REVIEW ARTICLE

Angiogenic and Osteogenic Properties of Fibrin in Bone Tissue Engineering

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ABSTRACT

The bone scaffold has become a promising alternative in bone tissue engineering due to the limitation associated with current bone treatments. However, the selection of scaffold material that could accurately mimic the extracellular matrix of native tissue remains challenging. Owing to its biological origin properties, natural materials including fibrin are widely used as scaffold materials as compared to synthetic materials. Fibrin has been recognized as one of the appealing natural biopolymers, which possesses unique characteristic due to its natural formed nano-scaffold, which provide a temporary matrix that facilitates cellular activities of cells. Fibrin has shown remarkable effects over other biomaterials in inducing angiogenesis and osteogenesis in bone regeneration owing to its mechanical and biological properties. In this article, we highlight the significance of fibrin materials in facilitating bone regeneration. We focus on the manipulation of fibrin composition and on the recent developments of fibrin composites in enhancing osteogenesis and angiogenesis for bone healing.

Keywords: Fibrin, Bone Scaffold, Angiogenesis, Osteogenesis, Bone Regeneration

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INTRODUCTION

Over two million bone grafting treatments have been performed annually worldwide, resulting in the second most common tissue transplantation after blood transfusion (1). There are two major types of bone graft which are autograft and allograft. Autograft is recognized as the gold standard among all available clinical grafts due to osteoconduction, osteoinduction and osteogenesis properties that are essentials in bone regeneration (1,2). However, donor site morbidity and long-term hospitalization have limited the use of autografts in bone grafting treatments (1-3). As an alternative, the allograft is widely used to overcome the donor site morbidity issue and then become the second preferable treatment in orthopaedic surgery (1).

Nevertheless, allograft bone treatment has some disadvantages including the lack of osteoinductive properties as well as the high risk of infection and immune rejection (1-3). In order to overcome these limitations, bone scaffold technology has been emerged as a new promising approach in the orthopaedic industry as the global scaffold technology market size worth 1.1 billion USD in 2020 with further growth expectations (4). There are four important properties of functional bone graft, which are biocompatibility, osteoconductive, osteoinductive and vasculogenic (2,5). Therefore, an ideal bone scaffold should provide a 3D microenvironment that mimics the mechanical properties and extracellular matrix (ECM) characteristic of native tissue which allows cells adhesion, cells proliferation and cells differentiation (3,5,6). Besides, one of the important mechanisms in bone regeneration is the coupling of angiogenesis and osteogenesis (7). Fig. 1 shows the growth factors signaling between osteoblasts and endothelial cells in bone repair. Therefore, material selection plays a vital role in scaffold fabrication to fulfil the ideal scaffold requirements that facilitating cells growth while maintaining biomechanical support.

Fibrin is one of the remarkable biomaterials with unique characteristics due to its naturally formed nano-scaffold, which provides a temporary matrix to facilitate cellular activities. Formation of fibrin matrix through clotting cascade does not only act as a blockade to prevent further blood loss at the injury site but also as a temporary scaffold for cells growth during tissue healing and remodeling (8,9). Besides fibrinogen and thrombin

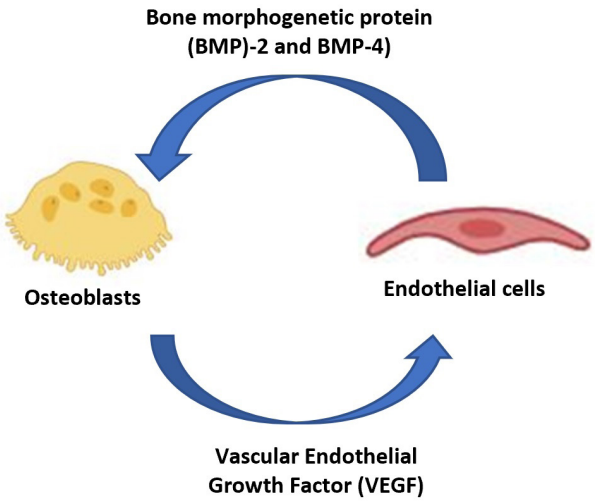

Figure 1: Growth factors signaling between osteoblasts and endothelial cells during bone repair

concentrations, the formation of the fibrin network is also regulated by other parameters such as salt concentration, pH, temperature and other plasma proteins (9). Various applications of fibrin such as microbeads, coating agent, injectable hydrogel and pre-formed scaffolds in bone tissue engineering have been investigated due to its versatile biological and mechanical properties. Here, we highlight the uniqueness of fibrin properties and various strategies of fibrin fabrication for bone osteogenic and angiogenic developments.

MECHANICAL AND BIOLOGICAL PROPERTIES OF FIBRIN

Bone mechanical properties play a crucial role in ensuring successful bone regeneration. The differentiation of precursor cells in osteogenesis is influenced by the local mechanical environment (3,10). One of the important bone mechanical properties is viscoelastic with different regions of bone has different viscoelastic properties (3,11). Bone exhibits viscoelastic behaviour that affects the ECM of native tissue in providing both mechanical stability and biochemical cues for cell growth (3,12). Thus, a scaffold with viscoelastic property closer to native bone has demonstrated good cellular viability and calcium secretion (13).

Therefore, the uniqueness of fibrin viscoelastic behaviour due to covalent cross-linking within fibrin networks that give clot rheological properties is becoming an advantage in mimicking the properties of specific native bone (8,14). A fibrin network is produced by cleaving the fibrinopeptides from fibrinogen to form the protofibrils or fibrin polymer, as shown in Fig. 2. Moreover, manipulating fibrinogen concentration affects the mechanical properties of fibrin by increasing the fibrinogen concentration resulted in doubling the elastic moduli and the maximum load which enhancing the

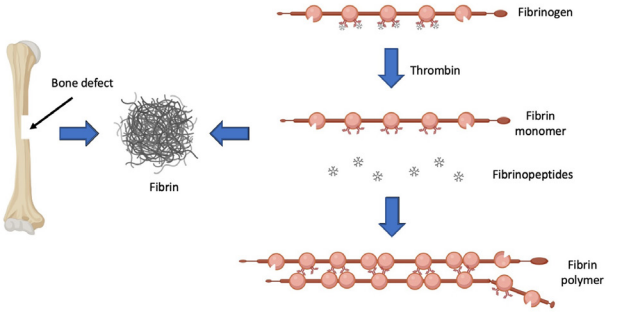

Figure 2: The transformation of fibrin from fibrinogen during the clotting process

rigidity and stiffness of the matrices (8,15). Mechanical stiffness of fibrin matrix that was affected by clot ligation gave great effect on the cell activity through the cell and ECM interaction (8). Interaction of cells with aligned topographical has been regulating the cells activity which mimics the microenvironment of native tissue (16,17). Fibrin matrix with lower fibrinogen concentration has shown low stiffness value but with enhanced colony-forming efficiency and maintained mesenchymal stromal cells (MSCs) differentiation potentials (18).

Therefore, manipulating the composition of fibrin during the polymerization process helps in predicting the mechanical properties of the fibrin matrix which significantly influencing the behaviour of the cells. Recently, autocalcification properties of a cell-free fibrin gel in an osteogenic medium have been observed thus showing the capability of fibrin for calcium deposition in the osteogenic microenvironment (19). Taking advantage of all unique characteristics, the fibrin matrix has been used to mimics the mechanical architecture of native bone tissue (20,21).

Natural fibrin that is derived from blood plasma is essential to many physiological processes such as tissue hemostasis and angiogenesis that involve in inducing cell signaling and cell adhesion (22,23). Natural fibrin provides a temporary matrix during the rebuilding and repair of tissue, making it an attractive pro-angiogenic biomaterial if is compared to chitosan which is an angiogenesis inhibitor (23). A study showed that autologous fibrin hydrogel has accelerated the revascularization and cells migration in humans mandibular by tuning the growth of fibroblasts and osteoblasts (24). Another study has shown that the usage of fibrin hydrogel for MSCs implantation at the femoral defect rats accelerated angiogenesis and bone regeneration of long bone healing (25). Besides, fibrin biopolymers have enhanced in-vivo vascularization together with bone formation (26-28).

Besides pro-angiogenic and bioactive properties, fibrin possesses excellent biocompatibility with minimal inflammation and foreign body reaction (29-31). Other natural material such as alginate does not have same

excellent biological properties due to uncrosslinked anionic polysaccharide structure (31). A study has indicated that fibrin is superior to collagen, a major bone organic component in terms of protein adsorption, osteoblast proliferation, and osteoblast differentiation (32). Fibrin has shown greater fibronectin-binding capacity of fibrin than collagen, even though fibronectin can interact with other cellular ligands, collagen (32,33).

Moreover, the intrinsic biological properties of fibrin have also allowed the binding with various growth factors such as Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF) and Insulin-like Growth Factor-1 (IGF-1) that are either secreted in normal tissue or delivered to injury site (8,9,34). The efficiency of fibrin as a growth factor delivery vehicle has been observed by the slow release of the bone morphogenetic protein (BMP-2) and VEGF from growth factors-doped fibrin glue within a porous titanium scaffold that enhanced the bone formation with angiogenesis inside the scaffold (35). Human fibrin concentrated growth factor membrane (CGF) acted as growth factor delivery for recombinant human bone morphogenetic protein-2 (rhBMP-2) that overcame the disadvantages CGF membrane's degradation properties (36).

Furthermore, fibrin gel has been identified as a cell delivery system due to degradation properties that allowed the cell migration from fibrin to calcium phosphate cement surface with the calcium phosphate cement improved the stability of the fibrin gel (37). Besides, fibrin microbeads have shown the capability in delivering the cells to a specific targeted area for the bone regeneration process (38,39). Moreover, alginate-fibrin microbeads with fast-degradable properties have shown the release of human umbilical cord mesenchymal stem cells (hUCMSCs) after a short period with high expression of alkaline phosphatase (ALP), osteocin (OC), collagen I, and Runx2 (38). Besides, two months of observation on implanted fibrin microbeads encapsulated mesenchymal stem cells into mouse skull showed the defected area was filled with bone-like tissue that similar to native bone (39). Fig. 3 summarizes the mechanical and biological properties of fibrin.

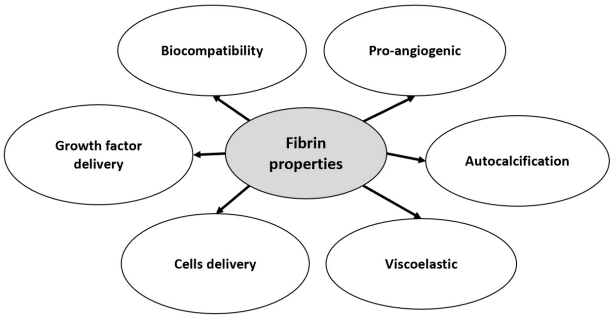

Figure 3: The mechanical and biological properties of fibrin

APPLICATIONS OF FIBRIN IN BONE TISSUE ENGINEERING

Manipulation of Fibrin Composition

The performance of natural fibrin depends on through clotting cascade of thrombin on fibrinogen originated from blood plasma (8,9,40). It has been reported that higher fibrinogen concentration significantly improved cell proliferation and cell adhesion due to enhanced surface roughness of the scaffolds (41,42). Enhancement of surface roughness is due to adsorption of fibrinogen or by the function of an epitope region of β15–42 found in fibrinogen (14,43). High fibrinogen concentration also affected intracellular signaling and cell differentiation that increased in the expression of ALP and osteocalcin (42,44).

It has been demonstrated that the presence of fibrinogen within a chitosan scaffold enhanced the bone formation of rat bone by inducing angiogenesis and osteogenic capacity in the defected area (45). Besides, a study showed higher concentration of fibrinogen demonstrated more in-vivo bone formations (46). However, a comparison of fibrin matrices with different fibrinogen showed that proliferation and osteogenic differentiation of rat mesenchymal stem cells (MSCs) was enhanced with lower fibrinogen concentrations which highlighted the importance of mechanical and topological properties of fibrin matrix in facilitating cell growth (18).

In another study, low thrombin concentration has been reported able to stimulate ALP's gene expression and angiogenic factors of osteoblastic cells on thrombin-coated biphasic calcium phosphate ceramic (47). Another finding demonstrated the role of thrombin in accelerating osteoblast differentiation with a high concentration of thrombin enhanced calcium deposition, ALP activity and Runx2 level of osteoblast cells (48). Therefore, high thrombin concentration has been suggested for altering the fibrin structure and thus enhancing the fibronectin-binding capacity (48).

Development of Fibrin Composite In Mimicking Bone Tissue

In recent years, the fabrication of nanofibrous scaffolds, which could mimic the native bone extracellular matrix has become prevalent in bone tissue engineering. Various strategies to develop an appropriate fibrin composite in improving the mechanical properties of the bone scaffold have been explored. One of the strategies to develop nanofibrous fibrin scaffolds is by using poly (methyl-methacrylate) beads to form an interconnected microporous network with enhanced mechanical properties (49). Besides, a study has demonstrated the ability of fibrin in improving the mechanical properties of the PCL fibres as a potential material for bone repair (50). Table I shows recent fibrin composites development for bone osteogenesis and angiogenesis. Fibrin based

Table I: Osteogenic and Angiogenic Properties of Fibrin/Fibrinogen Composites

Composite	In-vitro/In-vivo	Findings	References
Fibrin	Mesenchymal stem cells (MSC)	Changing fibrinogen concentration reversely affects MSC proliferation and osteogenic differentiation	18
Fibrin	TG2 gene-modified ectomesenchymal stem cells (TG2-EMSCs)	Enhancing osteogenic differentiation, extracellular matrix proteins deposition, bone matrix calcification	19
Fibrin with TG2-EMSCs	Cranial defects in rats	Bone regeneration after 12 weeks	19
Fibrin with human dental pulp stem cells (DPSC)	Mice with alveolar bone defect	Enhancement of bone formation and vascularization	26
Fibrin-based bioink	Rat with femoral defect	Enhancement of vascularization for critically-sized bone defect	27
Fibrin bioink	Immunodeficient mice	Formation of blood vessel formation and calcified bone matrix	28
Fibrin biopolymer	Rat femur	Accelerating bone regeneration by modifying inflammatory environment at the bone defect	29
Fibrin, alginate and calcium phosphate	Osteoblast cells (MC3T3-E1)	Enhancing osteogenic differentiation	31
Fibrin, alginate and calcium phosphate	Chorioallantoic membrane (CAM) assay	Enhancing in-vivo angiogenic properties	31
Human fibrin concentrated growth factor membrane (CGF)	Subcutaneous tissues of nude mice	Inducing several bony islands and the cartilage nodule after14 days	36
Fibrin and calcium phosphate cement (CPC)	Craniofacial defects in rats	Bone formation over 12 weeks	37
Fibrin and calcium phosphate granules	Human mesenchymal stem cells (hMSC)	High fibrinogen concentration affects hMSC proliferation and osteogenic differentiation	41
Fibrin in collagen scaffold	Osteoblast cells (MG-63)	Changing fibrinogen concentration improved cells adhesion, cells proliferation, and cells differentiation	44
Fibrinogen in chitosan scaffold	Critical size bone defects in rats	Improving bone regeneration, bone angiogenesis with eliciting immune response	45
Fibrin with calcium phosphate and glass ceramic	Swiss albino mice	Higher concentration of fibrinogen demonstrated more bone formation in the extraskeletal site of	46
Calcium phosphate ceramic coated with Fibrin	Osteoblast cells (MC3T3-E1)	Changing thrombin concentration increased the angiogenic potential of osteoblasts	47
Fibrin	Osteoblast cells (MC3T3-E1)	Changing thrombin concentration accelerated osteoblast differentiation	48
Fibrin- with polycaprolactone (PCL)	Human osteosarcoma cell line	Enhancing the mechanical properties, cell attachment and cell distribution	50
Fibrin based scaffold	Adult rabbits	Enhancement of bone repair of osteochondral defects with perfect restoration of tibial defects for young adult rabbits	51
Fibrin sealant	Human Muscle-Derived Stem Cell	Enhancement of bone Regeneration	52
Fibrin with graphene oxide, iron oxide nanoparticles and hydroxyapatite	Osteoblast cells (MG-63)	Enhancement of biocompatibility, alkaline phosphatase activity, and calcium deposit	53
Fibrin with graphene oxide, iron oxide nanoparticles and hydroxyapatite	Albino-Wistar rat	Bone healing potential of critical-size tibia defect	53
Fibrin biopolymer and biphasic calcium phosphate	Wistar rats	Acceleration of bone regeneration with the combination of photobiomodulation therapy (PBMT)	54
Fibrin biopolymer (FBP) and biphasic calcium phosphate	Rat femurs	Enhancing the bone matrix with incorporation with MSCs	55
Hydroxyapatite-gelatin-chitosan-fibrin-bone ash	Osteoblast cells	Excellent biocompatibility and cells attachment	56
Fibrin sealant with bovine mineral	Rabbit sinus	Reducing the bone healing period	57
Fibrin-platelet glue with bone fragments	Human with maxillary or mandibular problems	Bone healing with reduced infections and length of hospital stay	58
Fibrin with calcium carbonate scaffold	Human bone marrow stroma cells (hBMSCs)	Higher cells seeding efficiency for longer periods	59
Fibrin with nanocrystalline hydroxyapatite	Mouse with calvarial defect model	Enhancement of bone formation	60
Collagen-fibrin with hydroxyapatite	Endothelial cells and mesenchymal stem cells (MSC)	Regulating the endothelial network formation.	61
Fibrin with hydroxyapatite	Endothelial cells and fibroblasts	Regulating the angiogenesis formation	62
Collagen-fibrin hydrogel	MSC/HUVEC spheroids	Upregulating the osteogenic differentiation with pre-vascular network formation	63
Platelet-rich fibrin	Endothelial cells and osteoblasts	Formation of lumen structures and with higher expression of the proangiogenic factors	64
Platelet-rich fibrin, silicon and autologous bone	New Zealand rabbits	Bone mineralization after 3 weeks	67
Platelet-rich fibrin with Mg ring	Osteoblast cells (MC3T3-E1)	Enhancing the calcium deposition	58

Table I: Osteogenic and Angiogenic Properties of Fibrin/Fibrinogen Composites (continued)

Composite	In-vitro/In-vivo	Findings	References
Platelet-rich fibrin on titanium discs	Osteoblast cells (MG-63)	Enhancing alkaline phosphatase activity and bone mineralization	69
Platelet-Rich Fibrin in Titanium Mesh	Human with Vertical Maxillary Defect	Improving the quality and quantity of bone formation by combining with recombinant human bone morphogenetic protein 2 (rhBMP-2)	70
Fibrinogen-coated Titanium disk	Mouse muscle myoblast cell line (C2C12)	Enhancing the ALP activity and mineralization	71
Platelet-rich fibrin with tricalcium phosphate (TCP)	Rabbit osteoblasts	Enhancing cells attachment, cells proliferation, cells migration, ECM formation and bioactive release,	72
Platelet-rich fibrin with tricalcium phosphate (TCP)	New Zealand white rabbits	Bone regeneration after 8 weeks	72
Platelet-rich fibrin with polycaprolactone (PCL)	Human primary osteoblasts	Enhancing cell activity	73
Platelet-rich fibrin with PCL/chitosan	Human mesenchymal stem cells (HMSCs)	Enhancing osteogenic differentiation, ALP and calcium deposition	74
Platelet-rich fibrin with allogenic bone substitutes	Human osteoblast cell line	Enhancing cell activity	75
Platelet-rich fibrin with alloplastic and xenogeneic bone substitutes	Chorioallantoic membrane (CAM) assay	Enhancing in-vivo angiogenic properties	76
Platelet-rich fibrin with porcine-derived collagen matrices	Chorionallantoic membrane (CAM) assay	Enhancing in-vivo angiogenic properties	77
Platelet-rich fibrin with deproteinized bovine bone mineral	New Zealand rabbits	Enhancing vascular formation, bone remodeling and collagen formation	78
Platelet-rich fibrin with nanohydroxyapatite	Human with intra-bony defects (IBDs)	Increasing bone density and VEGF expression	79
Plasma-rich-fibrin with PCL	Rat with critical-sized calvaria defect	Enhancement of mineralization areas	80
Advanced platelet-rich fibrin (A-PRF) with autogenous iliac crest bone	Human with alveolar cleft	Enhancing bone regeneration	81
A-PRF with serum albumin-coated bone allograft	Human who required maxillary sinus augmentation (MSA)	Enhancement of implant placement with reduction in total treatment time	82
A-PRF with platelet-rich plasma, lyophilized bovine bone and atelocollagen type	Chronic marginal periodontitis was induced in sheep;	Facilitating alveolar bone regeneration	83
A-PRF with zirconia	Human with highest grade and stage periodontitis	Enhancement of bone regeneration and implant integration with the combination of Hyperbaric oxygen therapy (HBOT) before and after implantation	84
A-PRF with gold nanoparticles	Human Mesenchymal Stem Cells	Enhancement of osteogenic capacity	85
Leukocyte-platelet-rich fibrin with collagen and nano beta-tricalciumphosphate (nβ-TCP)	New Zealand white albino rabbits	Enhancing new bone formation	86
L-PRF with Multi-walled carbon nanotube/hydroxyapatite (MWCNT/HA)	Sheep	Enhancement of bone regeneration, biocompatibility and osteoconductivity	87

scaffold has enhanced the bone repair process with the perfect restoration of tibial defects for young adult rabbits (51). Fibrin sealant also has enhanced bone formation with the incorporation of Human Muscle-Derived Stem Cells (52). Besides, a composite of fibrin and graphene oxide also has improved the in-vitro osteogenesis properties and has shown bone healing potential of critical-size tibia defect (53).

Another approach to create bone environment is by incorporating mineralized components such as calcium phosphate and calcium carbonate in inducing osteogenesis (3,41,54). A combination of biocompatible synthetic mineralized materials and fibrin biopolymer has been suggested as the right scaffold material for bone repair therapies due to clotting formation that allowing cell adhesion and proliferation (55,66). The incorporation of fibrin with any type of bone components has reduced the bone healing period since only a small amount of bone substances were needed to achieve reliable bone formation (57). Additionally, significant improvement in the bone healing process with lessening in infections has been observed through a composite of fibrin-platelet bone fragments (58). Besides, fibrin coating on calcium carbonate scaffold has enhanced the attachment of human bone marrow stromal cells (hBMSCs) with high and uniform cell seeding (59). Moreover, the presence of fibrinogen into bone powder scaffold has accelerated bone regeneration at rabbit calvarial defected area proved the effect of fibrin on osteoinductivity properties (41). It was also observed that deposition of nanocrystalline hydroxyapatite on fibrin surfaces has increased the alkaline phosphatase activity and osteoblast gene expression with further in-vivo observation showed the bone formation in mice defected area (60).

Ideally, the incorporation of fibrin as bone biomaterials also facilitates the formation of angiogenesis (45,61,62). Besides, collagen-fibrin hydrogel composite has been proved to upregulated blood vessels formation together with osteogenic properties (63). Co-culturing the endothelial cells and osteoblast cells within platelet-rich fibrin resulted in higher expression of the proangiogenic factors by (64). Furthermore, the combination of fibrin and hydroxyapatite in mimicking highly porous and

interconnected scaffolds has enhanced the formation of newly formed vessel sprouting within the mineralized bone matrix (61,62). Previously, collagen-fibrin and hydroxyapatite composite has demonstrated the formation of endothelial networks in 24-well plates due to modulation in stiffness property (61). Additionally, fibrin has been utilized as one component for tumor microenvironment with results showed the direct interaction between angiogenic sprouts and tumor spheroids in the 3D bone-mimetic composite of hydroxyapatite and fibrin (65).

Recently, various composites of platelet-rich fibrin (PRF) with other biomaterials have been developed in making new regenerative bone materials (66-79). Folded PRF with human cortical bone matrix gelatin has shown better resistance against proteolytic digestion which is important in reducing the degradation rate of the bone matrices (66). Besides, a combination of PRF, silicon and autologous bone has sped up the in-vivo bone mineralization (67). Another interesting approach is the combination of PRF with alloy materials such as Magnesium (Mg) and Titanium that has enhanced the in vitro bone mineralization (68,69). By combining with recombinant human bone morphogenetic protein 2 (rhBMP-2), PRF in Titanium mesh has improved the quality and quantity of bone formation in humans with vertical maxillary defects (70). The integration of fibrin with alloy materials that have shown good osteogenic differentiation properties will be beneficial to the development of functional bone implants in bone regeneration (68-71).

Moreover, PRF is a natural biopolymer with bioactive components such as platelet cytokines and growth factors (68). Therefore, the incorporation of PRF is able to overcome the limitation of tricalcium phosphate (TCP) scaffold with the improvement of osteoblast cells morphology in-vitro as well as osteogenesis properties in-vivo (72). In the presence of PRF, the biocompatibility and bioactivity of non-active materials such as polycaprolactone (PCL) has been improved and subsequently has facilitated bone formation (73,74). Additionally, with the incorporation of PRF, bone tissue formation was enhanced with more calcium deposition and osteogenic differentiation due to the improvement of hydrophilicity and porosity of the PCL/chitosan scaffold (74). Besides, the combination of PCL with plasma-rich-fibrin has increased the mineralization areas of rats with critical-sized calvaria defects (80).

Not only osteogenic, but PRF composites also have shown significant angiogenic properties (76-79). Combination of PRF with alloplastic and xenogeneic bone substitutes have upregulated in-vivo vessels formation, which might be due to the release of growth factor through the fibrin polymer (76). Besides, a combination of PRF with collagen biopolymer has demonstrated in-vivo angiogenic potential (77). Furthermore, PRF composites have demonstrated their role in coupling angiogenesis and osteogenesis at the early stage of the in-vivo bone healing period (78-79). However, the stability of both PRF alone and PRF composites at long term studies are still unknown (79). Besides, advanced platelet-rich fibrin (A-PRF) and Leukocyte-platelet-rich fibrin (L-PRF) also have been used widely for bone tissue engineering (81-87). Fig. 4 summarizes the osteogenesis and angiogenesis potentials for various types of fibrin composites that will give useful insight in making a functional bone scaffold. However, more studies are needed specifically to observe long-term angiogenesis and osteogenesis within a fibrin bone scaffold.

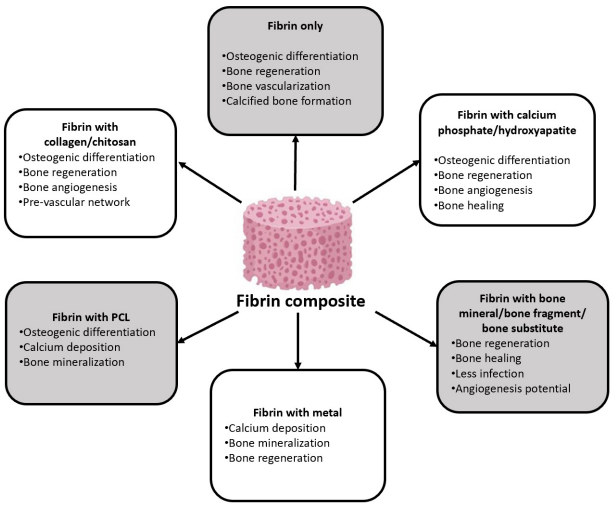

Figure 4: Osteogenesis and angiogenesis potentials for various types of fibrin composites

CONCLUSION

In this review we have summarized the unique traits owned by fibrin, making it an excellent choice for application in bone tissue engineering. Indeed, the combination of biocompatibility, biodegradability and intrinsic biological activity characteristics of fibrin appears to be an attractive choice for scaffold material with high potential in coupling both angiogenesis and osteogenesis which is crucial in ensuring successful bone regeneration. The addition of other materials such as calcium phosphate does not only improve the mechanical strength but mimicking the structure and function of the natural bone ECM. However, the degradation properties of fibrin should be considered when designing the experiments to ensure the prolonged stability of the fibrin scaffolds. Nevertheless, extensive studies are needed to tackle the degradation issue for optimal applications of fibrin as bone scaffolds.

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REFERENCES

1. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. Journal of Materials Science: Materials in Medicine. 2014;25(10):2445–2461.
2. Bauer TW, Muschler GF. Bone graft materials: an overview of the basic science. Clinical Orthopaedics and Related Research. 2000;371:10–27.
3. Lopes D, Martins-Cruz, Oliveira MB, Mano JF. Bone physiology as inspiration for tissue regenerative therapies. Biomaterials. 2018;185:240–275.
4. Scaffold Technology Market Size, Share & Trends Analysis Report By Type, By Application (Stem Cell, Regenerative Medicine, Drug Discovery), By Disease Type (Cancer, Dental, Neurology), By End-use, And Segment Forecasts, 2021 - 2028 (GVR-1-68038-995-1). (2021, May). Grand View Research [cited 2021 July 14]. Available from: https://www.grandviewresearch.com/industry-analysis/scaffold-technology-market
5. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Critical Reviews in Biomedical Engineering. 2012; 40(5):363–408.
6. Jin H, Zhuo Y, Sun Y, Fu H, Han Z. Microstructure design and degradation performance in vitro of three-dimensional printed bioscaffold for bone tissue engineering. Advances in Mechanical Engineering. 2019;11(10):168781401988378.
7. Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, Di Maggio N. It takes two to tango: coupling of angiogenesis and osteogenesis for bone regeneration. Frontiers in bioengineering and biotechnology. 2017;5:68.
8. Weisel JW. Structure of fibrin: impact on clot stability. Journal of Thrombosis and Haemostasis. 2007;5:116–124.
9. Brown AC, Barker TH. Fibrin-based biomaterials: Modulation of macroscopic properties through rational design at the molecular level. Acta Biomaterialia. 2014;10(4):1502–1514.
10. Lange JR, B Fabry. Cell and tissue mechanics in cell migration. Experimental Cell Research. 2013;319(16):2418-2423.
11. Chen X, Hughes R, Mullin N, Hawkins RJ, Holen I, Brown NJ, et al. Mechanical heterogeneity in the bone microenvironment as characterized by atomic force microscopy. Biophysical Journal. 2020;119(3):502–513.
12. Kim Y, Ko H, Kwon IK, Shin K. Extracellular matrix revisited: Roles in tissue engineering. International Neurourology Journal. 2016;20(2):168.
13. Shahin-Shamsabadi A, Hashemi A, Tahriri M, Bastami F, Salehi M, Mashhadi Abbas F. Mechanical, material, and biological study of a PCL/bioactive glass bone scaffold: Importance of viscoelasticity. Materials Science and Engineering: C. 2018;90:280–288.
14. Sproul E, Nandi S, Brown A. Fibrin biomaterials for tissue regeneration and repair. In Barbosa MA, Martins MCL, editors. Woodhead Publishing; 2018.
15. Montero A, Quílez C, Valencia L, Giryn P, Jorcano JL, Velasco D. Effect of fibrin concentration on the in vitro production of dermo-epidermal equivalents. International Journal of Molecular Sciences. 2021;22(13):6746.
16. Yao T, Chen H, Baker MB, Moroni L. Effects of fiber alignment and coculture with endothelial cells on osteogenic differentiation of mesenchymal stromal cells. Tissue Engineering Part C: Methods. 2020; 26(1):11–22.
17. Wang C, Huang W, Zhou Y, He L, He Z, Chen Z et al. 3D printing of bone tissue engineering scaffolds. Bioactive Materials. 2020;5(1):82–91.
18. Colley H, McArthur SL, Stolzing A, Scutt A. Culture on fibrin matrices maintains the colony-forming capacity and osteoblastic differentiation of mesenchymal stem cells. Biomedical Materials. 2012;7(4):045015.
19. Shi W, Que Y, Zhang X, Bian L, Yu X, Tang X et al. Functional tissue-engineered bone-like graft made of a fibrin scaffold and TG2 gene-modified emscs for bone defect repair. NPG Asia Materials. 2021;13(1):1-18.
20. Piechocka IK, Kurniawan NA, Grimbergen J, Koopman J, Koenderink GH. Recombinant fibrinogen reveals the differential roles of α- and γ-chain cross-linking and molecular heterogeneity in fibrin clot strain-stiffening. Journal of Thrombosis and Haemostasis. 2017;15(5):938–949.
21. Litvinov RI, Weisel JW. Fibrin mechanical properties and their structural origins. Matrix Biology. 2017;60–61:110–123.
22. Carrow JK, Kerativitayanan P, Jaiswal MK., Lokhande G, Gaharwar AK. Polymers for Bioprinting: Essentials of 3D Biofabrication and Translation. In: Atala A, Yoo JJ, editors. Academic Press;2015.
23. Dragostin OM, Tatia R, Samal SK, Oancea A, Zamfir AS, Dragostin I et al. Designing of chitosan derivatives nanoparticles with antiangiogenic effect for cancer therapy. Nanomaterials. 2020;10(4):698.
24. Tayapongsak P, O'Brien DA, Monteiro CB, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. Journal of Oral and Maxillofacial Surgery. 1994;52(2):161–165.
25. Seebach E, Freischmidt H, Holschbach J, Fellenberg J, Richter W. Mesenchymal stroma cells trigger early attraction of M1 macrophages and endothelial cells into fibrin hydrogels, stimulating long bone healing without long-term engraftment. Acta Biomaterialia. 2014;10(11):4730–4741.
26. Enukashvily NI, Dombrovskaya JA, Kotova AV,

Semenova N, Karabak I, Banashkov RE et al. Fibrin glue implants seeded with dental pulp and periodontal ligament stem cells for the repair of periodontal bone defects: a preclinical study. Bioengineering. 2021;8(6):75.
27. Nulty J, Freeman FE, Browe DC, Burdis R, Ahern DP, Pitacco P et al. 3D bioprinting of prevascularised implants for the repair of critically-sized bone defects. Acta Biomaterialia. 2021;126:154-169.
28. Rukavina P, Koch F, Wehrle M, Tröndle K, Björn Stark G, Koltay P et al. In vivo evaluation of bioprinted prevascularized bone tissue. Biotechnology and Bioengineering. 2020;117(12):3902-3911.
29. Creste CF, Orsi PR, Landim-Alvarenga FC, Justulin LA, Golim MD, Barraviera B et al. Highly effective fibrin biopolymer scaffold for stem cells upgrading bone regeneration. Materials. 2020;13(12):2747
30. Li Y, Meng H, Liu Y, Lee BP. Fibrin gel as an injectable biodegradable scaffold and cell carrier for tissue engineering. The Scientific World Journal. 2015; 2015: 685690.
31. Kohli N, Sharma V, Orera A, Sawadkar P, Owji N, Frost OG et al. Pro-angiogenic and osteogenic composite scaffolds of fibrin, alginate and calcium phosphate for bone tissue engineering. Journal of Tissue Engineering. 2021;12:204173142110056.
32. Oh JH, Kim HJ, Kim TI, Woo KM. Comparative evaluation of the biological properties of fibrin for bone regeneration. BMB Reports. 2014;47(2):110–114.
33. Marie PJ. Targeting integrins to promote bone formation and repair. Nature Reviews Endocrinology. 2013:9(5):288–295.
34. Breen A, O'Brien T, Pandit A. Fibrin as a delivery system for therapeutic drugs and biomolecules. Tissue Engineering Part B: Reviews. 2019;15(2):201–214.
35. Lv J, Xiu P, Tan J, Jia Z, Cai H, Liu Z. Enhanced angiogenesis and osteogenesis in critical bone defects by the controlled release of BMP-2 and VEGF: implantation of electron beam melting-fabricated porous Ti 6 Al 4 V scaffolds incorporating growth factor-doped fibrin glue. Biomedical Materials. 2015:10(3):035013.
36. Onji K, Kabir M, Zhu B, Yokozeki K, Saito T, Akazawa T et al. Human fresh fibrin membrane with bone morphogenetic protein-2 (BMP-2) induces bone formation in the subcutaneous tissues of nude mice. Materials. 2021;14(1):150.
37. Ahlfeld T, Lode A, Richter RF, Pradel W, Franke A, Rauner M et al. Toward biofabrication of resorbable implants consisting of a calcium phosphate cement and fibrin–a characterization in vitro and in vivo. International Journal of Molecular Sciences. 2021;22(3):1218.
38. Zhou H, Xu HHK. The fast release of stem cells from alginate-fibrin microbeads in injectable scaffolds for bone tissue engineering. Biomaterials. 2011;32(30):7503–7513.
39. Ben-Ari A, Rivkin R, Frishman M, Gaberman E, Levdansky L, Gorodetsky R. Isolation and implantation of bone marrow–derived mesenchymal stem cells with fibrin micro beads to repair a critical-size bone defect in mice. Tissue Engineering Part A. 2009;15(9):2537–2546.
40. Ducheyne P, Healy K, Hutmacher DW, Grainger DW, Kirkpatrick CJ. 2nd ed. Comprehensive Biomaterials II. Elsevier;2017.
41. Kim BS, Lee J. Enhanced bone healing by improved fibrin-clot formation via fibrinogen adsorption on biphasic calcium phosphate granules. Clinical Oral Implants Research. 2014;26(10):1203–1210.
42. Kim BS, Sung HM, You HK, Lee J. Effects of fibrinogen concentration on fibrin glue and bone powder scaffolds in bone regeneration. Journal of Bioscience and Bioengineering. 2014;118(4):469–475.
43. Guadiz G, Sporn LA, Simpson-Haidaris PJ. Thrombin cleavage-independent deposition of fibrinogen in extracellular matrices. Blood. 1997;90(7):2644–2653.
44. Kim BS, Kim JS, Lee J. Improvements of osteoblast adhesion, proliferation, and differentiation in vitro via fibrin network formation in collagen sponge scaffold. Journal of Biomedical Materials Research Part A. 2013;101A(9):2661–2666.
45. Santos SG, Lamghari M, Almeida CR, Oliveira MI, Neves N, Ribeiro AC et al. Adsorbed fibrinogen leads to improved bone regeneration and correlates with differences in the systemic immune response. Acta Biomaterialia. 2013;9(7):7209–7217.
46. Abiraman S, Varma HK, Umashankar PR, John A. Fibrin glue as an osteoinductive protein in a mouse model. Biomaterials. 2002;23(14):3023–3031.
47. Bluteau G, Pilet P, Bourges X, Bilban M, Spaethe R, Daculsi G et al. The modulation of gene expression in osteoblasts by thrombin coated on biphasic calcium phosphate ceramic. Biomaterials. 2006;27(15):2934–2943.
48. Oh JH, Kim HJ, Kim TI, Baek JH, Ryoo HM, Woo KM. The effects of the modulation of the fibronectin-binding capacity of fibrin by thrombin on osteoblast differentiation. Biomaterials. 2012;33(16):4089–4099.
49. Linnes MP, Ratner BD, Giachelli CM. A fibrinogen-based precision microporous scaffold for tissue engineering. Biomaterials. 2007;28(35):5298–5306.
50. Malikmammadov E, Tanir TE, Kiziltay A, Hasirci N. Preparation and characterization of poly (ε-caprolactone) scaffolds modified with cell-loaded fibrin gel. International Journal of Biological Macromolecules. 2019;125:683-689.
51. Feng X, Xu P, Shen T, Zhang Y, Ye J, Gao C. Age-related regeneration of osteochondral and tibial defects by a fibrin-based construct in vivo. Frontiers in Bioengineering and Biotechnology. 2020;8:404.
52. Gao X, Cheng H, Sun X, Lu A, Ruzbarsky JJ,

Wang B et al. Comparison of autologous blood clots with fibrin sealant as scaffolds for promoting human muscle-derived stem cell-mediated bone regeneration. Biomedicines. 2021;9(8):983.
53. Pathmanapan S, Periyathambi P, Anandasadagopan SK. Fibrin hydrogel incorporated with graphene oxide functionalized nanocomposite scaffolds for bone repair—In vitro and in vivo study. Nanomedicine: Nanotechnology, Biology and Medicine. 2020;29:102251.
54. Della Coletta BB, Jacob TB, Moreira LA, Pomini KT, Buchaim DV, Eleutério RG et al. Photobiomodulation therapy on the guided bone regeneration process in defects filled by biphasic calcium phosphate associated with fibrin biopolymer. Molecules. 2021;26(4):847
55. Cassaro CV, Justulin Jr LA, Lima PRD, Golim MDA, Biscola NP, Castro MVD et al. Fibrin biopolymer as scaffold candidate to treat bone defects in rats. Journal of Venomous Animals and Toxins including Tropical Diseases. 2019;25:e20190027.
56. Sathiyavimal S, Vasantharaj S, LewisOscar F, Pugazhendhi A, Subashkumar R. Biosynthesis and characterization of hydroxyapatite and its composite (hydroxyapatite-gelatin-chitosan-fibrin-bone ash) for bone tissue engineering applications. International journal of biological macromolecules. 2019;29:844-852
57. Choi WH, Kim YD, Song JM, Shin SH. Comparative study of bone regeneration using fibrin sealant with xenograft in rabbit sinus: pilot study. Maxillofacial Plastic and Reconstructive Surgery. 2021;43(1):1-6.
58. Giannini G, Mauro V, Agostino T, Gianfranco B. Use of autologous fibrin-platelet glue and bone fragments in maxillofacial surgery. Transfusion and Apheresis Science. 2004;30(2):139–144.
59. Zhu H, Schulz J, Schliephake H. Human bone marrow stroma stem cell distribution in calcium carbonate scaffolds using two different seeding methods. Clinical Oral Implants Research. 2010;21(2):182–188.
60. Osathanon T, Linnes ML, Rajachar RM, Ratner BD, Somerman MJ, Giachelli CM. Microporous nanofibrous fibrin-based scaffolds for bone tissue engineering. Biomaterials. 2008;29(30):4091–4099.
61. Rao RR, Ceccarelli J, Vigen ML, Gudur M, Singh R, Deng CX et al. Effects of hydroxyapatite on endothelial network formation in collagen/fibrin composite hydrogels in vitro and in vivo. Acta Biomaterialia. 2014;10:3091-3097.
62. Jusoh N, Oh S, Kim S, Kim J, Jeon NL. Microfluidic vascularized bone tissue model with hydroxyapatite-incorporated extracellular matrix. Lab on a Chip. 2015;15(20):3984-3988.
63. Heo DN, Hospodiuk M, Ozbolat IT. Synergistic interplay between human MSCs and HUVECs in 3D spheroids laden in collagen/fibrin hydrogels for bone tissue engineering. Acta Biomaterialia. 2019;95:348-356.
64. Dohle E, El Bagdadi K, Sader R, Choukroun J, James Kirkpatrick C, Ghanaati S. Platelet-rich fibrin-based matrices to improve angiogenesis in an in vitro co-culture model for bone tissue engineering. Journal of Tissue Engineering and Regenerative Medicine. 2018;12(3):598-610.
65. Ahn J, Lim J, Jusoh N, Lee J, Park TE, Kim, Y et al. 3D microfluidic bone tumor microenvironment comprised of hydroxyapatite/fibrin composite. Frontiers in Bioengineering and Biotechnology. 2019;7:168.
66. Csönge L, Bozsik Á, Tóth-Bagi Z, Gyuris R, Kónya J. Regenerative medicine: characterization of human bone matrix gelatin (BMG) and folded platelet-rich fibrin (F-PRF) membranes alone and in combination (sticky bone). Cell and Tissue Banking. 2021;1:1-7.
67. Hernández-Suarez A, Rizo-Gorrita M, Suárez-Vega D, Velazco G, Rodriguez Gelfenstein I, Vázquez-Pachón C et al. Effectiveness of silicon platelet-rich fibrin and autologous bone on bone regeneration in rabbit calvarian defects: a radiological and histological study. Applied Sciences. 2021;11(9):4074.
68. Wong PC, Wang CY, Jang JS, Lee CH, Wu JL. Large-pore platelet-rich fibrin with a Mg ring to allow MC3T3-E1 preosteoblast migration and to improve osteogenic ability for bone defect repair. International Journal of Molecular Sciences. 2021;22(8):4022.
69. Shah R, Thomas R, Gowda TM, Baron TK, Vemanaradhya GG, Bhagat S. In vitro evaluation of osteoblast response to the effect of injectable platelet-rich fibrin coating on titanium disks. The Journal of Contemporary Dental Practice. 2021;22(2):108.
70. Alraei K, Shrqawi J, Alarusi K. Application of recombinant human BMP-2 with bone marrow aspirate concentrate and platelet-rich fibrin in titanium mesh for vertical maxillary defect reconstruction prior to implant placement. Case Reports in Dentistry. 2021;2021:1-7.
71. Fayed O, van Griensven M, Tahmasebi Birgani Z, Plank C, Balmayor ER. Transcript-activated coatings on titanium mediate cellular osteogenesis for enhanced osteointegration. Molecular Pharmaceutics. 2021;18(3):1121-1137.
72. Wong CC, Yeh YY, Chen CH, Manga YB, Jheng PR, Lu CX et al. Effectiveness of treating segmental bone defects with a synergistic co-delivery approach with platelet-rich fibrin and tricalcium phosphate. Materials Science and Engineering: C. 2021;129:112364.
73. Al-Maawi S, Dohle E, Lim J, Weigl P, Teoh SH, Sader R et al. Biologization of PCL-mesh using platelet rich fibrin (PRF) enhances its regenerative potential in-vitro. International Journal of Molecular Sciences. 2021;22(4):2159.

74. Rastegar A, Mahmoodi M, Mirjalili M, Nasirizadeh N. Platelet-rich fibrin-loaded PCL/chitosan core-shell fibers scaffold for enhanced osteogenic differentiation of mesenchymal stem cells. Carbohydrate Polymers. 2021:118351.
75. Kyyak S, Blatt S, Pabst A, Thiem D, Al-Nawas B, Kämmerer PW. Combination of an allogenic and a xenogenic bone substitute material with injectable platelet-rich fibrin–A comparative in vitro study. Journal of Biomaterials Applications. 2020;35(1):83-96.
76. Blatt S, Thiem DG, Pabst A, Al-Nawas B, Kämmerer PW. Does platelet-rich fibrin enhance the early angiogenetic potential of different bone substitute materials? An in vitro and in vivo analysis. Biomedicines. 2021;9(1):61.
77. Blatt S, Burkhardt V, Kämmerer PW, Pabst AM, Sagheb K, Heller M et al. Biofunctionalization of porcine-derived collagen matrices with platelet rich fibrin: influence on angiogenesis in vitro and in vivo. Clinical oral investigations. 2020;24(10):3425-3436.
78. Mu Z, He Q, Xin L, Li Y, Yuan S, Zou H et al. Effects of injectable platelet rich fibrin on bone remodeling in combination with DBBM in maxillary sinus elevation: a randomized preclinical study. American Journal of Translational Research. 2020;12(11):7312.
79. Bahammam MA, Attia MS. Expression of vascular endothelial growth factor using platelet rich fibrin (PRF) and nanohydroxyapatite (nano-HA) in treatment of periodontal intra-bony defects-a randomized controlled trial. Saudi Journal of Biological Sciences. 2021;28(1):870-878.
80. Chen MC, Chiu HC, Kuo PJ, Chiang CY, Fu MM, Fu E. Bone formation with functionalized 3D printed poly-ε-caprolactone scaffold with plasma-rich-fibrin implanted in critical-sized calvaria defect of rat. Journal of Dental Sciences. 2021, 16(4): 1214-1221.
81. Lavagen N, Nokovitch L, Algrin A, Dakpe S, Testelin S, Devauchelle B et al. Efficiency of advanced-PRF usage in the treatment of alveolar cleft with iliac bone graft: a retrospective study. Journal of Cranio-Maxillofacial Surgery. 2021;49(10):923-928.
82. Trimmel B, Gyulai-Gaál S, Kivovics M, Jákob NP, Hegedűs C, Szabó BT et al. Evaluation of the histomorphometric and micromorphometric performance of a serum albumin-coated bone allograft combined with A-PRF for early and conventional healing protocols after maxillary sinus augmentation: a randomized clinical trial. Materials. 2021;14(7):1810.
83. Alexandru BC, Popa M, Oana L, Bondor CI, Georgiu C, Lazăr CS et al. Bone morphogenetic protein 7 expression in alveolar bone addition with autologous blood, lyophilized bone and atelocollagen. In Vivo. 2021;35(2):871-81.
84. Giacon TA, Giancola F, Paganini M, Tiengo C, Camporesi EM, Bosco G. Hyperbaric oxygen therapy and A-PRF pre-treated implants in severe periodontitis: a case report. International Journal of Environmental Research and Public Health. 2021;18(2):413.
85. Ghaznavi D, Babaloo A, Shirmohammadi A, Zamani AR, Azizi M, Rahbarghazi R et al. Advanced platelet-rich fibrin plus gold nanoparticles enhanced the osteogenic capacity of human mesenchymal stem cells. BMC Research Notes. 2019;12(1):1-6.
86. Fahimipour F, Bastami F, Khoshzaban A, Jahangir S, Eslaminejad MB, Khayyatan F et al. Critical-sized bone defects regeneration using a bone-inspired 3D bilayer collagen membrane in combination with leukocyte and platelet-rich fibrin membrane (L-PRF): An in vivo study. Tissue and Cell. 2020;63:101326.
87. Bastami F, Noori-Kooshki MH, Semyari H, Tabrizi R, Abrishamchian A, Mashhadi-Abbas F et al. Multi-walled carbon nanotube/hydroxyapatite nanocomposite with leukocyte-and platelet-rich fibrin for bone regeneration in sheep model. Oral and Maxillofacial Surgery. 2021;177:1-10.