International Journal of Nanomedicine

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REVIEW

A review of fibrin and fibrin composites for bone tissue engineering

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Abstract: Tissue engineering has emerged as a new treatment approach for bone repair and regeneration seeking to address limitations associated with current therapies, such as autologous bone grafting. While many bone tissue engineering approaches have traditionally focused on synthetic materials (such as polymers or hydrogels), there has been a lot of excitement surrounding the use of natural materials due to their biologically inspired properties. Fibrin is a natural scaffold formed following tissue injury that initiates hemostasis and provides the initial matrix useful for cell adhesion, migration, proliferation, and differentiation. Fibrin has captured the interest of bone tissue engineers due to its excellent biocompatibility, controllable biodegradability, and ability to deliver cells and biomolecules. Fibrin is particularly appealing because its precursors, fibrinogen, and thrombin, which can be derived from the patient's own blood, enable the fabrication of completely autologous scaffolds. In this article, we highlight the unique properties of fibrin as a scaffolding material to treat bone defects. Moreover, we emphasize its role in bone tissue engineering nanocomposites where approaches further emulate the natural nanostructured features of bone when using fibrin and other nanomaterials. We also review the preparation methods of fibrin glue and then discuss a wide range of fibrin applications in bone tissue engineering. These include the delivery of cells and/or biomolecules to a defect site, distributing cells, and/or growth factors throughout other pre-formed scaffolds and enhancing the physical as well as biological properties of other biomaterials. Thoughts on the future direction of fibrin research for bone tissue engineering are also presented. In the future, the development of fibrin precursors as recombinant proteins will solve problems associated with using multiple or single-donor fibrin glue, and the combination of nanomaterials that allow for the incorporation of biomolecules with fibrin will significantly improve the efficacy of fibrin for numerous bone tissue engineering applications.

Keywords: fibrin, fibrinogen, injectable hydrogel, fibrin preparation, fibrin beads, fibrin coating, nanofibrous scaffold, bone repair

Introduction

There are over 200 bones of different shapes, sizes, and functions in the human body. They provide the weight-bearing structure for the body and play several important roles such as protection of the most vital organs, movement and locomotion of the body, production of blood cells, and acting as a storehouse for growth factors and minerals.[1] Therefore, loss of this multifunctional tissue adversely affects the patient's quality of life and represents a burden for the health care system. Fortunately, bone exhibits unique regenerative capacity and can heal without structural or functional impairment. However, if the defect size is greater than the healing capacity of osteogenic tissues, the site will not regenerate spontaneously. Furthermore, diseased bones are incapable of complete healing. In this situation, orthopedic surgeons have different biomaterial

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http://dx.doi.org/10.2147/IJN.S124671

possibilities: autogenous bone grafting, allogenic bone grafting, or the use of synthetic biomaterials.

Autogenous bone grafts that involve harvesting the bone from one site (usually from the iliac crest) of the patient and transplanting it into a damaged area of the same patient comprise ~58% of bone substitutes and remain the gold standard for the reconstruction of small bone defects.[2] They possess osteoconductive, osteoinductive, and osteogenic characteristics due to the presence of bone chips, osteogenic cells, and growth factors, respectively.[3] Nevertheless, their use is associated with disadvantages including donor site morbidity, limited graft supply, bleeding, chronic pain, infections, and poor cosmetic outcomes.[3]

The allograft, which involves transplanting donor bone tissue, often from a cadaver, constitutes ~34% of the bone substitutes.[2] Allogeneic bone grafts are not associated with donor site morbidity and are available in various forms and sizes. However, several drawbacks are associated with allografts: risk of transmission of infectious diseases, possibility of immunological rejection, and loss of biological and mechanical properties due to graft sterilization. Moreover, the demand for allograft tissue far exceeds the available supply.[3–5]

Bioinert materials such as alumina, stainless steel, and poly(methyl methacrylate) (PMMA) have been used in a wide range of bone surgeries. The considerable advantages of these implants over biological grafts are their availability and reproducibility. However, these biomaterials do not integrate well with the host bone and are encapsulated by fibrous tissue after implantation in the body. Production of wear debris and a high mismatch in stiffness between load-bearing implants and the adjacent bone are additional limiting factors.[6,7]

Tissue engineering has emerged as a new therapeutic approach for bone repair and regeneration, seeking to overcome such potential problems related to the aforementioned approaches. The optimal tissue-engineered construct relies on three essential components: a suitable cell source, growth and differentiation factors, and an appropriate scaffold to support cell-based regeneration of tissue. So, scaffolds play a pivotal role in bone tissue engineering and the selection of an appropriate biomaterial is crucial. Scaffold materials must be biocompatible, biodegradable, facilitate cell penetration and bone ingrowth, provide biomechanical support until the cells regenerate bone, be inexpensive, readily available and easy to produce and handle.[8,9] To fulfill these requirements, numerous biomaterials have been widely tested with varying success. Details of their advantages and disadvantages are found in many other comprehensive reviews.[10–12]

Fibrin possesses remarkable advantages over other biomaterials, which makes it an ideal candidate for bone tissue engineering applications. It is nature's nano-scaffold following tissue injury to initiate hemostasis and provide a temporary structure that facilitates cellular activities and also deposition of a new extracellular matrix (ECM).[13,14] Fibrin gel precursors, fibrinogen and thrombin, can be derived from a patient's own blood, which enables the fabrication of completely autologous and inexpensive scaffolds. Moreover, owing to its multiple interaction sites for cells and other proteins, fibrin acts as a bioactive matrix and is suitable for cell and biomolecule delivery systems.[13] In addition, the structural properties of fibrin substrates can be easily controlled by changes in fibrinogen and/or thrombin concentrations in the precursor solutions.[15] Furthermore, fibrin can be injected as a liquid and it solidifies thereafter in situ; thus, it can fill bone gaps with any shape or geometry. However, because of the rapid degradation and weak mechanical properties of fibrin, many investigators use fibrin in combination with other materials to overcome such limitations. Nanomaterials have become a popular material to use with fibrin in order to promote bioactivity of fibrin and to further emulate the natural nanostructured features of bone itself.

Difficulties involved in assessing the literature

Despite the ever-increasing volume of publications regarding the use of fibrin in bone tissue engineering, the usefulness of fibrin remains debatable. Much of the contradiction among the literature arises from using a variety of different fibrin glues (Table 1). Fibrin glue can be prepared either from pooled plasma, for example, commercially available fibrin sealants, or from single-donor plasma. Isolation techniques (eg, cryoprecipitation and ethanol precipitation), batch to batch variability, and composition (eg, concentration of thrombin, fibrinogen, factor XIII, and antifibrolytics) lead to different mechanical and biological properties of the generated clots.[16,17] For example, Dickneite et al showed clear differences in hemostatic and clot strength properties of 12 commercially available fibrin sealants.[17] Furthermore, differences in component origin may change the biochemistry of the clot, like interspecies or pooled and single donor.[18,19] Variations in experimental design, implantation site, and animal models as well as fibrinogen and thrombin concentrations are other parameters that make direct comparison of the research findings difficult. Moreover, to achieve the desired biological and/or physical properties for bone tissue engineering applications, fibrin must be reinforced, and there is a board range of nano- and other materials such as autogenous bone grafts,[20,21] allogenic bone grafts,[22,23] xenogeneic bone grafts,[24] metals,[25–27] ceramics,[28–30]

Table 1 Diversity in fibrin sealants and experimental design seen in the literature

Trade name	Fibrinogen (mg/mL)	Thrombin (IU/mL)	Factor XIII (U/mL)	Aprotinin (KIU/mL)	Adjacent agent	Implantation site	References
Biocol®	127	558	11	3,000	Calcium carbonate granules and TGF-β	Parietal (rabbit)	145
Quixil®	60–100	1,000	None	Transexamic acid	Autologous bone graft	Alveolar bone (human)	125
Bolheal®	80	250	75	1,000	β-TCP and BMSc	Subcutaneous (rat)	197
Beriplast®	65–115	400–600	40–80	1,000	Allogenic periosteal cell and PLGA	Ulna (rabbit)	104
Tisseel®	75–115	500	10–50	3,000	MSCs/HA-coated titanium	Tibia (sheep)	114
Tisseel	75–115	500	10–50	3,000	Biphasic calcium phosphate ceramic	Different locations (human)	140
Tisseel	75–115	500	10–50	3,000	Biphasic calcium phosphate ceramic	Femur (rabbit)	138
Tisseel	75–115	500	10–50	3,000	MDSCs	Calvarium (mouse)	171
Greenplast®	100	500	66	1,100	Apatite-coated PLGA/HA particulates and BMP-2	Calvarium (rat)	169
Bolseal®	50–75 (porcine fibrinogen)	400	10–70		Gelatin/HA nanoparticles and (BMP-2)	Radial diaphysis (rabbit)	36
Sigma	200	100			HA nanoparticles/BMP-2	Calvarium (mouse)	186
Calbiochem	20	2.5			Apatite-coated PLGA microspheres	Calvarium (rat)	198
	Single-donor human	Bovine thrombin (Merck®)			HA and HABGS	Intramuscular (mouse)	19
	Autologous	Autologous		Tranexamic acid	Autogenous bone grafts	Maxillary sinus (dog)	128
	Single-donor (rat origin)	Single-donor (rat origin)		Tranexamic acid	Osteogenic cells and BMP-2	Subcutaneous of dorsum (mouse)	199

Abbreviations: TGF-β, transforming growth factor-β; β-TCP, β-tricalcium phosphate; PLGA, poly(lactide-co-glycolide); HA, hydroxyapatite; MDSC, muscle-derived stem cells; BMSC, bone marrow-derived stromal cell; MSC, mesenchymal stem cell; BMP-2, bone morphogenetic protein 2; HABGS, Calcium phosphate calcium silicate system.

polymers,[31–33] and composites.[34–36] Biological components including (but not limiting to) mesenchymal stem cells (MSCs) derived from various tissues such as bone marrow,[18] adipose tissue,[37] umbilical cord,[32] muscle,[38] skin,[39] tooth,[40] and osteoblasts from different regions[41–43] and also several biomolecules and growth factors like bone morphogenetic protein (BMP),[37] basic fibroblast growth factor (bFGF),[41] and vascular endothelial growth factor (VEGF)[44] can be used in combination with fibrin. Different properties of these adjacent materials led to different results both in vitro and in vivo.[45]

However, all fibrin that is used in the literature is the same in that it is a natural biopolymer with unique physical and biological properties. These qualities have attracted much attention for the use of fibrin glue in bone tissue engineering. With these conflicting results in mind, the following sections explore the role of fibrin in these studies (Table 1).

Composition, structure, and properties of fibrin

Composition and structure of fibrin

Fibrin is a natural biopolymer formed in the last step of the clotting cascade by the action of thrombin on fibrinogen.[46] Fibrinogen is a large, complex, fibrous glycoprotein essential to many biological processes such as hemostasis, wound healing, inflammation, angiogenesis, and so on.[47] It is composed of two sets of three polypeptide chains (Aα, Bβ, and γ), including the fibrinopeptides A and B, which are joined together by 29 disulfide bonds within the E domain. The α and γ chains are linked to the same chains of the opposite subunit by one and two interchain disulfide bond(s), respectively. The two β chains are not directly linked to each other: the β chain of one subunit is linked to the α chain of the other subunit via a disulfide bond, while an additional disulfide bond connects the second β chain to the γ chain of the opposite subunit (Figure 1).[48] The rod-shaped fibrinogen protein is ~45-nm long containing two identical outer D domains each connected to the central E domain, which contains the two pairs of fibrinopeptides, by a coiled-coil segment.[49]

In the last step of the coagulation cascade, thrombin cleaves off two sets of peptides, fibrinopeptides A and B, from the amino terminal ends of the Aα and Bβ chains which exposes knobs that can interact with holes that are always exposed at the ends of the molecule. Hence, each E-site interacts with a complementary binding site located on the D domain of adjacent molecules.

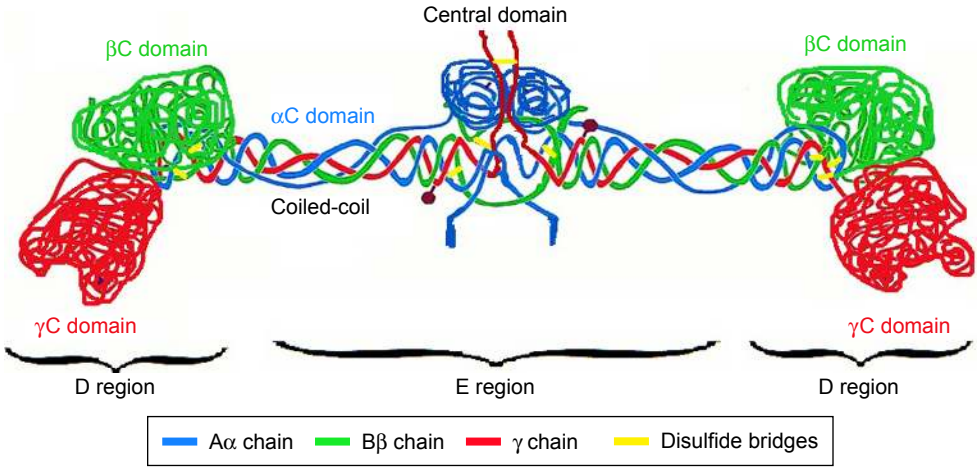

Figure 1 Fibrinogen structure. Aα chains are shown in blue, Bβ chains are shown in green, and γ chains are shown in red. Disulfide bridges stabilizing the coiled-coil regions are shown in yellow.

Such "E:D" associations result in the spontaneous formation of half-staggered, double-stranded protofibrils. These protofibrils then aggregate and branch, yielding a three-dimensional (3D) clot network, as shown in Figure 2.[47,48] Such structures are inherently weak, but the covalent cross-linking of fibrin by factor XIIIa leads to the formation of a stable structure.

The fibrin structure can be described by variables such as thickness of the fibers, number of branch points, porosity, and permeability of the gels.[47] These parameters vary to a very large extent, depending on the conditions of polymerization such as fibrinogen and thrombin concentrations, salt concentration, pH, temperature, and the presence of other plasma proteins (eg, fibronectin and albumin).[48,50–52] Clots with thick fibers, few branch points, and large pores are formed at lower concentrations of thrombin while higher thrombin concentrations tend to yield clots with thin fibers, many branch points, and small pores.[15,53]

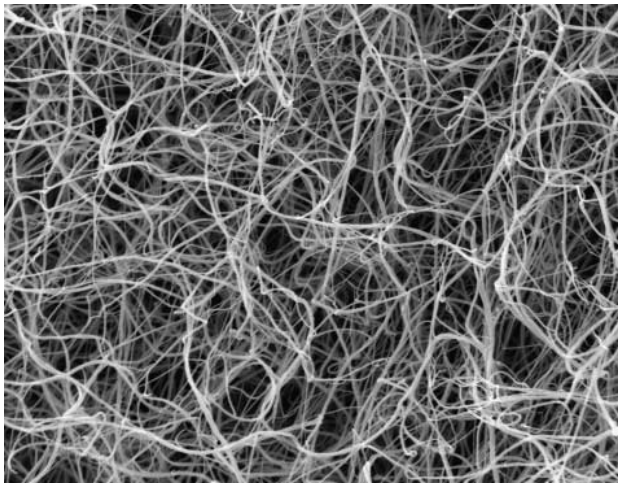

Figure 2 Scanning electron micrograph of 1 mg/L human fibrinogen polymerized by 1 U/mL thrombin at pH 7.4 and 150 mM NaCl. Full width of the figure is 62.5 mm. Janmey PA, Winer JP, Weisel JW. Fibrin gels and their clinical and bioengineering applications. *J R Soc Interface*. 2009;6(30):1–10, by permission of The Royal Society.[15]

Biological properties of fibrin

The fibrin matrix not only acts as a barrier preventing further blood loss but also provides a temporary scaffold needed to support tissue healing and remodeling.[49] Moreover, fibrin specifically binds numerous proteins and growth factors resident in normal tissue or is released into the wound in response to wound healing.[13,48,49] These proteins include ECM proteins such as fibronectin and vitronectin, many growth factors including FGF, VEGF, insulin-like growth factor-1, and enzymes like plasminogen as well as tissue plasminogen activator (tPA). This complex mixture of proteins bind to the fibrin matrix, enabling it to play an active role in wound healing through specific receptor-mediated interactions with cells.[13,49]

Mechanical properties of fibrin

Fibrin demonstrates unique viscoelastic behavior, that is, the mechanical response depends on the rate and duration of loading.[54] Stiffness and deformation provide information about the elastic and inelastic behavior of fibrin, respectively.[53] Fibrin stiffness behavior is a function of the experienced strain, a phenomenon which is called "strain hardening": at low strains, stress is directly proportional to strain, but at large strains, fibrin stiffness rises up to 20-fold with increasing strain. The study of fibrin deformation reveals that the fibrin network expresses irreversible deformation, but surprisingly, it completely recovers its stiffness after removing the stress. In other words, fibrin is

an experienced structure, such that there are no net changes in its structure during creep experiments.[55] Kim et al found that fibrin exhibits a nonlinear mechanical response to external compression and that its stress–strain curve has three distinct regimes.[56] First, a linear viscoelastic response to compression can be observed, in which most fibers are straight. Then, a stress plateau follows in which more and more fibers buckle and collapse. Finally, network densification occurs with a stress–strain response that is markedly nonlinear and dominated by bending of fibers after buckling and inter-fiber contact.[56] The authors in their next study showed that deformation of the fibrin matrix occurs nonuniformly and that as a downward force was applied on the top surface of the clot, the top layers compressed earlier and stronger than the lower network portions.[57]

Fibrin degradation properties

When the clot is no longer needed, fibrinolysis is activated to efficiently dissolve clots and avoid thrombosis. Plasmin that circulates in the blood as the precursor plasminogen is considered to be the main fibrinolytic enzyme.[58] Plasminogen adheres to the fibrin clot and is activated to plasmin by the action of plasminogen activators such as tPA that also binds to fibrin. Hence, fibrin has a dual role during fibrinolysis, functioning as both a cofactor and a substrate for the fibrinolytic enzyme plasmin.[59] Following activation, plasmin cleaves fibrin at specific sites and releases soluble fibrin digestion products into the circulating blood. There are several factors affecting the effectiveness of fibrinolysis in vivo, including the fibrin network structure, the clot hemodynamic environment, the kinetic properties of the fibrinolytic enzymes, and the balance of their formation and inactivation.[58]

Importance of fibrin composition, structure, and properties in bone tissue engineering

Each component of fibrin may have an important contribution in bone tissue engineering research. Bluteau et al noted that low thrombin concentrations (0.5–5 U/mL) upregulated the expression of osteoblastic markers and elicited an enhanced angiogenic response of osteoblasts by VEGF expression.[60] El-Hakim found that injection of factor XIII into the tail of diabetic rats significantly affected the healing process of bone defects that were created in the inferior border of mandible bone probably by stabilizing blood clots or forming stable bonds between the chains of collagen molecules.[61] Kim and Lee reported that coating of fibrinogen on the surface of biphasic calcium phosphate (BCP) noticeably enhanced human mesenchymal stem cell (hMSC) proliferation and adhesion, probably due to the surface roughness that was created by the adsorption of fibrinogen and/or by the function of an epitope region of β15–42 contained in fibrinogen. Furthermore, in vivo implantation of granules in a rabbit calvarial model revealed a significant improvement in bone healing through the addition of fibrinogen on the BCP surface. This improvement was attributed to the promotion of native fibrin clot formation, enhancement of initial stability of ceramic particles, and the effect of the fibrin matrix on fibroblast and vascular cell migration into the injury site.[62] Similarly, Santos et al reported that incorporation of fibrinogen into chitosan scaffolds improved bone formation in a critically sized bone defect in rats, which correlated with the elicited immune response.[63] In another study,[64] the authors seeded human monocytes on naked chitosan substrates or fibrinogen-coated chitosan and differentiated them into macrophages for 10 days. Results indicated that compared to pure chitosan substrates, fully differentiated macrophages on chitosan/fibrinogen films produced significant amounts of important factors involved in bone and wound healing and angiogenic mediators such as platelet-derived growth factor-BB (PDGF-BB), BMP-5, BMP-7, and FGF-7. The authors concluded that the adsorption of fibrinogen on chitosan films regulated macrophage responses toward a bone remodeling/regenerative phenotype.[64] Moreover, Linsley et al precoated cell culture plates with fibrinogen (10 mg/mL) and observed that these substrates were able to support hMSC attachment, proliferation, and early and late osteogenic differentiation especially in osteogenic medium.[65] Colley et al reported that MSCs that were precultured for 7 days on fibrin and then re-plated onto tissue culture plastic, exhibited superior proliferative, and osteogenic capacity compared to those of cells that were cultured directly on tissue culture plastic. These improvements were more obvious when cells were grown on lower fibrinogen concentrations. The authors provided evidence of better maintenance of the stem-like nature of MSCs that were cultured on fibrin gels made of lower concentrations of fibrinogen. The authors also emphasized the role of mechanical and topological properties as well as ligand density of fibrin gels on their properties.[66]

A change in fibrin properties dramatically influences the behavior of the cells inside it (Table 2). For example, Bensaid et al[67] reported that MSCs seeded in fibrin scaffolds containing low concentrations of fibrinogen and thrombin (1.8, 9, and 18 mg/mL of fibrinogen and 10, 50, and 100 U/mL of thrombin) were able to spread, proliferate, and express alkaline phosphatase (ALP) while in scaffolds

Table 2 Control of cell functions within fibrin by changing structural variables

Fibrinogen concentration	Thrombin concentration	Dependent variables	Main findings	References
1.8–90 mg/mL	10–500 IU/mL	Change in fibrinogen and thrombin concentration	Component concentration dramatically affects cell activity and gel degradation	67
5–50 mg/mL	2–250 IU/mL	Change in fibrinogen and thrombin concentration	Changing fibrinogen concentration reversely affects hMSC proliferation and osteogenic differentiation	68
5, 10, or 20 mg/mL	10 IU/mL	Change in fibrinogen concentration	In the presence of ceramics, greatest cell growth is detected in highest fibrinogen concentration	72
10–80 mg/mL	5 U/mL	Change in fibrinogen concentration	In the presence of bone powder, hMSC proliferation and osteogenic differentiation are increased by increasing fibrinogen concentration	73
5 mg/mL	0.25–4 U/mL	Change in thrombin concentration	Thrombin dose-dependently increased osteoblast differentiation by modulation of the fibronectin-binding capacity of fibrin	74
40 mg/mL	5 U/mL	Increasing ionic strength of the fibrinogen solution	Both osteogenic differentiation of entrapped hMSCs and physical properties of the final gel were improved	50
4 mg/mL	2.5 U/mL	Tensile stress applied to fibrin gels	BMSCs were cultured in a strained fibrin gel, exhibited a specific orientation which was identical to the strain direction	78

Abbreviations: hMSC, human mesenchymal stem cell; BMSC, bone marrow-derived stromal cell.

containing higher concentrations of respective components (45 and 90 mg/mL for fibrinogen and 250 and 500 U/mL for thrombin), the cells failed to do so. Researchers explained that at high concentrations of precursors, the fibrin gel may be too dense to allow for cell activities.[67] In another study, Catelas et al[68] noted that the proliferation of hMSCs increased in clots formed from a low fibrinogen concentration, while their osteogenic differentiation potential increased in formulations containing high fibrinogen concentrations. The authors suggested that the higher proliferation of cells is a result of a more open structure for the low fibrinogen concentration, and promotion of the differentiation of the cells at higher fibrinogen concentrations perhaps is caused by the presence of more growth factors contained in the fibrinogen component,[68] although other investigators have argued that there are no growth factors in commercial fibrin sealant components.[69] The same group in a separate study detailed the effects of the gel component concentration on the morphology of hMSCs.

Confocal microscopy images of hMSCs revealed that cells in gels with low fibrinogen concentrations (5 mg/mL) were elongated and spindle-like, but at high fibrinogen concentrations (50 mg/mL), the cells mostly appeared rounded. At intermediate concentrations, both elongated and rounded morphologies were observed.[70] Migration of cells from a fibrin matrix also depends on its formulation. Hale et al documented that MSC migration from fibrin hydrogels increased by the dilution of the fibrinogen component for both single-donor and commercial fibrin glues.[71] However, in vitro and in vivo outcomes may be different when fibrin is used in combination with other biomaterials rather than fibrin alone. For example, Linsley et al showed that while hMSC growth in pure fibrin scaffolds decreased with increasing fibrin concentration, in the presence of BCP ceramics, the trend was reversed and greatest cell growth was detected in the highest fibrin concentration.[72] Kim et al mixed demineralized bone powder and various fibrinogen concentrations (10, 20, 40, or 80 mg/mL) by 5 U/mL thrombin and showed that the proliferation and expression of osteoblastic markers of hMSCs were enhanced in scaffolds prepared from higher fibrinogen concentrations.[73] However, it is necessary to point out that there was a noticeable decrease in the growth rate of cells cultured on the scaffolds fabricated with 80 mg/mL fibrinogen, probably due to lower porosity of the scaffolds and the results may vary if the cell culture duration time increased. In agreement with this study, Abiraman et al reported that when bioactive glass or calcium phosphate calcium silicate granules that were coated by 5 mg fibrin glue were implanted into the muscles of mice, greater bone formation was observed than when granules were coated by 2.5 mg fibrin glue.[19]

To understand the mechanism of such cellular behavior, Oh et al[74] prepared fibrin matrices by mixing 0.25–4 U/mL of thrombin and 5 mg/mL of fibrinogen. The authors differentiated MC3T3-E1 osteoblast-like cells inside matrices and observed that thrombin dose-dependently increased osteoblast differentiation. This behavior was correlated to thrombin-induced alterations in the fibrin structure. They also provided evidence suggesting that thrombin modulated the fibronectin-binding capacity of fibrin through enlargement of surface area and/or exposure of the cryptic fibronectin-binding sites in fibrinogen.[74] The key role of the fibronectin-binding capacity of fibrin on cellular functions is supported by outcomes from other studies. Oh et al[75] showed that although the level of the total serum protein adsorption to fibrin was similar to that of collagen, the fibrin glue adsorbed

fibronectin ~6.7 times more. Their result also revealed that fibrin stimulated both the proliferation and differentiation of osteoblasts at higher levels compared to collagen.[75]

In addition to the mechanism of action, it should be pointed out that decreasing fibrinogen and thrombin concentrations negatively affects the mechanical and degradation properties of fibrin hydrogels. For instance, Bensaid et al[67] found that the gels prepared from 9 mg/mL fibrinogen and 50 U/mL thrombin resorbed quickly and completely disappeared within 15 days in vitro. In contrast, gels prepared with 90 mg/mL fibrinogen and 500 U/mL thrombin concentrations did not exhibit any detectable macroscopic degradation during the same time period.[67] Hence, in a different approach and to enhance both physical and biological properties of a fibrin gel, Davis et al increased the ionic strength of the fibrinogen solution through supplementation with sodium chloride (NaCl) before gelation and successfully improved both osteogenic differentiation of entrapped hMSCs and physical properties of the final gel.[50] Physiological concentrations of fibrinogen and thrombin also provided a slow gelation time, which prevented mechanical stress and osmotic shock of the entrapped cells. Noticeably, these low concentrations of fibrin constituents can be prepared from an autologous origin. In another study, the authors used such procedures to simultaneously improve the pro-angiogenic and osteogenic potential of entrapped MSCs in fibrin gels.[76] Furthermore, because human umbilical cord blood endothelial colony forming cells (ECFCs) produce BMP-2 as a paracrine signaling molecule, the addition of ECFCs to the system resulted in increased osteogenic potential compared with a monoculture of MSCs.

An alternative approach to manipulate structure and control cell functions within fibrin gels is to use mechanical force. It is well documented that fibrin, in a state of nature, exhibits a mesh-like structure composed of fibrils with no specific orientation.[53] However, Matsumoto et al reported that after applying a continuous mechanical force, clots displayed bundle-like structures with fibrils that were oriented parallel to the strain direction.[77] In a later work by the same group, bone marrow-derived stromal cells (BMSCs) were cultured in a strained fibrin gel and exhibited a specific orientation which was identical to the strain direction, suggesting that structural changes in the gel provided contact guidance cues to promote cell alignment. Moreover, the direction of the cell proliferation as well as mineral deposition in the strained gel was restricted to the same direction. The authors attributed this observation to tightly aggregated fibrin fibrils having no spaces or pores within its bundles and therefore inhibited random cell migration and mineral deposition. However, control of cell function was not limited to structural features, as the authors mentioned that a more rigid gel enhances osteoblast proliferation, whereas less rigid fibrin gels promotes differentiation.[78] In another study, they noted that these fibrin gels induced the formation of cortical bone-like tissue after subcutaneous implantation into mice, while implantation of control gels induced bone tissues with disordered structures (Figure 3).[79] Numerous proactive nanoscale surface features can be noticed after straining.

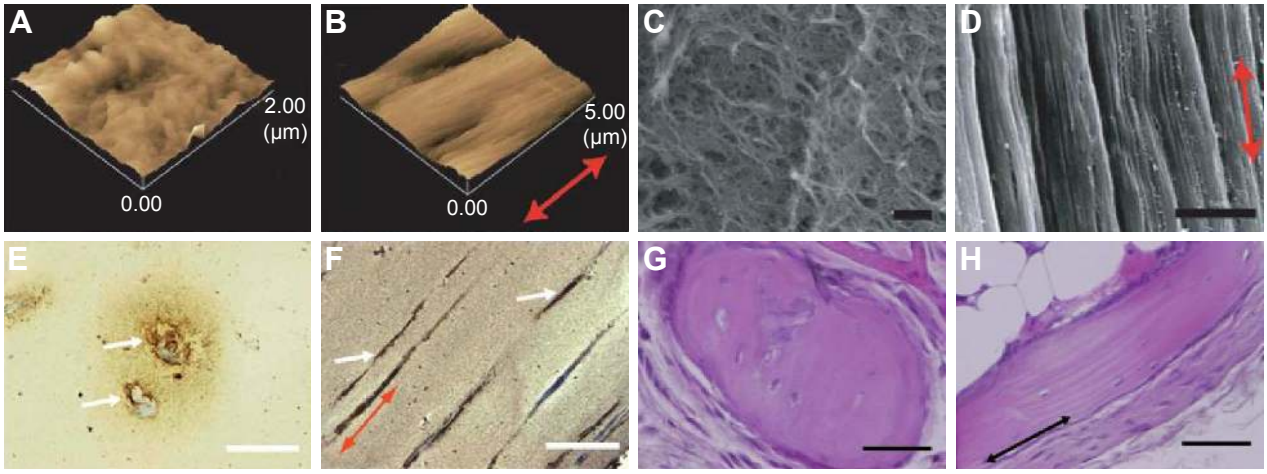

Figure 3 AFM images of a control fibrin gel (**A**) and a strained fibrin gel (**B**). Scanning electron microscopy images of a control fibrin gel (**C**) (bar: 1 μm) and bundle-like structures formed in a strained fibrin gel (**D**) (bar: 5 μm). Reproduced from Matsumoto T, Sasaki J-I, Alsberg E, Egusa H, Yatani H, Sohmura T. Three-dimensional cell and tissue patterning in a strained fibrin gel system. *PLoS One*. 2007;2(11):e1211.[77] Mineral depositions of contained mouse BMSCs detected by von Kossa staining of a control gel (**E**) and strained gel (**F**) (bar: 50 μm). Reproduced from[78] with permission of The Royal Society of Chemistry. H&E stained images of ectopic bone formation by nonstatic (**G**) and static (**H**) fibrin gels. Implanted fibrin gels were harvested at 6 weeks after implantation in mice (bar: 50 μm). Reproduced from Sasaki JI, Matsumoto T, Imazato S. Oriented bone formation using biomimetic fibrin hydrogels with three-dimensional patterned bone matrices. *J Biomed Mater Res A*. 2015;103(2):622–627.[79] The arrows indicate the mechanical force direction.

Abbreviations: AFM, atomic force microscopy; BMSCs, bone marrow-derived stromal cells; H&E, hematoxylin and eosin.

Another issue that requires attention is fibrin degradation rate. It has been shown that faster degrading of fibrin increases the expression of osteogenic markers by both MSCs and MG63 cell line while decreasing their proliferation.[80] However, fast degradation of fibrin does not give enough time for osteogenic cells to differentiate and produce their own matrix and fill the defect site. The addition of protease inhibitors into the matrices is a common method to prolong fibrin degradation time. Le Guehennec et al provided evidence that a minimal concentration of aprotinin, a potent competitive inhibitor for the active site of plasmin, is necessary for obtaining a stable fibrin scaffold onto which the bone cells can colonize and generate new bone.[81] However, because of its small size (58 amino acids, 6.5 kDa), aprotinin can readily diffuse out of the fibrin resulting in the loss of gel protection.[82,83] Researchers have tried to circumvent this problem by chemically conjugating aprotinin to fibrinogen[82] or by covalently crosslinking an engineered aprotinin variant into the fibrin matrix.[83] Despite these promising efforts, the suitability of aprotinin as a fibrinolysis inhibitor is debatable because of its heterologous origin that has occasionally been associated with adverse reactions or even death in humans.[84] To avoid these potential risks, tranexamic acid has been proposed as a safe alternative to this fibrinolysis inhibitor. Tranexamic acid is a synthetic derivative of the amino acid lysine that competitively inhibits the activation of plasminogen to plasmin.[84] Demol et al observed that supplementation of 0.5 mg/mL of tranexamic acid in culture medium successfully stabilized the fibrin hydrogels without affecting the activity of the encapsulated cells.[85] Jegoux et al compared the efficiency of aprotinin and tranexamic acid and concluded that both the inhibitors led to equivalent bone regeneration both qualitatively and quantitatively.[86]

Fibrin preparation

Fibrin sealants can be prepared at industrial scales by the fractionation of large pools of plasma or from single plasma donations by blood establishments or hospital blood banks.[87] The procedure for the preparation of the majority of commercial fibrin involves either thawing of the frozen plasma at 2°C–4°C (cryoprecipitate) or precipitation of plasma proteins with 10% ethanol (Cohn fraction I).[69,88] The manufacturing process may lead to co-purification of other plasma proteins such as fibronectin, factor XIII, and von Willebrand factor (vWF).[88] Adjustment of the concentration of the fibrinogen, typically to >80 g/L, is achieved by ultrafiltration.[69]

Thrombin is also generated at industrial scales from pooled plasma in several steps. First, prothrombin is isolated from plasma by cryoprecipitation and several chromatography steps. Prothrombin is then activated to thrombin, purified by chromatography and concentrated to 500–1,000 IU/mL by ultrafiltration.[69,89]

Fibrin can also be prepared from single-donor plasma either from autologous or allogeneic origin. A number of different methods can be used to isolate fibrinogen from plasma (Figure 4). These include cryoprecipitation or chemical precipitation with ethanol, ammonium sulfate, or poly(ethylene glycol).[16,90] The principal advantage of cryoprecipitation over other procedures is that no potentially cytotoxic chemicals are required for this process; however, it is time-consuming and the fibrinogen yield is extremely low. Cryoprecipitation is time-consuming but chemical precipitation is rapid. However, purity of the final product is a concern.[16,90] There are also methods and devices to generate thrombin from a single donation of plasma.[87,91]

Several considerations must be taken in account when selecting the appropriate fibrin glue for scaffold fabrication (Table 3). Commercially produced fibrin glue is available at a standardized quality, whereas single-donor fibrin glue suffers from batch-to-batch variability. Moreover, single-donor fibrin glues offer lower fibrinogen concentrations, less cross-linking of fibrin fibers, and poorer resistance to clot lysis compared to commercial fibrin glues.[92] But a single-donor fibrin glue does have some major advantages over commercial ones. First, the possibility of viral transmission is greatly reduced with the use of an autologous fibrin glue.[93] Furthermore, the steps used to inactivate pathogens may affect the biological properties of the final product.[94] The cost of a single-donor glue is another factor that is much lower than commercial fibrin.[93]

In addition, single-donor plasma can be prepared from platelet-rich plasma in which platelets present huge reservoirs of growth factors that may enhance bone repair.[95] In this regard, Xuan et al showed that fibrin prepared from autologous platelet-rich plasma, as an adjunct to Bio-Oss® particles for maxillary sinus augmentation of dogs, significantly increased bone formation in the graft sites compared to the case of Tisseel®.[96]

Different forms of fibrin applications in bone tissue engineering

In bone tissue engineering, fibrin glue is often used as beads and microbeads, coating agents on other scaffolds, pre-formed scaffolds, or injectable hydrogels. In the following sections, these different applications are described.

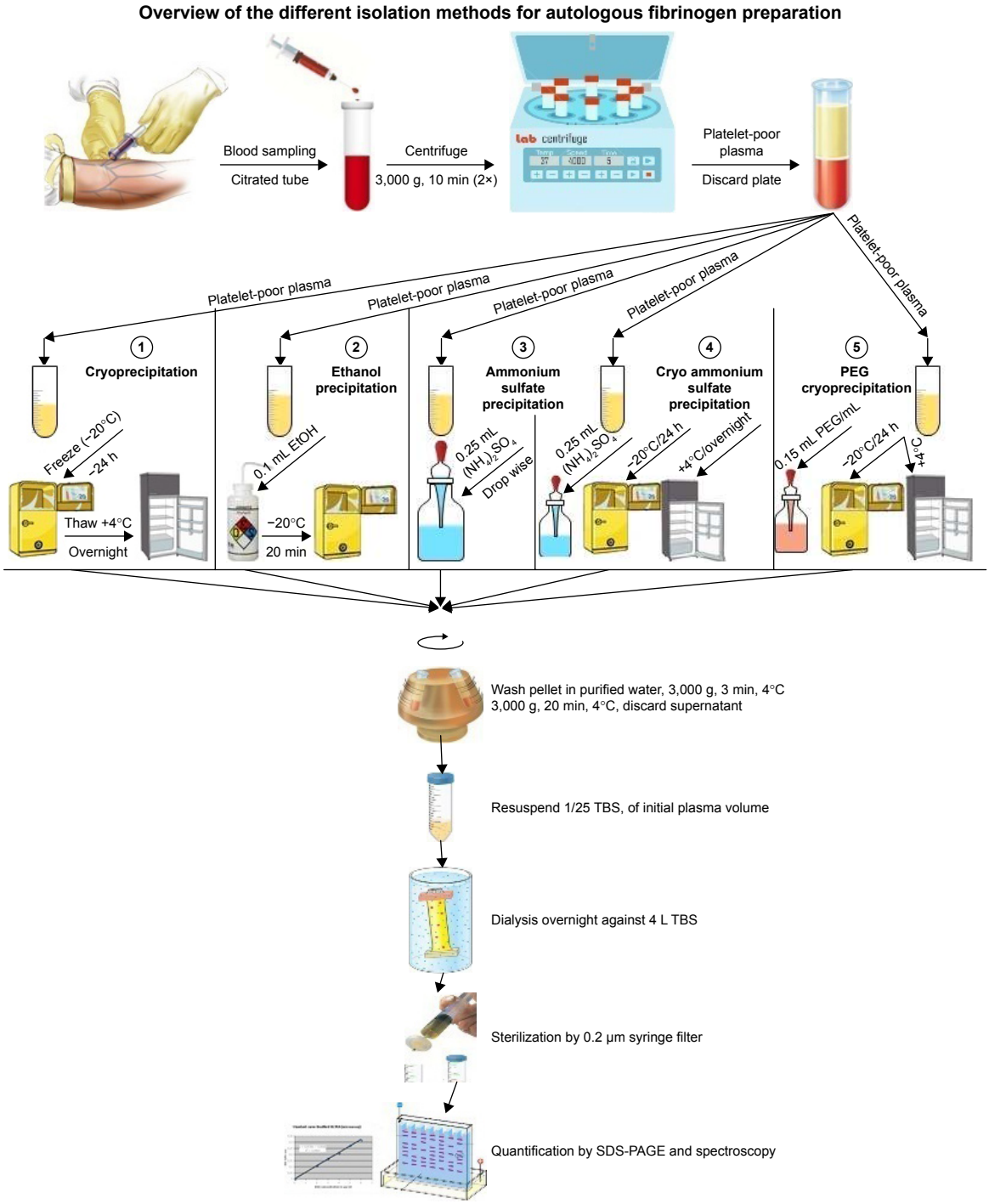

Figure 4 Different methods used to isolate fibrinogen from plasma.
Abbreviations: EtOH, ethanol; PEG, poly(ethylene glycol); SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris-buffered saline.

Fibrin beads for bone tissue engineering

Made of extensively cross-linked and partially denatured fibrin, fibrin microbeads (FMBs) have been used to improve the biodegradability of matrices while retaining their high cell-adhesion properties.[97] In a typical procedure, FMBs are synthesized by oil-stirring of a fibrinogen and thrombin mixture at 65°C–75°C for 6–8 hours and stored as a dry powder.[98] At this high temperature, fibrinogen denatures while factor XIIIa can remain active and cross-link proteins. Due to the conformational transformations of fibrinogen at these high temperatures, FMBs are highly haptotactic to various cell types, including osteogenic bone marrow-derived progenitors.[99,100]

For bone tissue engineering studies, the obtained FMBs were incubated for 2 days in a suspension containing whole

Table 3 Advantages and disadvantages of single-donor and commercial fibrin glue

	Pros	Cons
Commercial fibrin glue	• Standardized quality • High tensile strength • Available	• Risk of viral transmission • Expensive • Reduced bioactivity
Single-donor fibrin glue	• Cheap • Safe • Can be prepared from platelet-rich plasma	• Batch-to-batch variability • Less cross-linking of fibrin fibers • Low tensile strength

bone marrow (from mice or rats) and subsequently a high number of mesenchymal cells attached to them. In vitro and in vivo analysis demonstrated the osteogenic potential of the isolated cells.[97,98,101] Next, authors cultured FMB-MSCs constructs in osteogenic medium and obtained a bone-like structure which was implanted into a mouse skull defect. After 2 months, results showed that defects filled with bone-like tissue had a Ca/P ratio similar to that of the native bone while no repair was seen in the control animals without implants or when the defect was filled with FMBs alone.[102] In addition to efficiently repairing bone, the use of FMBs successfully eliminated stages of culturing the MSCs on tissue culture plastics, trypsinizing the cells that can damage their cellular membrane receptors, performing cell passages, and finally loading the cells onto the scaffolds.[102] Furthermore, FMBs may be used as a matrix onto which cells can be maintained for a long time at room temperature. The matrix preserves their proliferation and bone differentiation potential, which in turn facilitates cellular transformation between different research and/or clinical centers.[103]

Despite these promising results, in these studies, cells were seeded onto the surface of the beads rather than being encapsulated inside the microbeads.[32] In an effort to encapsulate periosteal cells inside fibrin, Perka et al fabricated cell-encapsulating fibrin beads ~3 mm in diameter.[104,105] The constructs were then implanted into ulna defects of rabbits. After 28 days, analysis revealed greater bone healing in cell-encapsulating fibrin bead groups compared to control groups with fibrin beads alone or untreated defects.[104] The same authors in their future study noted that the addition of α-TCP particles to fibrin beads improved both proliferation and differentiation of osteoblasts.[106] However, injectability of these large beads is an important concern.[32] In another approach, Zhou et al[107] developed alginate-FMBs with sizes of several hundred micrometers to encapsulate human umbilical cord mesenchymal stem cells (hUCMSCs). They demonstrated that the addition of a small amount of fibrin to the alginate microbeads greatly enhanced their degradation. The authors explained that fibrin may lose the alginate structure resulting in mechanically weaker microbeads that were readily degradable and/or fibrin enhanced cell attachment and proliferation in the microbeads which in turn increased the internal pressure of microbeads and accelerated their breakdown. Moreover, the released hUCMSCs exhibited excellent proliferation, osteogenic differentiation, and bone mineral synthesis features.[32,107] The same group, in a series of subsequent studies, mixed stem cell-encapsulating microbeads with a calcium phosphate cement (CPC) paste, where the microbeads could protect the cells from the mixing and injection forces. They noted that alginate-FMBs embedded in a CPC surface[107] or completely wrapped inside the CPC paste[108] and were able to quickly release the hUCMSCs that were attached to CPC. They also showed that incorporation of fibronectin and Arg-Gly-Asp into the CPC paste greatly improved cellular functions. Later, the released cells differentiated into an osteogenic lineage as indicated by the synthesis of bone minerals.[107,109]

Fibrin as a coating agent in bone tissue engineering

Due to the poor mechanical properties of fibrin sealants, many authors have frequently coated fibrin on more mechanically stable materials such as metals, polymers, and bioceramics. In these systems, the main function of fibrin was to facilitate cell/growth factor attachment and distribution within the entire scaffolds.

Zhu et al[110] showed that the addition of fibrin to the culture medium during seeding of hBMSCs into $CaCO_3$ scaffolds resulted in higher seeding efficiency as well as a more uniform distribution of cells throughout the scaffolds compared to seeding in culture medium alone. The polymerization of fibrinogen during seeding which prevented the cells from exiting the scaffold and the capacity of the fibrin glue to bind to cells possibly resulted in these effects.[110] The same group in a different study showed that the use of fibrin positively affected the osteogenic differentiation and angiogenic activity of the seeded cells by sustaining the expression of osteocalcin (OC) and VEGF, respectively. However, the maximum level of expression of OC and VEGF in hBMSCs did not increase through the use of fibrin.[111] Kneser et al[42] immobilized osteogenic cells in fibrin and seeded them into a processed bovine cancellous bone (PBCB) matrix. Later, scaffolds with and without cells were implanted into a rat calvarial defect. After 1 month, a low survival rate of transplanted cells especially in the central parts of scaffolds was observed, and no significant

differences in the process of bone healing was seen between the two groups.[42] In another study, Steffens et al co-seeded human endothelial cells and primary osteoblasts into PBCB-based scaffolds to investigate whether a blood vessel network which was created by endothelial cells may enhance survival of osteogenic cells.[43] Despite some preliminary promising results toward the formation of a 3D network of perfused human neovessels in chick embryo chorioallantoic membrane and subcutaneous mouse models, promotion of bone repair was not detected in the calvarial bone defects in mice. The authors postulated that endogenous neovascularization in the defects may have been sufficient to sustain osteogenesis.[112]

Nair et al[113] cultured BMSCs on bare or fibrin-coated bioactive ceramics made of hydroxyapatite (HA) and silica-coated HA cylinders for up to 28 days. Analysis of cell viability, morphology, proliferation, cell cycle assay, and differentiation showed that the fibrin considerably enhanced cellular functions.[113] Abiraman et al reported that bone formation occurs in quadricep muscles of mice only when fibrin is coated on bioactive glass and calcium phosphate calcium silicate granules.[19] Kalia et al[114] sprayed autologous MSCs, suspended within fibrin glue, onto HA-coated collars of Ti-Al (6%) V (4%) prostheses and inserted the implants into the tibia of sheep. After 6 months, radiographical and histological analyses showed that bone growth and bone contact to the implant surface increased by using MSCs and fibrin.[114]

Fibrin has also been coated on bioinert metallic implants to enhance cell distribution and response and to release growth factors. Kopf et al[115] noted that preincubation of Ti surfaces with whole human blood led to an increased number of attached cells, enhanced ALP and collagen-I protein expression, and promoted mineralization. These improvements in cell behavior were attributed to the creation of a 3D fibrin network on Ti surfaces and/or the release of cytokines that were embedded within the platelets in the fibrin matrix.[115] Spear et al[25] showed that fibrin deposition on porous networks composed of 444 ferromagnetic stainless steel fibers significantly influenced infiltration of human osteoblasts within the scaffolds. For control samples without fibrin, cells with well-spread morphologies were observed only on the metallic fiber surfaces and junctions, while fibrin-containing samples showed cell attachment over and between the fibers.[25] In another work, BMP-2 containing fibrin gels were used to fill porous Ti implants and repair critical-sized segmental femoral bone defects in rats. It is important to note that bone regeneration did not occur in control groups wherein defects were filled with empty Ti or fibrin-filled scaffolds. In other words, fibrin alone did not improve the bone regeneration potential of Ti implants but served as a delivery vehicle for BMP-2 that improved the bone repair process.[26] Fibrin can also be employed to simultaneously deliver osteogenesis and angiogenesis growth factors when incorporated into porous metallic implants.[27] Some other researchers have argued that constructing 3D scaffolds of natural polymers on metallic implants, rather than providing a thin coating, resembles the architecture of the native bone matrix more closely. Soumya et al created a 3D fibrin/alginate scaffold on Ti plates and noted that this modification improved hMSCs adhesion, proliferation, and subsequent differentiation into osteoblasts (Figure 5).[34]

In the case of synthetic polymers, Kang et al[37] coated the surface of poly caprolactone (PCL)/poly(lactic-co-glycolic acid) PLGA scaffolds with a mixture of fibrin and hyaluronic acid and used the obtained constructs as a delivery system for both BMP-2 and adipose-derived stromal cells (ASCs). Results showed that the coating of a scaffold with fibrin/hyaluronic acid significantly enhanced initial cell attachment without affecting the porosity of the scaffold. In addition,

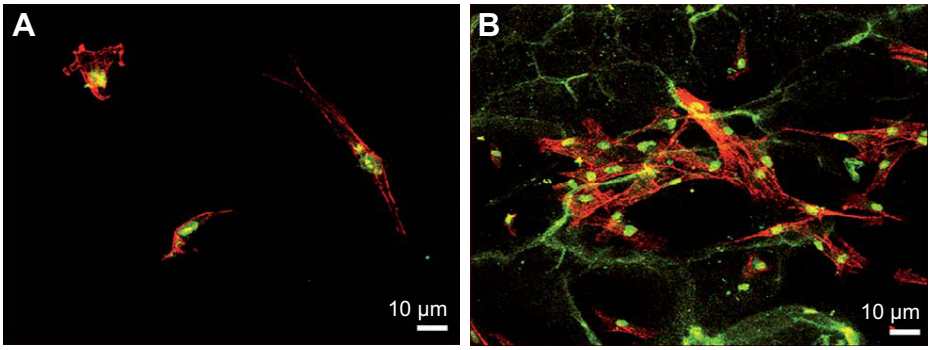

Figure 5 Confocal microscopic images of actin-stained hMSCs on (**A**) control Ti and (**B**) modified Ti after 6 h of incubation. The interpenetrating network of fibrin alginate scaffolds can be clearly seen on the modified Ti plate with the cells exhibiting a well spread morphology with a highly organized actin cytoskeletal arrangement than for the control plate. Reproduced from Soumya S, Sreerekha P, Menon D, Nair SV, Chennazhi KP. Generation of a biomimetic 3D microporous nano-fibrous scaffold on titanium surfaces for better osteointegration of orthopedic implants. *J Mater Chem*. 2012;22(5):1904–1915 with permission of The Royal Society of Chemistry.[34]
Abbreviation: hMSC, human mesenchymal stem cell.

ALP activity of ASCs seeded on the scaffold and ectopic bone formation in the mouse model was significantly improved by the coating.[37]

Schantz et al,[116] in a different study, seeded BMSCs or calvaria-derived osteoblasts within a fibrin matrix into the PCL scaffolds. Cell culture analysis revealed continuous cell proliferation, osteogenic differentiation of the cells, and homogeneous cell distribution within the PCL scaffolds.[116] Furthermore, PCL scaffold–cell constructs showed greater bone regeneration compared to non-seeded PCL scaffolds when implanted into critically sized calvarial defects in rabbits.[117] The same group further demonstrated that incorporation of osteoconductive calcium phosphate micro-particulates into PCL/fibrin scaffolds significantly improved cell attachment, proliferation, and differentiation into mineralized tissue.[35] However, some other researchers argued that in addition to osteoconductive scaffolds (composites of PCL and tricalcium phosphate) and osteogenic cells (BMSCs), a minimal amount of osteoinductive growth factors (BMP-2) is necessary to achieve successful bone formation in some situations such as defects in a posterolateral spinal fusion model.[118]

Fibrin may also be useful to improve the behavior of cells on other natural polymers. Conjunction of fibrin on chitosan/nano β-TCP composites markedly improved cellular functions on scaffolds including MSC attachment, density, proliferation, differentiation, and mineralization.[119] Kim et al[31] added different fibrin concentrations on collagen sponge scaffolds and noted that although the porosity and water-uptake ability were slightly reduced at high fibrinogen concentrations, the attachment, proliferation, and differentiation of cells were enhanced as evaluated by scanning electron microscopy (SEM) images, DNA content assays, and ALP activity, respectively. Authors attributed these improvements to an enlargement of surface area as a result of fibrin fibril formation and increased confluence between the cells (Figure 6).[31] Another explanation may be increases in fibronectin adsorption that in turn enhanced cell functions as shown by Oh et al.[74] However, these results are in contrast to Weinand et al who reported a superior bone formation ability of collagen I compared to fibrin glue, when these hydrogels were coated on porous β-TCP scaffolds.[18]

Some researchers have tried to combine tissue engineering approaches with genetic therapy to improve bone repair. In this context and to trigger a continued localized presence of an endogenously produced BMP-7, Schek et al[120] seeded transduced fibroblasts on solid free form-fabricated scaffolds by a fibrin gel or a poly-lactic acid sponge. The constructs were implanted subcutaneously into mice, and 4 weeks later, fibrin gels had a superior ability as a carrier by producing 2–4 times as much bone as the polymer sponge.[120] Yuan et al[121] developed a hybrid scaffold of nanocalcium sulfate (nCS) discs and fibrin containing BMP-2-producing BMSCs. To promote angiogenesis during bone formation, they also engineered two fusion proteins, VEGF and FGF9, with the fibrin binding sequence NQEQVSP to enable covalent binding of growth factors to fibrin by the action of FXIII during polymerization. The obtained constructs were implanted subcutaneously into mice, and 8 weeks later, results showed that the conjugation of FGF9 and VEGF in fibrin enhanced neovascularization and bone formation >4-fold compared to nCS with BMSC.[121]

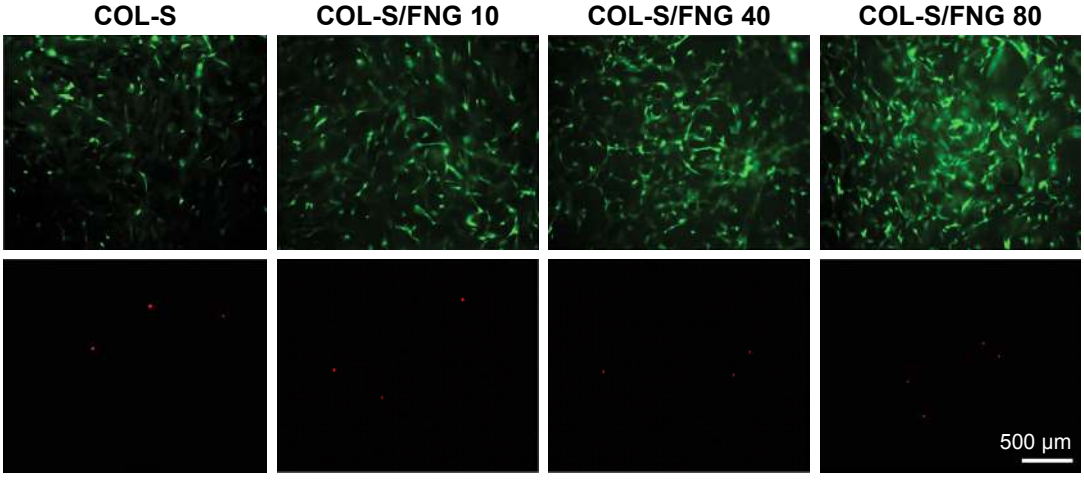

Figure 6 Live/dead fluorescence imaging of MG-63 cells cultured on collagen sponges (COL-S), COL-S/fibrinogen (FNG) 10, COL-S/FNG 40, and COL-S/FNG 80 scaffolds in normal growth media. The viability/cytotoxicity assay was performed after 5 days of culture. Live and healthy cells were stained green by calcein acetoxymethyl (Calcein AM), and dead cells were stained red by ethidium homodimer-1 (EthD-1). Copyright © John Wiley and Sons. Reproduced from Kim BS, Kim JS, Lee J. Improvements of osteoblast adhesion, proliferation, and differentiation in vitro via fibrin network formation in collagen sponge scaffold. *J Biomed Mater Res A.* 2013;101(9):2661–2666.[31]

Pre-formed fibrin scaffolds

Despite many beneficial features of injectable fibrin hydrogels, the precise control over the microarchitecture of these materials is not possible. Hence, some researchers have focused on developing fibrin scaffolds with a controlled microstructure. Since these scaffolds are often nanofibrous, they are discussed in detail in the "Application of nanotechnology in fibrin-based bone tissue engineering" section.

Injectable fibrin-based tissue engineering

Injectable fibrin hydrogels have been extensively investigated for applications in bone tissue regeneration due to their minimally invasive implant procedure, shortened healing time, reduced patient discomfort, and complications along with a decrease in health care costs. Moreover, they can easily fill irregular-shaped defects. Although fibrin possesses fascinating features, fibrin alone is not capable to heal bone defects,[26,152,169] and it is necessary to mix fibrin with other components such as biological bone grafts, osteoconductive biomaterials, osteogenic cells, and bioactive molecules.

The combination of a fibrin glue with an autologous bone graft to repair bone defects has been shown to improve surgical handling of graft particles and prevent their displacement or movement during the surgery.[122,123] Giannini et al provided evidence of a favorable improvement in bone regeneration, along with reduced infections and length of hospital stays upon the combination of autologous bone fragments with a mixture of platelet concentrates and a cryoprecipitate fibrin glue.[124] Tayapongsak et al also noted acceleration of revascularization and stimulation of the growth of both fibroblasts and osteoblasts, when using an autologous fibrin glue in mandibular defects of humans.[122] Segura-Castillo et al[125] also noted a considerable elimination of bone graft resorption in the reconstruction of alveolar clefts when using fibrin glue. This phenomenon allowed for the formation of a bridge between the healthy segments of the maxilla and the graft, which in turn improved integration of the graft through facilitating cellular migration.[125] Huh et al used autologous platelet-enriched fibrin glue (in which fibrinogen and thrombin were concentrated from platelet-rich plasma instead of the whole plasma) to improve bone regeneration capacity of the autogenous particulate bone when repairing the mandible defects in mongrel dogs.[126] The same authors, in their next studies, showed that the addition of platelet-enriched fibrin glue to autogenous bone grafts also effectively enhanced the vertical alveolar ridge[127] and maxillary sinus floor augmentation in dogs.[128] Despite these positive effects on the healing process, there are few reports on the negative effect of the combination of fibrin with an autologous bone graft on bone repair. Gerngross et al noted a negative impact on bone remodeling in the cortical tibia of a sheep model upon the addition of a fibrin sealant on autologous cancellous bone grafts.[20] Lappalainen et al[21] also observed a similar result upon the association of a fibrin glue with autologous particulate bone in rabbit critical-sized calvarial defects. The negative effect was explained by the potential role of fibrin in limiting the ingrowth of vessels into the grafted bone or its role as a void filler which prevents the formation of the natural fibrin.[21]

Due to the limitations related to the use of autologous bone graft, some authors have focused on combining autologous bone grafts with osteoconductive bioceramics to, at least partially, overcome such problems. Hallman et al[129,130] augmented the maxillary sinus floor of 20 patients with a mixture of bovine hydroxyapatite (BH), autogenous bone particles, and fibrin glue. Histological evaluation 6 months and 3 years after the procedure showed bone formation on the surface of the grafted materials. After 6 months, the bone that was formed around the BH particles was immature woven bone, while observations after 3 years showed that the mature lamellar bone replaced the woven bone.[129,130] Such grafts were also used to enable the insertion of Ti implants into the maxillary sinus floor of 20 patients with acceptable long-term results.[131]

On the other hand, the use of pure bioceramics in combination with fibrin glue has shown promising results in terms of handling characteristics of materials and adherence to the surrounding tissues.[132] Woo et al reported injectability of calcium sulfate ceramic powders was enhanced when combined with fibrin glue.[133] Similarly, Lopez-Heredia et al reported that the fibrin glue improved porosity, pore size, pore size distribution, and interconnectivity of the CPCs paste. These factors can be easily controlled by the ratio of fibrin glue to ceramic powders.[30] On the other hand, adding sufficient amounts of long setting fibrin, obtained using low thrombin concentrations, changed the fracture behavior of the ceramics, so that composite could maintain its shape even after reaching the maximal load and failure.[30]

It is important to note that in bone tissue engineering, the total mechanical properties of the implants including stiffness, toughness, and strength should be as close as possible to that of the normal bone. Ono et al implanted apatite-wollastonite containing glass ceramic (A-W.GC) granules with/without a fibrin sealant in the femoral defects of rabbits and after 24 weeks reported a better match of the mechanical properties of the implant site to those of normal

cancellous bone in the fibrin-containing group. In addition, they noted that as the volume ratio of fibrin to granules increased from 1:1 to 4:1, the mechanical properties of the implant site came closer to those of normal cancellous bone.[29] Dong et al also showed that the fibrin glue effectively enhances the mechanical properties of CPC nanoparticles[134] and microparticles[135] compared to the CPC/water group both in vitro and after implantation in the femurs of rabbits.

However, the benefit of fibrin glue on the bone repair capacity of bioceramics is a matter of controversy. Some reports show that fibrin glue not only enhances the physical and mechanical properties of the ceramics but also improves their biological performance, while others claim that fibrin glue jeopardizes the regeneration of bone tissue. Moreover, some findings indicate that fibrin glue enhances handling characteristics of ceramics but does not promote or inhibit the process of osteogenesis. Several reports highlighted the benefit of fibrin glue on the repair capacity of bioceramics. Kania et al found that adding commercial or single-donor fibrin to coral granules stimulates the early rate of bone regeneration.[136] Woo et al observed enhanced proliferation and differentiation of the osteoblasts upon the addition of a fibrin sealant to calcium sulfate cements.[133] Ono et al[28] reported that the addition of fibrin glue to the A-W.GC granules improved both early vascularization and bone formation of the mixtures after implanting them into the defects located in the proximal metaphysis of the rat tibia. Higher ratios of fibrin to ceramics, possibly by increasing the bone formation space, further enhances the bone regeneration.[28] Similarly, Dong et al added fibrin gels to CPC nanoparticles and implanted the mixture into the femoral defects of rabbits and observed greater regenerated bone in the fibrin/CPC group compared to CPC alone.[134] The CPC/fibrin glue system can also act as a carrier for BMPs that remarkably enhances the bone regeneration potential of the composite.[137] Guehennec et al noted a potential osteoinductive role for a fibrin sealant when added to BCP ceramics, while pure BCP showed an osteoconductive phenomenon.[138] Furthermore, deep bone colonization appeared to be slower for the fibrin/ceramic group compared to the ceramic alone group; probably because fibrin in the composite group filled the space between the granules.[138] The same group also showed that following sinus lift augmentation in the sheep models, the BCP/fibrin grafting was effective as equal or even superior to autologous bone grafting.[139] They also successfully used this composite for the treatment of bone defects that resulted from tumor resection or curettage in 51 patients.[140] Recently, van Esterik et al achieved success in improving osteogenic and vasculogenic differentiation potential of BCP/fibrin scaffold by modifying the composition of BCP from a HA/β-tricalcium phosphate (HA/β-TCP) ratio of 60/40 (BCP 60/40) to HA/β-TCP ratio of 20/80 (BCP 20/80).[141]

As mentioned earlier, some researchers noted a negative impact of fibrin on bone repair. For example, Carmagnola et al[142] noted that adding fibrin to Bio-Oss particles resulted in 4 times less tissue contact between bone and biomaterials in mandibular defects of dogs. Besides, in contrast to Bio-Oss alone group, no soft connective tissue replacement with newly formed bone was observed in Bio-Oss/fibrin treated defects.[142] A fibrin sealant was also reported to inhibit bone induction in the abdominal muscles of rats when used in combination with demineralized bone particles. The inhibited bone formation was perhaps due to the immunological response to xenogeneic proteins (fibrinogen and thrombin were from humans and aprotinin was of bovine origin).[143] Similarly, Bösch et al found that while heterologous fibrin impaired the bone healing process, due to a local immune response, homologous fibrin accelerated the growth of capillary vessels and connective tissue cells, which resulted in rapid new bone formation.[144] Other researchers argued that the addition of a fibrin sealant to coral granules limited the infiltration of osteoprogenitor cells into the implantation area.[145] This was further confirmed by an observed delay in bone formation in femoral defects of rats implanted with fibrin-containing PLGA/calcium phosphate scaffolds. The authors stated that high concentrations of fibrinogen (35–55 mg/mL) used for their experiments probably impeded cell invasion into the defects and caused delayed bone healing.[33]

Fibrin glue as an injectable delivery system in bone tissue engineering

Investigations have shown that the incorporation of osteogenic cells and/or biological factors into a fibrin matrix promotes bone healing. On the other hand, it has been documented that when osteogenic cells were mixed with fibrin glue, more bone formation occurred compared to a combination of cells with liquids, such as medium liquid[146] and phosphate-buffered saline.[38] Hence, here it is stated that both scaffolds and cells have critical roles in bone regeneration. Table 4 presents some of these applications of fibrin in bone tissue engineering which are explained in more detail below.

Cell delivery

Seebach et al[147] provided evidence that fibrin supports the early action of implanted BMSCs, such as host cell recruitment, modulation of the early inflammatory reaction, acceleration of new vessel formation and bone regeneration in the femoral defects of rats. Therefore, the authors suggested

Table 4 Delivery of cells and/or biomolecules to a bone defect site by fibrin

Cell	Biomolecule	Adjacent biomaterial	Implantation site	References
BMSCs			Femur of rat	147
MSCs and ECFCs				200
BMSCS or periosteal cells or alveolar bone cells	BMP-2		Subcutaneous tissue of mouse	149
BMSCs		Particulate mineralized bone	Tibia of rabbit	151
	BMP-2		Femur of rats	157
	Non glycosylated form of BMPs		Calvarium of rat	158
	BMP-2	Heparin conjugated fibrin system	Muscle of rat	163
	Substance P (fast release) and BMP (slow release)	Heparin conjugated fibrin system	Calvarium of mouse	170
	BMP-2	β-TCP	Calvarium of rats	159
AMSCs	BMP-2 and PDGF		Calvarium of mouse	177
Osteoblasts	bFGF		Subcutaneous tissue of rat	41
BMSCs	Vancomycin	Alginate beads	Tibia of rabbit	184
Stromal vascular fraction	BMP-2	Silicated calcium-phosphate granules	Subcutaneous tissue of mouse	154

Abbreviations: BMP-2, bone morphogenetic protein-2; β-TCP, β-tricalcium phosphate; PDGF, platelet-derived growth factor; substance P, a small peptide that effectively recruits MSCs to damaged tissues; BMSC, bone marrow-derived stromal cell; MSC, mesenchymal stem cell; ECFC, endothelial colony-forming cell; AMSC, adipose mesenchymal stem cell; bFGF, basic fibroblast growth factor.

that a fibrin hydrogel can serve as an attractive carrier for MSC-based tissue engineering approaches.[147]

Kim et al used fibrin-containing osteoblasts (obtained from the bone marrow of the iliac crest) to regenerate radial shaft defects of rabbits and noted that results were comparable to an autologous iliac bone graft.[148] In addition to BMSCs, researchers mixed several other cell types with fibrin to accelerate bone repair. Park et al proposed skin-derived mesenchymal stem cells (SMSCs) as an alternative to BMSCs after confirming the bone formation capacity of SMSCs both in vitro and in a mouse subcutaneous model. In these experiments, cell-embedded fibrin gels were injected into a demineralized bone matrix (DBM) scaffold.[39] Moreover, Zhu et al showed that periosteal cells have more bone formation potential compared to BMSCs or alveolar bone cells when mixed with single-donor fibrin glues and were injected into the subcutaneous space on the dorsum of nude mice.[149] However, McDuffee et al reported that BMSCs are more osteogenic than muscle-derived stem cells (MDSCs) when implanted intramuscularly into mice.[38] Despite this promising outcome, bone tissue engineering can always benefit from a more efficient cell/fibrin construct. For instance, Zhao et al demonstrated that the incorporation of β-tricalcium phosphate nanoparticles in a fibrin glue significantly increased both proliferation and ALP expression of entrapped MSCs.[150] Therefore, fibrin composites, such as particulate allogenic bone/fibrin[151] and gelatin/nano HA/fibrin,[36] are preferred for cell delivery purposes over fibrin alone. The co-delivery of growth factors and cells is another attractive approach to enhance the efficiency of a fibrin/cell mixture. Ito et al[152] filled fibrin, fibrin/autologous BMSCs, and fibrin/BMSCs/autologous platelet-rich plasma around metallic implants in a mandible defect of dogs. Histological and histomorphometric analyses after 4 and 8 weeks showed more bone–implant contact in the fibrin/cell group compared to fibrin alone, which was enhanced even more significantly when the fibrin/cells/platelet gel was used altogether.[152] Liu et al found that the delivery of MSCs and rhBMPs using fibrin glue was more effective in posterolateral spinal fusion compared to the delivery of single MSCs or rhBMPs.[153] Similarly, Mehrkens et al found that addition of 250 ng of rhBMP-2 inside the fibrin gel in conjunction with freshly isolated stromal vascular fractions (SVFs) of human adipose tissue induced ectopic bone tissue formation in mice,[154] while in the absence of incorporated BMP-2, the construct did not display any evidence of bone tissue formation.[154,155] However, recently, the authors noted that at orthotopic sites, exogenous BMP was not necessary and SVF could form bone tissue and vessel structures in a critically sized rat femoral defect. After that, autologous SVF cells were used to successfully treat low-energy proximal humeral fractures in 8 patients.[156]

BMP delivery

BMPs, which belong to the transforming growth factor (TGF) superfamily, are the most potent osteoinductive proteins. BMPs induced MSCs to migrate to bone-forming sites, differentiate into an osteoblast lineage, and regenerate bone.[157] Local pharmacokinetics are very important for the therapeutic efficacy of BMPs. For example, when

BMP-2 in saline is placed at the site of a bone defect, it is quickly cleared from that site, resulting in minimal bone formation and healing.[158] Besides this minimal effect, the rapid release of BMP-2 can cause adverse effects and will require more frequent administration which is costly.[157] Therefore, it is necessary to develop a delivery system that can release BMPs locally for a sufficient time period and concentration. Among several different BMP carriers, a fibrin matrix is considered as an excellent carrier. Schützenberger et al[157] added BMP-2 to fibrinogen and then mixed it with thrombin. Their in vivo study in a rat femoral defect showed that although compared to a commercially available absorbable collagen sponge-BMP-2 system, a 7-fold lower dose of BMP-2 used in fibrin gels, the fibrin-BMP-2 system would result in equal or superior bone formation versus a collagen/BMP-2 system.[157] As in the case of cell delivery, the addition of osteoconductive agents such as β-tricalcium phosphate to a fibrin carrier intensifies the efficacy of a delivery system.[159] Fibrin glue was also used to encapsulate collagen sponges loaded with BMPs and block bone formation in undesired areas by limiting BMP diffusion.[160] However, due to the relatively small size of BMPs compared to the pores of fibrin and their low affinity to interact with fibrin, BMPs were released quickly from the fibrin glue.[158] Hence, efforts have been made to enhance the delivery capacity from a fibrin glue. Schmoekel et al found that a less soluble form of BMPs, a non-glycosylated form (nglBMP-2), resulted in a more gradual release of growth factors in a Tris-buffered saline and therefore more bone healing compared to conventional soluble BMPs.[158] The utility of ng1BMP-2 was also confirmed in a series of prospective veterinary clinical trials.[158,161] The same group in another strategy designed a tripartite fusion protein, referred to as TG-pl-BMP-2 with TG denoting a transglutaminase-sensitive binding domain and pl the plasmin-sensitive linking domain. TG-pl-BMP-2 can be covalently incorporated into a fibrin matrix under the influence of the blood transglutaminase factor XIIIa, while a plasmin substrate provides a cleavage site for the local release of the attached growth factor under the influence of cell-activated plasmin. The authors observed that this system noticeably enhanced the bone healing process in the rat calvaria model compared to wildtype BMP-2 and also pancarpal fusion in dogs compared to a cancellous bone autograft.[162]

In another approach, Yang et al, conjugated heparin to fibrin and bound BMPs to heparin via electrostatic interactions between negatively charged sulfate groups of heparin and positively charged amino acid residues of the BMP-2 proteins.[163,164] In addition to the BMP-2 interaction with heparin in heparin-conjugated fibrin (HCF) systems, the release of BMP-2 could also be affected by the in vivo slower degradation of HCF compared to regular fibrin.[164] The efficiency of the HCF system for bone regeneration has been assayed in different animal studies including the hind limb muscle of rats,[163] calvarial defects in mice,[165] and spinal fusion in rabbits.[166] Hong et al[166] found that a very low dose (37.5 μg) of commercial BMP-2 applied in an HCF carrier promoted bone formation comparable to that of an autograft in a rabbit spinal fusion model. However, the authors emphasized that human host tissue is much less responsive than that of rodents; hence, before clinical utilization, large animal models should be used to test an HCF system with various doses of BMP-2.[166] This gradual release of BMP-2 can also reduce the potential side effects of excessive fatty marrow formation.[167] However, results from other studies of the same group demonstrated that heparin-conjugated PLGA nanospheres provide more control on protein release in vitro and bone formation in vivo compared to HCF systems.[163,168] The BMP-2 release rate was further slowed down by using a delivery system of apatite-coated PLGA/HA particulates as a carrier for the controlled release of BMP-2 which was suspended in the fibrin gel.[168,169] The adsorption and desorption of BMP-2 on HA occur through the physicochemical and electrostatic interactions between amino groups of proteins and a negatively charged site of HA crystals. Moreover, BMP-2 is released from a fibrin gel by a simple diffusion mechanism, which causes a short-term release of BMP-2. Hence, the long-term release of BMP-2 from apatite-coated PLGA/HA particulates suspended in a fibrin gel may be attributed to the combination of the two release mechanisms.[169] Figure 7 represents the bone formation ability of an apatite-coated PLGA/HA particulate/fibrin system in a critical-sized calvarial defect in rats.[169] The HCF system can be also employed to release multiple factors at different rates. In this scenario, the authors co-delivered a substance P (SP), a small peptide that is quickly released from the HCF gel and effectively recruits MSCs to damaged tissues and BMPs to further enhance the bone regeneration capacity of the HCF systems. The HCF gels with SP and/or BMP-2 were implanted into mouse calvarial defects for 8 weeks, and the results showed that the efficacy of bone regeneration was significantly higher in the SP/BMP-2 dual delivery group.[170]

As mentioned in the previous sections, the use of genetically engineered cells and viruses is another approach to deliver BMPs to a desired site. Usas et al used transduced

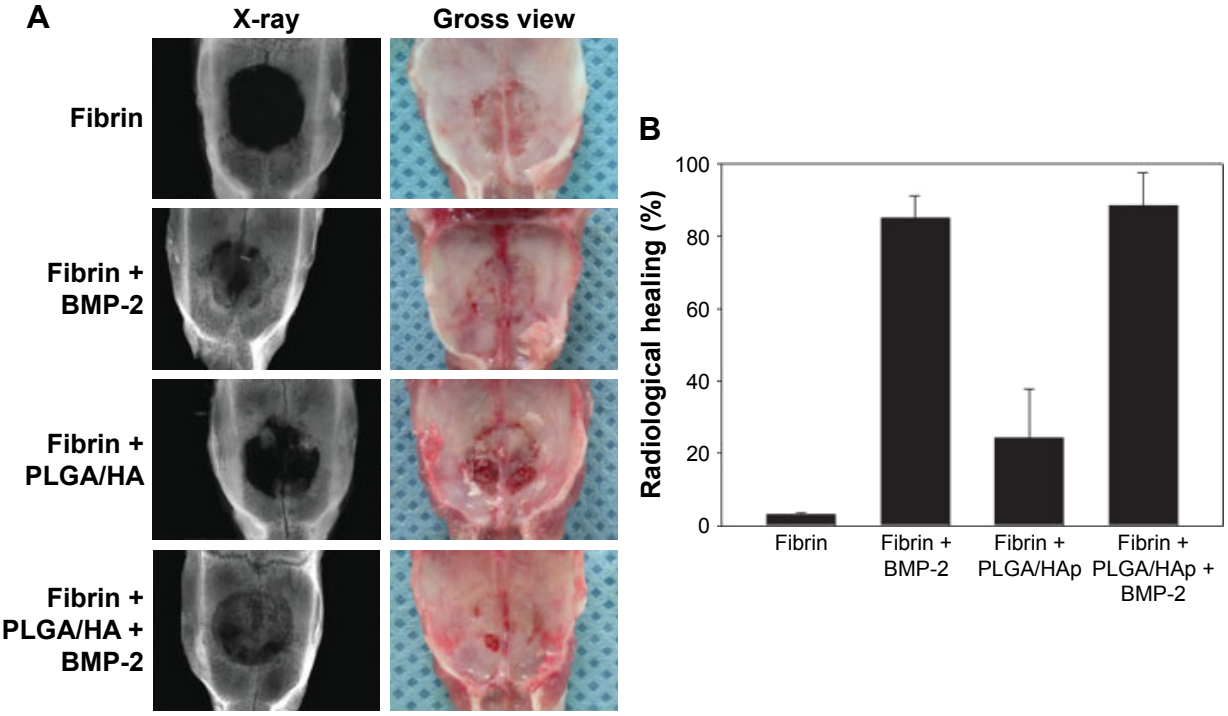

Figure 7 (**A**) Gross examination and soft X-ray examination of the fibrin gel group, fibrin gel apatite-coated PLGA/HA particulate group, BMP-2-loaded fibrin gel group, and BMP-2-loaded apatite-coated PLGA/HA particulates suspended in a fibrin gel group at 8 weeks after implantation into critical-sized calvarial defects of rats. (**B**) Radiological defect healing at 8 weeks. Reproduced from Kim SS, Gwak SJ, Kim BS. Orthotopic bone formation by implantation of apatite-coated poly (lactide-co-glycolide)/hydroxyapatite composite particulates and bone morphogenetic protein-2. *J Biomed Mater Res A*. 2008;87(1):245–253.[169]
Abbreviations: PLGA, poly(lactic-co-glycolic acid); HA, hydroxyapatite; BMP-2, bone morphogenetic protein 2; HAp, hydroxylapatite particulates.

MDSC secreting BMP-4 in hydrogels, such as fibrin, to promote bone regeneration in the calvarium of mice.[171] Sheyn et al showed that genetically modified porcine ASCs which overexpress rhBMP6 can induce vertebral defect regeneration in rodents[172] and in a clinically relevant, large animal pig model.[173] Immunohistochemical analyses also indicated that ASCs not only secrete BMPs but also differentiate into bone-forming cells in response to osteogenic induction and are actively involved in tissue regeneration itself.[172] However, there is some argument concerning the superior osteogenic potential of genetically modified BMSCs compared to that of modified ASCs both in vitro and in vivo.[174] In another study, Schek et al[146] mixed BMP-7 expressing adenovirus with collagen, fibrin, or cell culture medium and implanted them intramuscularly into nude mice. After 4 weeks, radiological and histological analyses showed a superior ability of fibrin glue to deliver the virus. Interestingly, in vitro culturing of mouse fibroblasts on a fibrin-contained virus showed that fibrin gel concentrations considerably affected viral infections of cells since lower fibrin concentrations resulted in a higher transduction levels of cells. The authors explained that the difference in fibrin structures led to differences in the diffusion rate of a virus which directly affected the infection of cells.[146]

Biomolecules other than BMP delivery

Many studies used fibrin for the delivery of other biomolecules. bFGF is a 16 kDa polypeptide that has osteogenic properties in addition to well-known angiogenic properties.[175] Jeon et al[176] demonstrated that by increasing thrombin or fibrinogen concentrations, a fibrin gel with a dense structure and also decreased degradation rate can be created to slow down bFGF diffusion. On the other hand, the incorporation of heparin to fibrin significantly retarded the release of bFGF from the hydrogel because heparin interacts with both bFGF and fibrin, and this specific interaction resulted in a slow release of growth factors.[176] In their next study, the researchers subcutaneously implanted fibrin-contained bFGF and/or osteoblasts in rats and found that compared to the delivery of osteoblasts alone, co-delivery of bFGF and osteoblasts resulted in two and nine times more bone formation and calcium content, respectively. The authors attributed these effects to enhanced osteogenic gene expression of the transplanted cells and neovascularization of the transplants.[41]

Zhang et al showed significantly improved revascularization as well as quality and speed of bone healing upon the delivery of VEGF to femoral defects, using fibrin-suspended PLGA microspheres.[44] PDGF is a growth factor secreted

by activated platelets which stimulates the repair mechanisms in wounded tissues such as bone. It has been shown that the addition of PDGF to cell culture medium of fibrin gels containing aggregated multicellular spheroid adipose mesenchymal stem cells significantly improves both vascularization and mineralization by cells. Vila et al also used fibrin glue for the controlled delivery of an engineered form of PDGF and BMP-2 to enhance bone and vasculature formation in mouse calvarial defects.[177] The same approach is involved in the delivery of parathyroid hormone (PTH1–34) which increases bone turnover via a direct and indirect effect on osteoblasts and osteoclasts. In a study by Arrighi et al,[178] an inactive prodrug TG-pl-PTH1–34 was immobilized to fibrin as in the case of TG-pl-BMP-2 (see section BMP delivery). After plasmin-induced cleavage, the released active PTH1–34 promoted bone regeneration at the desired site. The bone-forming capability of the PTH1-fibrin system was demonstrated in the femoral and humerus defects in sheep.[178] Zoledronate (ZOL) is a bisphosphonate used to treat osteoporosis by inactivating osteoclastic functions and promoting osteoclastic apoptosis. Jing et al encapsulated ZOL plus BMP-2 in a fibrin sealant and used the obtained system to repair the femoral necks of osteoporotic rabbits.[179] Fibrin scaffolds were also used for the delivery of heparan sulfate (HS), which is another bioactive factor enhancing bone repair. The uniform distribution of HS in the fibrin scaffolds was achieved by adjusting the concentration of thrombin, which demonstrates significant improvements in bone formation when injected into rat calvarial defects.[180]

Fibrin glue has also been used for efficient and adequate delivery of antibiotics to the desired location. Tofuku et al reported that the use of an antibiotic-impregnated fibrin sealant (AFS) significantly reduced the rates of deep surgical site infections (SSIs) in patients who underwent implantation of orthopedic hardware surgery; 11 of the 188 patients acquired a deep SSI in the group treated without AFS while none of the 196 patients acquired a deep SSI in the other group.[181]

Another research area is the treatment of bone defects that suffer from infection. Co-delivery of antibiotic and osteogenic cells via a fibrin scaffold may be an ideal option. To this end, Hou et al[182] embedded vancomycin alginate beads in a fibrin gel to supply sustained antibiotics at the graft site, while BMSCs seeded in the gel promoted bone regeneration. In vitro results showed that cell morphology did not change with exposure to the antibiotic beads but that cell proliferation and differentiation were enhanced in fibrin scaffolds containing antibiotic beads. The more porous fibrin structure resulting from the inclusion of the beads and presence of alginate were suggested as possible reasons for cell function improvement.[182] In their future in vivo study, they showed that inclusion of vancomycin alginate beads/fibrin in a DBM exhibited no negative impact on the process of osteogenesis and wound healing in the femoral defects of goats.[183] In addition, they successfully treated chronic osteomyelitis of rabbit tibia by a vancomycin alginate bead/BMSCs/fibrin gel delivery system.[184]

Application of nanotechnology in fibrin-based bone tissue engineering

In recent years, nanotechnology has become more prevalent and has found widespread use in the field of bone tissue engineering, especially in the fabrication of nanoparticles and nanofibrous scaffolds which mimic the structural hierarchy of native ECM. The diameter of fibrin fiber ranges between tens and a few hundreds of nanometers; therefore, all fibrin discussed in the previous sections are natural nanostructures. However, researchers have tried to develop methods by which they can precisely control the nano- and microstructure of fabricated fibrin scaffolds. Linnes et al[185] developed a method by which fibrin scaffolds of defined pore size, porosity, and microstructure can be fabricated. Such procedures involved pouring a fibrinogen and thrombin solution around an interconnected template made of PMMA beads. After polymerization of fibrin, the PMMA beads were dissolved in acetone through several rinses using acetone and the scaffolds were resolvated in 70% ethanol overnight.[185] Next, the authors incorporated calcium phosphate nanoparticles into the matrices and observed that in vitro culturing of osteoblast-like cells on modified scaffolds resulted in significantly higher ALP activity as well as osteoblast marker gene expression compared to fibrin scaffolds, while higher cell proliferation was detected on fibrin alone scaffolds. An in vivo study was performed by the implantation of disc-shaped scaffolds in cranial defects of mice, and the results demonstrated the promotion of bone formation in defects treated with fibrin/calcium phosphate scaffolds. In addition, scaffolds could serve as a delivery system for BMP-2 to further improve bone regeneration.[186] In their next study, the authors covalently immobilized ALP on the fibrin scaffolds using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. In vitro and in vivo results showed a superior bone-forming ability of immobilized ALP scaffolds compared to the fibrin alone group. However, the outcome was not robust, and the authors suggested that additional factors and/or procedures are required to achieve better results in vivo.[187]

Electrospinning is a unique technique that uses electrostatic forces and produces polymer fibers with diameters ranging from a few nanometers to a few microns. For the first time, Perumcherry et al[188] uniformly electrospun fibrin into nanofibers. Briefly, fibrinogen and thrombin solutions were separately mixed in a PVA solution. PVA was used as a carrier of fibrin to aid in electrospining. These two solutions were taken separately in two syringes and connected to a delivery device having a common needle at the end of the syringes. By applying a high voltage, the flow rate of the solutions was adjusted in such a way that the fibrin formation took place just before the formation of a fiber jet. SEM images revealed the formation of bead-free fibers with a dimension ranging from 50 to 500 nm.[188] Next, the authors fabricated a fibrin-based multiscale hybrid scaffold by sequential electrospinning of fibrin and PCL (Figure 8) or simultaneous electrospinning of fibrin and PLGA[189] to combine the benefits of biological properties of the fibrin and the excellent mechanical properties of the synthetic polymers.

Beside nanofibrous fibrin scaffolds, fibrin nanotubes and fibrin nanoparticles (FNPs) can be prepared by a water-in-oil emulsification–diffusion route. These constructs showed excellent stability against aggregation and high temperatures and also had the ability to deliver drug and growth factors in a controlled manner.[190] These nano-fibrin scaffolds were also used to enhance angiogenesis in chitin/poly (butylene succinate)/bioactive glass[191] and chitin/calcium sulfate gels.[192] Vedakumari et al[193] developed a wet precipitation method for synthesizing FNPs. In this technique, first, a crude form of fibrin was isolated from bovine blood by stirring the blood with a glass rod. Next, the obtained fibrin was washed, treated by 0.5 M sodium acetate, and 30% hydrogen peroxide and grounded. Later, fibrin was dissolved in NaOH (1 N), and then HCl (1 N) was added dropwise under vigorous stirring which eventually led to the formation of FNPs at pH 5.5. Biocompatibility of the FNPs was demonstrated by in vitro and in vivo analyses.[193] The authors, in their next study, prepared magnetic FNPs (MAG-FNPs). To this, they incinerated red blood cells of defibrinated blood and dissolved the residue in 5 N HCl to obtain an iron solution which was added dropwise to a fibrin/NaOH solution as mentioned earlier. The MAG-FNPs were crystalline and spherical with sizes ranging from 12 to 15 nm which improved the ALP activity of Saos-2 cells. Therefore, the authors concluded that MAG-FNPs could be widely used for magnetic field assisted bone tissue engineering applications.[194] Nanocomposites of iron–fibrin nanohydroxyapatite (IF–nHAp) were another novel biomaterial developed by them. IF–nHAp was needle shaped with an average width of ~30 nm and length of 80 nm. When an MG-63 cell line was cultured in the presence of this composite, its osteogenic potential improved as noted by the upregulated expression of osteogenic genes and enhanced calcium deposition and mineralization.[195]

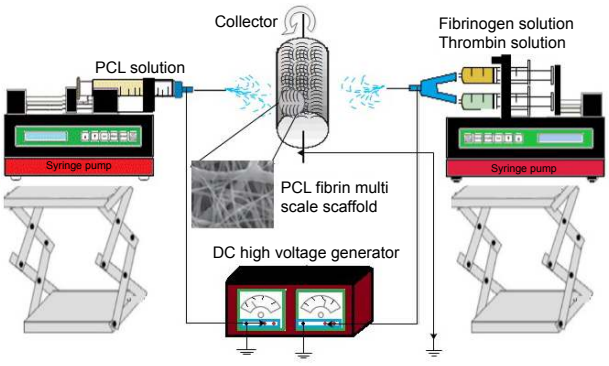

Figure 8 Schematic representation of the electrospinning of PCL fibrin multiscale nanoscaffolds. The arrows indicate steps that are repeated sequentially to obtain a composite scaffold.
Abbreviations: DC, direct current; PCL, poly caprolactone.

In this manner, as one can easily determine, the efficacy of fibrin was improved through its incorporation with nanoscaffolds and nanomaterials. It has been speculated that such nanomaterials improve the bioactivity of fibrin and availability of fibrin to interact with cells. It is important to mention in this context that nanomaterials have more surface area for interactions with fibrin, and since fibrin itself is a nanostructured protein, its bioactivity can be manipulated through nanostructures.

Conclusion and future trends

Fibrin is one of the most promising biopolymers used for bone tissue engineering applications, and therefore, the literature on the use of fibrin in this field is extensive and continues to evolve. Indeed, the combination of excellent biocompatibility, biodegradability, intrinsic bioactivity, and many other unique characteristics make fibrin an attractive biomaterial for bone tissue engineering. As mentioned in previous sections, commercial fibrin glues are expensive and are associated with the risk of disease transmission while single-donor fibrin glues suffer from batch-to-batch diversity. In the future, the development of fibrinogen and thrombin as recombinant proteins may solve these problems. Further studies have shown that fibrin alone is incapable of healing bone defects, and so, addition of other materials and/or biomolecules is unavoidable. The combination of fibrin with osteoconductive ceramics that allows the incorporation of growth factors, drugs, and metallic ions may enable the fabrication of multifunctional scaffolds that stop bleeding by

homeostasis features, deliver therapeutic agents depending on pathological situations, and promote bone tissue remodeling. Another issue that deserves further investigation related to fibrin involves its preparation from platelet-rich plasma. Although platelets are considered as a huge reservoir of growth factors, their specific role(s) in the repair of bone is poorly understood. Considering problems, like a very limited range of platelet concentrations in which they exhibit positive effects in bone healing[14] and interspecies variation in the growth factors.[196] Therefore, more work is needed to translate the possible beneficial features of platelets to clinical practice. Lastly, the combination of nanomaterials with fibrin has shown tremendous potential in order to maximize its interactions with cells due to the increased surface area of nanomaterials and their ability to manipulate its bioactivity.

Acknowledgments

Financial support from the Iranian Nanotechnology Initiative Council is gratefully acknowledged. Special thanks also to Dr Javad Saberi for his consultation.

Disclosure

The authors report no conflicts of interest in this work.

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