging issue for orthopedic patients. The challenge especially depends on the limited regeneration capability and inflammatory responses of cartilage tissue. Scar formation also hinders healing. At that point, bone tissue engineering offers promising strategies for prochondrogenic, anti-inflammatory, and facilitated healing for native regeneration.[66] In vitro studies have shown that Mel is an enhancer agent for chondrogenesis marker genes and major components of the ECM, such as COL2A1, SOX9, and aggrecan, facilitating the chondrogenesis of human BMSCs.[67] Mel-loaded PCL membranes inhibited macrophage infiltration, increased chondroid formation, enhanced collagen maturation, and reduced fibrovascular tissue formation. They improved the biomechanical strength of the enthesis following implantation in a rat acute rotator cuff tear model, which suggests that Mel-loaded PCL membranes have excellent clinical application potential for tendon-to-bone healing.[30] In another study, Mel was loaded into albumin nanoparticles and incorporated with PCL membranes, and 3D bioprinting was achieved. Mel was released from the scaffolds for 22 days. Albumin nanoparticles are used to carry and prevent Mel from rapidly degrading. These results indicate that Mel has a chondrogenesis effect in vitro by increasing glycosaminoglycan levels and collagen deposition when applied to a human chondrocyte cell line.[68] **Table 2** presents 3D bioprinted polymer-based Mel blends.

PCL has a robust physiochemical structure, improved biological properties, and high mechanical strength, and it is biocompatible and biodegradable. PCL blends allow the binding of different pore sizes of cell types on PCL scaffolds. Mel-incorporated

 

**Table 2.** Overview of 3D bioprinted polymer-based Mel composites.

| 3D Bioprinted Mel Composites | Application | Structural Properties | Major Outcomes | References |
|---|---|---|---|---|
| PLGA NPs, mixed with sodium alginate hydrogel with PCL/$\beta$-TCP | • In vitro (MC3T3-E, bEnd.3 cell lines)<br>• In vivo diabetic calvarial bone defect | • FDM technique enhanced sustainability<br>• Higher encapsulation efficiency Mel: PLGA ratio (1:3)<br>• Ideal pores within the scaffolds, $\approx$ 200 µm | • Enhanced cell adhesion and biosafety<br>• Enhanced antioxidant activity and activated the release of HO-1 to stimulate VEGF activation to enhance angiogenesis and following the osteogenesis. | [40] |
| 3D Bioprinted PCL scaffolds | • In vitro Schwann cell proliferation and neural expression<br>• In vivo promoted nerve regeneration | • Mechanical stability and drug release were adequate<br>• The Young's moduli of the scaffold were 47.06 MPa | • Increased regenerative effects and Mel prevented oxidative stress and inflammation | [70] |
| 3D-printed Mel-loaded Mg-PCL scaffolds | • In vitro<br>• In vivo Osteosarcoma rabbit model | • Good biodegradability, biocompatibility, and nontoxicity | • Inhibited the key cell-in-cell (CIC) pathway, which addresses an increased number of living cells in adjacent living cells and promotes tumor metastasis through Rho/ROCK signaling<br>• Scaffold inhibited osteosarcoma cell proliferation and metastasis | [63] |
| 3D Fibrous scaffolds based on polymer blend solution containing PCL-loaded Mel diatom frustules | • In vitro Saos-2 cells | • Higher porosity and has successfully induced osteogenic activity by controlled Mel delivery | • Significantly improved the ALP activity of the human primary sarcoma cell line (Saos-2 cells). | [65] |
| Albumin nanoparticles incorporated with PCL membranes | • In vitro | • Good biodegradability, biocompatibility | • Chondrogenesis effect in vitro by increasing glycosaminoglycan levels and collagen deposition when applied to the human chondrocyte cell line | [68] |

PCL scaffolds appear to be promising options for bone tissue engineering.

## 2. Conclusion and Future Directions

Bone healing has immense healthcare concerns and economic burdens worldwide. Despite several surgical methods and medical treatment options, rapid and effective bone regeneration is still challenging for clinicians. In bone tissue engineering, promising developments have been achieved, including the fabrication of cell-seeded scaffolds, drugs, or molecule encapsulation strategies. Sustained release systems are among the most prominent innovations in the use of biodegradable polymers for drug delivery, and synthetic polymers are the most preferable owing to their biocompatibility and tunable design.

3D bioprinting applications of Mel-loaded polymers in the field of bone tissue engineering are still at an experimental level. The drawbacks arise from the light sensitivity and insolubility of Mel in aqueous solutions. Indeed, polymer technology offers a promising channel for the use of Mel in 3D printing. However, the printing process, printability, mechanical load bearing after printing, and storage conditions constitute the main drawbacks of 3D printing Mel in studies and lead to difficulties in clinical translation. Therefore, 3D printing strategies may reduce rapid degradation and enhance the encapsulating efficiency and feasibility of Mel in the bone regeneration process.

3D bioprinting technology has become a novel tissue engineering system over the past twenty years and has attracted considerable scientific attention in all pathological treatment fields. Integrating 3D printing methods with polymers as bioinks offers patient-specific options for creating a graft material. However, not only 3D bioprinted polymer scaffolds but also the addition of regenerative medicines and anti-inflammatory or antioxidant agents improve the results. Mel has been reported to be associated with many biological functions, such as acting as an antioxidant, an inhibitor of tumor proliferation, an immunomodulator of cell signaling, a neuroprotective agent, and a regulator of most body systems as an internal clock hormone. Nevertheless, bioavailability, half-life, and rapid degradation through the administration route limit the therapeutic potential of Mel. It is clear from this review that Mel-entrapped PLGA composite scaffolds have increased encapsulation efficiency and exhibit increased ECM mimicking in bone regeneration applications, with no side effects. In addition, PCL offers better mechanical stability and slower degradation. There are phase 2 studies of 3D-printed PCL scaffolds, and they are potentially promising candidates for translation into clinical applications. Further studies are needed to eliminate the scarcity of studies on the effects of 3D bioprinted Mel-based natural polymers in bone regeneration. Overall, this review attempts to shed light on bone healing strategies in the field of bone tissue engineering from the view of sustained release methods for Mel.

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## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

All the authors contributed equally to the manuscript. M.U. and A.O. designed the scope of the review; D.A. and C.A. wrote and grammar-checked the entire manuscript.

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