DOI: 10.15825/1995-1191-2020-1-196-208

FIBRIN – A PROMISING MATERIAL FOR VASCULAR TISSUE ENGINEERING

V.G. Matveeva, M.U. Khanova, L.V. Antonova, L.S. Barbarash

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation

This review looks at the use of fibrin in vascular tissue engineering (VTE). Autologous fibrin is one of the most affordable biopolymers because it can be obtained from peripheral blood by simple techniques. A description and comparative analysis of the methods and approaches for producing fibrin gel is provided. The ability of fibrin to promote cell attachment and migration, survival and angiogenesis, to accumulate growth factors and release them in a controlled manner, are unique and extremely useful in VTE. Fibrin gels can serve as a three-dimensional matrix molded in different sizes and shapes to be applied in a variety of ways, including as a scaffold, coating, or impregnation material. Fibrin's high porosity and biodegradability allows controllable release of growth factors, yet fibrinolysis must be tightly regulated to avoid side effects. We discuss the main methods of regulating the rate of fibrinolysis, as well as possible side effects of such exposure. Low mechanical strength is the main limitation in using fibrin as a scaffold for vascular tissue engineering. Possible options for increasing the strength properties of fibrin matrix and evaluating their effectiveness are presented. We propose that unique biocompatibility and ideal biodegradation profile of fibrin justify its use as a scaffold material for developing an ideal fully autologous small-diameter tissue-engineered vascular graft.

Keywords: vascular tissue engineering, fibrin, cell carrier, biopolymer, autologous tissue-engineered vascular graft, fibrinolysis, mechanical strength, implantation.

BACKGROUND

Active search for an ideal material to be used in vascular tissue engineering (VTE) is continually going on. Developed synthetic materials, such as dacron or polytetrafluorethylene (PTFE), polyethylene terephthalate (PET) are successfully used in large diameter vessel reconstruction, however their applicability for creating low diameter vascular prostheses (less than 6 mm) is low because of intima hyperhrophy and thrombosis due to incomplete endothelization, low blood flow velocity and mismatch in compliance (vessel diameter and its adaptive reactions to arterial blood pressure changes) [1]. Lack of effective low diameter vascular grafts facilitates the search for innovative strategies, new materials and their modifications.

Understanding the significance of normal physiological reactions of the vascular wall in the prevention and control of thromboses, hyperplasia and inflammation created a whole new area in VTE devoted to imitating the structure and function of the native arterial wall while developing new generation vascular grafts. Among the most potentially advantageous approaches in VTE related to maximal imitation of the extracellular matrix (ECM) in combination with biological functionality of the product, biogel cell colonization and the technology of biodegradable frames should be noted. In these areas fibrin presents a special interest for researchers, as it possesses a set of unique characteristics which make it a practically ideal natural biological material for VTE [2].

Fibrin is formed at the final stage of the coagulation cascade and presents a natural biodegradable matrix. Fibrin-based biomaterials are ideally biocompatible, possess high affinity to various biological surfaces, controlled biodegradation by means of fibrinolysis, the biodegradation products of which are non-toxic [3].

Currently fibrin gel is actively used at the clinic for hemostatic purposes, closure of the wound surface and as a sealant. Search is going on in tissue engineering for fibrin matrixes in ophthalmology (sclera [4] and lens [5] reconstruction), in neurology (plexus and peripheral nerve reconstruction [6]), in traumatology and orthopedics (cartilage reconstruction [7]), during artificial skin development [8], etc. Being a natural physiological frame, it supports angiogenesis and tissue reparation [9]. Fibrin fibers contain cell adhesion sites which create a platform for cell adhesion, migration and proliferation, as well as conditions for adequate tissue formation [10]. The 3D porous structure of fibrin supports cell migration into the frame and their vital activity due to nutrient and oxygen diffusion into the fibrin frame and waste removal [11]. Fibrin is the most available of all polymers. Fibrin gels can be easily and promptly obtained from the patients' own blood. Autologous scaffolds formed on its basis do not have the risk of transferring various pathogen transfer or launching the immune reactions of the organism to

Corresponding author: Vera Matveeva. Address: 6, Sosnoviy blv., Kemerovo, 650002, Russian Federation.
Tel. (906) 927-66-01. E-mail: matveeva_vg@mail.ru

a foreign body. The properties and special features of fibrin which have crucial significance for VTE will be described further in more detail.

MECHANISMS OF FIBRIN GEL FORMATION

Fibrin is a product of fibrinogen polymerization as the final stage of the coagulation cascade. Fibrinogen is a dimer of glycoprotein which consists of 3 pairs of identical chains (α, β and γ). At the first stage active single molecules of fibrin monomer are formed, facilitated by thrombin, which are capable of polymerization and forming fibrin fibers (Fig. 1).

Calcium ions are key co-factors in enzymatic transformation of fibrinogen into fibrin. At this stage fibrin is dissolved in 5M urea, therefore it was called soluble fibrin.

At the same time thrombin activates factor XIII which in the presence of calcium ions (Ca^{2+}) forms lateral covalent bindings between D-domains of the fibrin monomers. Lateral aggregation of fibrin fibres leads to their thickening and enhancement of their resilient and non-rigid properties [12]. Fibrin's general mechanical properties are determined by their structural peculiarities at the level of molecules, individual fibers, as well as branching of the fibers in the fibrin network [13, 14]. Introducing factor XIII in sufficient concentration enables to obtain fibrin with good mechanical properties [15, 16]. High factor XIII concentration also catalyses cross-linking of cell adhesion proteins which enhances the adhesion properties of fibrin [17].

WAYS OF OBTAINING FIBRINOGEN PRECIPITATE AND FIBRIN GEL

Autologous fibrinogen precipitate is obtained from blood plasma by means of physical and chemical methods. Cryoprecipitation is one of the physical precipitation methods and has been described in many sources. However protocols vary largely by freezing time and temperature, as well as melting temperature and time and the number of freezing-melting cycles. Chemical methods include primarily precipitation by means of ammonium sulfate and ethanol; their combination is also used [18, 19]. Preferable precipitation methods (ethanol, ammonium sulfate and cryoprecipitation) vary according to data from various sources as the criteria of evaluation are also different. In some cases the ethanol method is preferable as there is a higher fibrinogen concentration in the precipitate and the time required to obtain it is less [20]. In other cases cryoprecipitation is called a leader as in case of sufficiently high fibrinogen concentration the

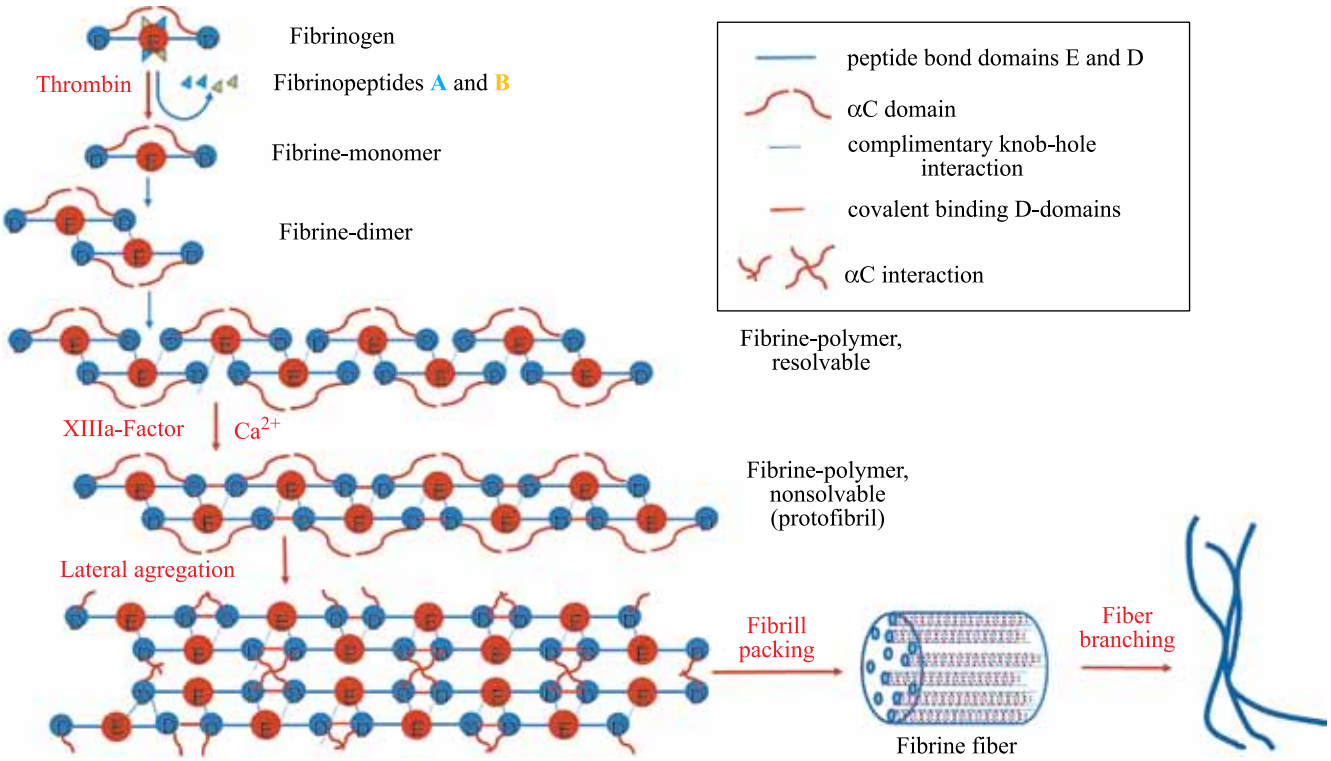

Fig. 1. Schematic diagram of the fibrin polymerization. Thrombin cleaves fibrinopeptides A and B from fibrinogen, after which the complementary regions on domains E and Dopen for knob-hole interaction. At this stage, the oligomers lengthen and form two-stranded soluble fibrin polymers. XIIIa factor in the presence of calcium ions covalently cross-links the D domains of neighboring fibrin monomers. Factor XIIIa crosslinks or ligates γ-chains more rapidly than α-chains. At this stage, the fibrin polymer becomes insoluble and obtain mechanical strength. Further, two-stranded fibrin molecules (protofibrils) laterally aggregate to make fibers, the process is enhanced by αC-interactions of D-domains. Elongation and thickening of fibrin fibers occurs at the same time with branching, as a result a three-dimensional fibrin network is formed

mechanical properties of the obtained fibrin are higher [21], which is related to partial protein denaturation during addition of ethanol or ammonium sulfate with subsequent formation of incomplete connections between fibrin monomers and fibrin clot dyshesion. Therefore different technologies for obtaining fibrinogen precipitate are used depending on the final goal.

In the majority of the studies fibrin gel is obtained from fibrin precipitate by means of introducing autologous or commercial thrombin and $CaCl_2$ [22, 23]. Tomomi Hasegawa et al. have developed and presented a non-thrombin technology of obtaining fibrin from fibrinogen [24]. In this case a polymerizing mixture is used which has low thrombogenicity as compared to thrombin, and during comparison with commercial thrombin does not activate an immune response [25].

NATURAL AND CONTROLLED FIBRIN BIODEGRADATION

Fibrin gels possess the advantage of a fully biodegradable polymer with a possibility of regulating the speed of degradation. In a normal biological environment fibrin fibers are degraded by means of fibrinolysis. In the process of fibrinolysis a non-active plasminogen, influenced by external (tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA)) and internal (XIIa factor, kallikrein) enzymatic activation is transformed into plasmin, a proteolytically active enzyme [26] (Fig. 2).

Plasmin asymmetrically cleaves fibrin into separate fragments: 'early' large molecule fibribolysis products – fragments X and Y, and 'late' fragments D and E. Fibrinolysis facilitates fibrin gel degradation with formation of non-toxic products, however the internal instability of fibrin is viewed as the main difficulty regarding its use as the main frame material or coating for TE constructions [27]. Apart from plasmin, various proteases take part in fibrin degradation, in particular an important role in this process belongs to membrane type matrix metalloproteinases (MT-MMP) [28].

Success in regulating the speed of VTE implantation is directly related to the possibility of regulating the speed of fibrinolysis. Under natural conditions fibrin decomposes within several days or weeks, the speed of fibrinolysis depends on fibrin structure, cross-linking features and protease inhibitor content [29]. Long term stability and mechanical sustainability of the matrix play an important role for the cells which require certain time and sufficient frame durability in order to remodel the matrix. Fibrin matrixes are gradually substituted by mature collagen that is synthesized by cells which have populated the full thickness and surface of the matrix (fibroblasts, EC/EPC and SMC) [27, 30].

In order to control the process of fibrin degradation two approaches are applied: additional transverse cross-linking of the fibrin fibers and use of fibrinolysis inhibitors [31]. Additional transverse cross-linking of the fibrin fibers is achieved by means of XIII factor which, apart from lateral covalent binding between fibrin γ- and α-chains, achieves transverse cross-linking with the fibrin of such molecules as α2-antiplasmin, TAFI (thrombin activated fibrinolysis inhibitor), PAI-2 (type 2 plasminogen activation inhibitor) which stabilizes the fi-

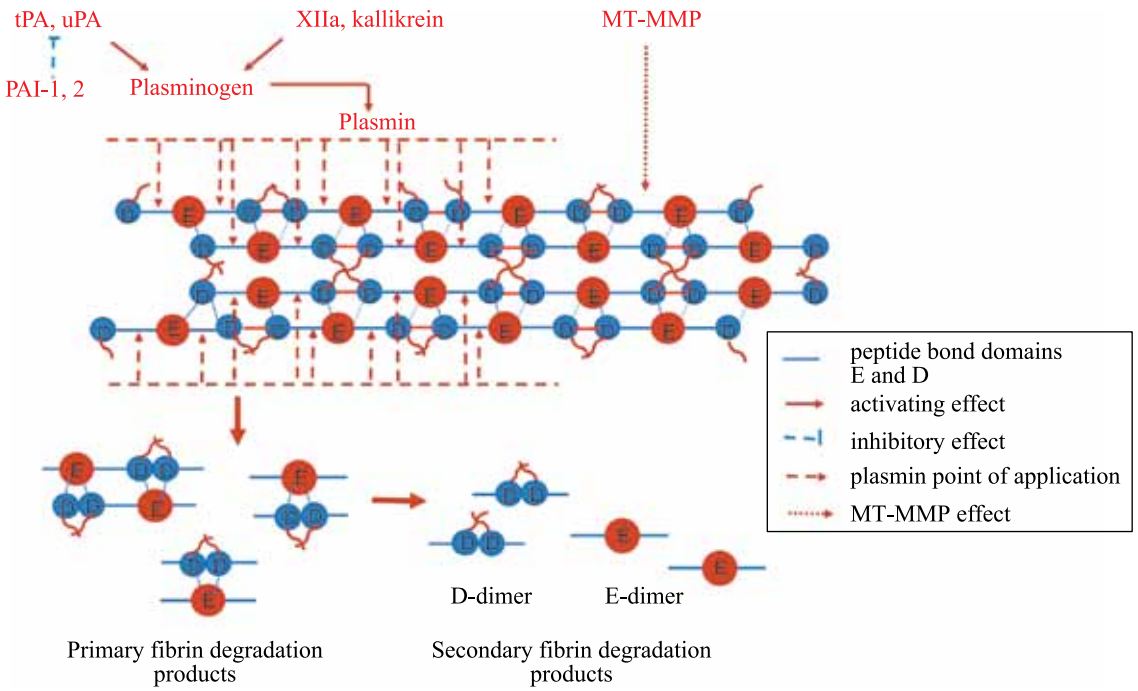

Fig. 2. Schematic diagram of fibrinolysis. MT-MMP – membrane-type matrix metalloproteinases; tPA – tissue plasminogen activator; uPA – urokinase plasminogen activator; PAI – plasminogen activator inhibitor; D – D-domain; E – E-domain

brin network and increases the resistance of cross-linked fibrin to fibrinolysis [32, 33].

In the course of developing fibrin coatings and frames on the basis of fibrin aprotinin is usually used as a proteolytic inhibitor and ε-aminocaproic acid (EACA) as a fibrinolytic inhibitor. The issue regarding the influence of these inhibitors on cell vital, proliferative and functional activity is extremely important as it determines the effectiveness of collagen synthesis and adequate tissue formation. Unfortunately there is no decisive answer to this question. There is evidence both of positive influence of the inhibitors on the vital activity of the cells [34] and of unfavourable results [35]. A study by Grassl et al. tested a wide range of EACA concentrations and absence of impact on collagen synthesis has been shown for SMC colonized on the fibrin tubular frame [35]. Opposite results have been obtained by Mol et al. [30] in whose study use of EACA inhibited ICM formation but did not hinder myofibroblast (MFB) proliferation in a human vein. These conclusions were based on a histological evaluation of tissue constructions after cultivating samples in static conditions for 2 to 6 weeks. Collagen fibers in the samples with EACA have not been detected throughout the cultivation period. Researchers believe that the inhibiting effect of EACA on collagen fiber formation is due to the fact that EACA is a synthetic analog of lysine and may play the role of competitive residue which blocks covalent cross-linking of the collagen molecules.

Nor does there exist a common opinion among scientists regarding the impact of aprotinin on cell vital activity. Both increased MFB collagen synthesis and tissue development potentiation have been described after increased aprotinin concentration [34], as well as the absence of its influence on the formation of ICM by the cells, in particular on type IV collagen and laminin synthesis [36]. In the opinion of Mühleder et al. [36], aprotinin-induced fibrin stabilization leads to a disruption in angiogenesis and formation of tubular 3D structures. The authors relate the mechanism of this effect to fibrinolysis inhibition. Nevertheless, no direct impact of aprotinin on the angiogenic properties of EC, as in the case of cultivating cells without fibrin the presence of aprotinin in the growth medium facilitates angiogenesis [37]. The impact of various aprotinin concentrations on the vasoreactive (contractility/dilatation) and mechanical properties of fibrin-based small diameter TE vascular prostheses has been described [38]. According to Swartz et al. aprotinin increases the cell colonization density and the physico-mechanical properties of TE vessels, but only owing to preserving the fibrin matrix structure. A diverse impact of aprotinin on vasoreactivity in VTE has been noted. On the one hand, it increases the capability of vasodilatation in VTE, but at the same time it decreases the receptor-mediated and non-receptor vasoconstriction [38].

Such diverse opinions regarding the impact of fibrinolysis inhibitors on fibrin properties and cell behaviour do not enable to form unanimous conclusions and require further comprehensive investigation.

FIBRIN AS A CELL CARRIER, OPPORTUNITIES FOR REGULATION OF ITS PROPERTIES

An important issue in VTE is the development of a 3D frame imitating the ICM which would facilitate cell adhesion, proliferation, migration and adequate tissue development. Fibrin imitates the ICM and not only provides physical support to the cells but also acts as a biomimetic niche, inducing biochemical and biophysical signals which regulate cellular behaviour. A 3D fibrin matrix has a nanometric fibrous structure which includes microporosity and macroporosity. The pores provide a supply of nutrients to the cells as well as migration and colonization opportunity [39]. It is known that fibrin supports expansion, migration and proliferation of various cell types, including EC [40], EPC [39], fibroblasts [41], MFB [11], mesenchymal stem cells (MSS0 [42]. The structure and physico-mechanical properties of the fibrin gel depend on fibrinogen and thrombin concentration [39, 43]. In case of low activity or concentration of thrombin there is no full separation of A and B peptides from the fibrinogen, correspondingly adequate fibrin fibers are not formed and polymerization time is increased which impacts the matrix durability significantly. In case of sufficient concentration or activity of thrombin the size of the pores and permeability of the fibrin carrier depends on the concentration of fibrinogen. Thus, increasing fibrinogen concentration within the range of 5 to 20 mg/ml decreases the size of the pores and the permeability of the matrix but increases its durability and stiffness [39, 43]. However too small pores prevent cell migration into the matrix thickness and have a negative influence on their growth and survival capability due to decreased permeability and nutrient perfusion. For the EC located on the surface of the matrix changes of the pore size do not have such a significant influence on their vital activity. Therefore depending on the goals and purposes the optimal concentration of fibrinogen should be selected individually.

Kurniawan et al. have shown that the buffer which was previously considered inert has a significant influence on fibrin self-assembly and gel permeability [44]. Buffer agents which control the pH of the media do not impact initial assembly of the fibrin monomers into protofibrils, however they hinder greatly further lateral protofibril association into thicker fibrin fibers. Buffer-mediated interruption of protofibril binding leads to a noticeable decrease in the fibrin network permeability, but does not have any significant effect on the elasticity modulus [44].

Apart from fibrinogen concentration, fibrin and pH of the buffer another factor that influences the structural

properties of fibrin is the concentration of calcium ions (Ca^{2+}). Fibrin gels with fibrinogen end point concentration 25 mg/ml and above, Ca^{2+} concentration 20 mM and pH between 6.8 and 9 possess transparency and stability properties [27]. When fibrin components are used outside these ranges a cloudy gel is formed which fully degrades within several weeks.

An optimal fibrinogen/thrombin ratio enables to obtain fibrin which supports EPC growth and their differentiation towards an endothelial phenotype with high angiogenic properties [39]. An advantage of the fibrin carrier has been noted in comparison with fibronectin. It has been shown that EPC viability, differentiation and angiogenic properties with fibrin are higher as compared to fibronectin. Besides, fibrin can support a larger number of cells functionally active for a longer time [39].

Cell distribution and proliferation on the matrixes is an important aspect for effective tissue formation. It is known that high homogeneity of cell distribution determines high ICM protein synthesis [30]. Therefore hierarchy and uniformity in cell distribution throughout the scaffold volume are of crucial significance. The work performed by T. Aper et al. showed [11] that human EC and MFB cultures, when colonized onto a composite matrix consisting of fibrin and a polyglactin frame, formed a hierarchic structure with a uniform EC monolayer on the surface and the MFB penetrating into the thickness of the frame correspondingly to the structure of the native vessel. Migration of the cells while retaining their viability, according to data presented by the authors, amounts to 519 ± 27 micron. In deeper fibrin layers no live cells have been discovered, which is related to insufficient oxygen and nutrient supply. Other research found 3D distribution of colonized fibroblasts in fibrin within a depth up to 3 mm [30, 45]. The average thickness of a human coronary artery varies between 390 to 1300 micron; therefore, maximal depth providing for MFB viability in a fibrin matrix will enable to create a small diameter vessel. Thus, fibrin gel as a cell carrier, depending on the cell type, ensures their hierarchic and uniform distribution, facilitates free migration and proliferation on the surface and within the matrix. This will enable to quickly and effectively colonize fibrin and form a compact formula. Also, cellular synthesis of ICM proteins within the matrix prevents washout of the soluble components and provides for ICM maturation in a short timeframe.

Good elasticity of fibrin gel is another important aspect of its effectiveness as a cell carrier. Elasticity determines the functional characteristics of the cells, influences the course of angiogenesis, regulates cell migration and traction force [46]. Fibrin possesses both elastic and viscous properties which are directly related to displacement deformation. Displacement deformation, in its turn, induces the launching of the signal mechanotransduction complex which regulates the adaptation of the cells to their physical environment. Fibrin fibers are exceptionally distensible and elastic [47], which sets fibrin advantageously apart from other protein fibers [48].

FIBRIN AS A DIRECT ANGIOGENESIS REGULATOR

In the process of wound healing the fibrin clot not only limits blood loss but also ensures release of a number of factors which stimulate new blood vessel formation. The classic model of wound healing accepts the active role of cells which influence the behaviour of other cells by means of signal transduction, however at the same time a passive role of a basis is assigned to the ICM [49, 50]. It has currently been proved that the fibrin matrix takes active participation in the regulation of angiogenesis, colonization and cell invasion [51].

The matrix structure contains not only cell integrin binding sites but also determines the speed and degree of the proteolytic degradation of the matrix induced directly by the cells themselves [52]. The factor regulating and controlling EC fibrin colonization and invasion is fibrinolysis which in turn is determined by metalloproteinase and plasmin system activity [51]. Hemostasis and angiogenesis are two interrelated physiological processes which act in a balanced and concordant way in order to restore microcirculation after vascular disruption [50]. Directly after the injury it is important to prevent excessive bleeding, and effective coagulation is needed for this. Starting angiogenesis at this stage is unproductive and premature, as newly formed vessels are delicate and unstable [53]. Therefore thrombogenesis and angiogenesis processes are strictly controlled, and angiogenesis is launched only after hemostasis has been successfully completed. Proof of direct impact of fibrin on angiogenesis is presented in a paper by Hadjipanayi et al. [54]. Fibrin binds many pro- and antiangiogenic factors which are released after coagulation (transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), platelet factor 4 (PF4), thrombospondin 1 (TSP1)) [55], and it also takes part in controlled release of these factors and angiogenesis regulation. The fine regulation mechanism is closely related to fibrinolysis and hemostasis processes. At early stages the fibrin clot performs a hemostatic function and initiates controlled release of angiogenic inhibitors, at the same time it facilitates EC attraction and migration by means of chemotactic factors. At the stage of a formed fibrin clot and activation of fibrinolytic processes fibrin gradually increases the release of proangiogenic factors and decreases the release of antiangiogenic ones, thus creating conditions for effective angiogenesis.

FIBRIN AS A MEANS OF GROWTH FACTOR DELIVERY

Fibrin is an independent cell behaviour regulator. In the course of morphogenesis and tissue healing the release of growth factors (GF) from the ICM plays a key role for the attraction of cells, regulation of their distribution zones and signal transfer [56, 57]. In this process GF release should be carefully balanced in order to achieve proper tissue formation.

Fibrinogen and fibrin are able to bind and retain GFs from different groups (PDGF, VEGF, FGF, TGF-β) via a heparin-binding domain nonspecifically with high affinity [55, 58]. GFs which are not bound to fibrinogen/fibrin are quickly eluted from the fibrin matrix, while those which are bound are retained for a prolonged period and released slowly. Nonspecific high affinity of fibrin to GF plays an important role in damaged tissue restoration, because fibrin acts as a reservoir for the delivery of growth factors, facilitating their reparation and healing.

Slowing down of the GF release process may be achieved by means of pressing fibrin, which at the same time increases frame density and improves the physicomechanical properties of the construction [53]. Another way of increasing GF content in fibrin is related to the use of plasma rich in platelets while manufacturing the matrix. Platelets contain a large number of GFs, such as PDGF, TGF-b1 and TGF-b2, FGF, VEGF and insulin-like growth factor (IGF), which stimulate cell proliferation, matrix remodeling and angiogenesis [54]. Use of platelet concentrate during fibrin matrix formation is an interesting option for tissue healing facilitation [55], however it is not suitable for creating vascular prostheses as it increases the thrombogenicity of the construction. Apart from the physiological ability of fibrin to bind and release GF, the fibrin gel/matrix enables to incorporate growth factors, bioactive peptides and proteins [56] which opens additional opportunities for focused differentiation and influence on the vital activity of colonized cells.

THE VASOREACTIVITY OF FIBRIN-BASED VTE

An ideal VTE graft should adapt to changing conditions of the blood flow, i.e. manifest vasoreactivity. The main elements in vasoreactivity are the smooth muscle cells which are able to respond to contractile and dilatation stimuli. Owing to contractile properties and proper radial location of vascular smooth muscle cells conditions are created not only to increase frame strength and stability but also to achieve vasoreactivity for the whole construction. Swartz et al. have been studying the properties of fibrin-based small diamteter VTE colonized with SMC and EC [32]. The constriction and relaxation ability of VTE have been evaluated after exposure to various vasoactive substances on the circular segments of the construction. The VTE achieved significant mechanical performance and vasoreactivity already after 2 weeks of maturation under static conditions. After implantation the process of remodeling was continued with an increase in mechanical performance and vascular reactivity.

FIBRIN INCREASES CELL RESISTANCE TO SHEAR STRESS

EC play a decisive role in vascular biology and perform various functions: serve as a selective permeability barrier, take part in thrombogenesis regulation and thrombolysis, platelet adhesion, vascular tone modulation, immune and inflammatory response regulation, mechanotransduction. EC loss induces local activation of a pathophysiological cascade leading to neointimal hyperplasia [59]. Therefore formation and support of a functional athrombogenic endothelial monolayer is a key requirement in VTE development.

Under physiological conditions EC are subject to complex mechanical exposure which includes shear stress, pressure and radial distention. It is known that over 70% of the EC colonized on a synthetic frame are washed off within the first 20 minutes after implantation and this process is intensified in case the shear stress level is increased [60]. Covering the construction with adhesive proteins enables the EC to withstand the flow. The adhesive proteins used for this include fibronectin, collagen, gelatin, whole ICM and fibrin. The most comprehensive comparative characteristic of cell retention on the surface of a vascular prosthesis covered with various types of protein is presented in the work by Chlupáč et al. [23]. The researchers studied retention properties for human EC on commercial prostheses made from PET with type I collagen and similar prostheses additionally coated with laminin, fibrin and fibrin/fibronectin. The effectiveness of cell colonization after 4 hours of interlaminary cultivation under rotation conditions amounted to 22–30% of the initially introduced amount and did not vary significantly in the prostheses with or without coating. After 3 days of maturation in static conditions the colonized prostheses were placed into a bioreactor. A significant and progressing with time loss of cells was observed on the commercial and laminin-coated prostheses. Under the same conditions a less significant loss of cells was taking place from the surface of prostheses coated with fibrin and fibrin/fibronectin, and by 120 min even an increase in their number was noted. The researchers relate the increase in the number of EC on a surface coated with fibrin or fibrin/fibronectin to the beginning of cell proliferation.

Adhesion to fibronectin and fibrin involves integrin receptors αvβ3 and α5β1 [61], which play a decisive role in adapting EC to hemodynamic efforts; their activation launches intercellular signal transfer and a change in the expression of genes which activate angiogenesis and proliferation [62]. The abovenamed receptors are not involved in EC adhesion to collagen and laminin [61], which emphasizes the advantage of EC interaction with

fibrin in order to form the desired cellular responses. The ability of fibrin to retain EC under shear stress conditions does not depend on the type of polymer basis (PET, PTFE, ePTFE) nor on the type of fibrin use (as a polymer coating or as an independent frame) [63]. Totally fibrin scaffolds also enable to obtain high density endothelization and support the monolayer under physiologically significant shear stress conditions [63].

Unfortunately coating of the graft with adhesive proteins (ICM, plasma or fibronectin) apart from improving EC adhesion also facilitates platelet adhesion and aggregation and increases the risk of thrombogenesis. As compared to whole ICM and gelatin, fibrin demonstrates lower platelet activation and adhesion on the surface and consequently lower thrombogenesis [64]. Thus, fibrin is the material of choice in supporting VTE endothelization under pulsing blood flow conditions.

FIBRIN MOLDING

Fibrin polymerization takes place during a certain period of time which has its positive aspects in the creation of TE constructions. There exist various technologies of scaffold manufacturing: mold casting in order to create a 3D porous structure and frame saturation for modification or for the creation of a fibrin coating.

In the first case fibrin components are poured into the desired mold, they are allowed to polymerize and then the ready construction is extracted from the form. Due to initial fluidity of the component mixture during fibrin molding it is possible to obtain precise and complex forms, for example valves and complex vessels with side branches [65]. The fluidity property enables to saturate different types of molds which creates a stable fibrin layer on the surface and imparts new desirable properties to the construction [23]. Moreover, introducing cell culture into the suspension or solution before mixing and polymerization facilitates uniform cell distribution in the inundated matrixes. Owing to the properties of fibrin which are extremely favourable for cell colonization and further remodeling in the body, technologies for manufacturing fibrin small diameter vessel prostheses by molding are being actively developed [15].

However using exclusively fibrin as the basis for VET encounters the main problem – insufficient mechanical performance of the fibrin gels in order to withstand physiological dynamic stress *in vivo*.

LOW MECHANICAL PERFORMANCE OF FIBRIN, OPTIONS FOR PROBLEM SOLUTION

Commonly used techniques aimed at improving mechanical performance of fibrin gels include two areas: improving the performance (strength) properties of the fibrin itself and creating a frame or hybridization with polymers/tissues.

Impacting on fibrin in order to improve its performance and durability characteristics includes several options: increased fibrinogen and thrombin concentration (described above); cross-linking of the fibrin fibres by XIIIa factor; densification of the fibrin structure by means of centrifugal force or compression; colonizing fibrin with cells during subsequent maturation in a bioreactor or in static mode. In studies aimed at VTE creation both separate methods are used as well as a combination of several methods [15, 16].

Cross-linking with XIIIa factor not only prevents fibrinolysis but also improves the performance characteristics of the fibrin clot. XIIIa factor enables to improve the resilience modulus for the fibrin fibers by over 8 times, and the ultimate resistance by 4.5 times [12, 16].

Improvement of the physicomechanical properties of the fibrin clot is to a certain extent facilitated by mechanical stimulus during polymerization. After being subject to continuously fluctuating forces of sufficient intensity a more rigid and durable fibrin matrix is formed as compared to similar matrixes which have not experienced mechanical pressure [66]. Thomas Aper et al. have presented a technology of high speed centrifugal molding, which enabled to sufficiently improve biomechanical stability of the fibrin tubular frames when the speed of rotation for the press mold was increased from 1000 to 1500 rpm [15]. The main principle of centrifugal molding is related to removing excess fluid which amounts to over 80% of the fibrin matrix volume; the densification of the fibrin clot induced by this facilitates cross-linking of the fibrin fibers. It has been noted that isolated centrifugal molding is less effective than a combination of similar parameters of centrifugal force and XIIIa factor cross-linking. Moreover, introducing EC and SMC into the fibrin content along with adding XIIIa factor additionally improves the performance of the tubular fibrin construction [15].

Colonizing the fibrin construction with fibroblasts, SMC and EC facilitates strengthening of the wall by means of forming cell interactions and ICM protein synthesis. However in order to improve the physicomechanical properties after *in vitro* cell colonization the construction should undergo a period of maturation under static or dynamic conditions. Pulsing flow conditions speed up and increase the effectiveness of fibrin construction maturation. Conditioning of the fibrin frame colonized with fibroblasts under conditions of a pulsing bioreactor improve the performance of the construction within 7 weeks to the parameters of the native artery [67].

Insufficient durability and rigidity of the fibrin matrixes is closely related to the problem of fibrin shrinkage [65]. The impact of the pulsing flow on the cell-colonized fibrin construction decreases the percentage of fibrin shrinkage [68]. A pulsing flow stimulates expression of cytoskeletal proteins, aligns the colonized cells along the direction of the flow, and also significantly increases ICM protein synthesis and accumulation; this improves durability and resiliency properties of the matrix, acti-

vates remodeling and creates a resistant frame which prevents shrinkage [68].

Forming a strengthening fibrin-based polymer frame or reinforcing net not only improves durability characteristics but also decreases the period of maturation for the fibrin construction. Thus, creation of a quickly degrading frame made of polyglycolic acid with poly-4-hydroxybutyrate on a fibrin basis colonized by MFB decreased the preparation time to 4 weeks [69]. After 4 weeks of *in vitro* dynamic deformation the mechanic properties of VTE obtained indicators comparable to those of the internal mammary artery (burst pressure level 903 ± 123 mm Hg). A shorter period of preparation is described in the case of strengthening the wall of a MFB-colonized autologous fibrin vascular prosthesis by a polyvinildenfluoride (PVDF) reinforcing net [70]. During 2 weeks while the construction was placed into bioreactor conditions with physiological flow velocity and pressure gradient parameters it was possible to increase the average suture retention durability to 6.3 N, and the tensile breaking strength to 236 mm Hg. At the same time the fibrin gel matrix showed good tissue development and high concordance with native vessels.

Reinforced small diameter fibrin VTE show very promising results after implantation: high patency, no calcifications or aneurysms, signs of active wall remodeling (presence of SMC and mature autologous ICM proteins) and EC monolayer formation on the inner surface of the graft [71].

The electrospinning method is gaining increasing popularity in the making of vascular prostheses. It enables to form nanosize fibers and to create a porous frame structure. An option has been suggested to create a fibrin frame strengthened by an ultrathin fibrous cover made of poly-ε-caprolactone (PCL) with the use of electrospinning, which enables to get a small diameter vascular prosthesis ready for implantation within 1 week [72]. A week after implantation to mice these prostheses obtained performance properties similar to those to be found in native arteries. Fibrin-mediated cell remodeling, stable intima, massive matrix accretion with organized collagen layers and elastin fibers were formed already at week 4 after implantation. Grafts showed high patency, low thrombogenicity and an inclination to calcification. This technology has a promising development prospect, however further research is required using larger animals and increasing the implantation period.

The concept of hybrid scaffolds which combine the advantages of natural and synthetic materials is very attractive and is believed to be effective. Creating coatings on the surface of polymers and decellularized tissues are meant to set up cell adhesion sites and increase biocompatibility; in this context a lot of attention is devoted to fibrin coating. In this technology form molding and the main mechanical stress is borne by the polymer frame or the decellularized tissue, and the fibrin coating creates a biocompatible layer on the surface which makes cellular invasion and growth easier. Good penetration power of the fibrin gel, good linkage with textured or porous surfaces after polymerization enable to use fibrin as a coating or for the saturation of frames made from various polymer materials or xenogenic tissue [23, 71, 73]. As a polymer basis for biomimetic hybrid frames with biological properties of fibrin the following are used: porous PCL [74], polylactide [71], PET [23], PTFE/dacron [75]. In order to retain mechanical properties and improve biocompatibility biological composite frames are developed on the basis of decellularized arteries covered by fibrin gel [73]. It has been suggested to manufacture a hybrid frame by applying aerosol fibrin gel on the inner and outer surfaces of decellularized arteries with successful cell colonization (MFB and EC). In this case the decellularized fibers of the carotid artery are closely interwoven with fibrin gel fibers and the layers are firmly bound to each other. The obtained hybrid frames / vascular grafts and native arteries demonstrate similar physicomechanical properties [73].

Thus densified and reinforced (frame-mounted) fibrin, as well as hybrid frame options can be potentially bases for creating effective small diameter vascular prostheses.

OPPORTUNITIES FOR CREATING FIBRIN-BASED AUTOLOGOUS GRAFTS

One of the main goals in VTE is the creation of a fully autologous prosthesis for small diameter vessels. The discovery of vascular EPC enables to obtain pure cultures of autologous EC even from patients with CVDs. EPCs can be obtained from the patient's own peripheral blood. An increase in the number of EPCs in response to a mechanic vascular damage, hypoxia and ischemia expand opportunities for EC recovery from blood which presents the most available, continuous and sustainable source of autologous endothelial colony forming cells (ECFCs) [76, 77]. Besides, it is possible to isolate colony-forming late outgrowth smooth muscle cells (LOSMC) from blood [78, 79], which are necessary for colonization of the vascular prosthesis wall.

Aper et al. have been successful in obtaining a fully autologous VTE prosthesis by having united all the three components: fibrin frame, ECFC and LOSMC [15]. In order to get a highly compact fibrin matrix with satisfactory physicomechanic properties cross-linking by XIIIa factor, high speed centrifugal molding, colonization with ECFC and LOSMC which had been isolated from peripheral blood were used. The construction was implanted to sheep for 1 and 6 months. As a result of active remodeling of the prosthesis wall after 6 months a structure similar to the native artery was formed. Fibrin was replaced by newly synthesized ICM proteins, cell and capillary ingrowth from surrounding tissue into the implanted segments was noted; as a result the biomechanical properties began to correspond to the properties of

the native artery. Despite evident success the durability properties of the prostheses before implantation did not quite correspond to those of the native artery, and one of the sheep died from prosthesis rupture in the process of the operation. A possible solution could be additional introduction of a maturation stage for the colonized fibrin construction in the conditions of a bioreactor, which would somewhat increase the time for preparing the construction before implantation. Nevertheless the results are encouraging and there is hope that functional, fully autologous vascular prostheses may be obtained from available sources of the patient's own cells and tissues.

CONCLUSION

Fibrin is a promising material in VTE. It has a great potential for molding which enables to obtain complex 3D shapes. A fibrin frame possesses unique tools for cell adhesion, migration and retention under shear stress; it is able to control angiogenesis, to accumulate and gradually release growth factors. The opportunity to control biodegradation, as well as the morphobiological properties of fibrin, enable to select and save the required characteristics. The listed advantages of fibrin in combination with availability of the source and lack of immune response after implantation make it an ideal frame for developing VTE grafts. Fibrin gel possesses an inner potential, largely exceeding other matrix materials with the exception of mechanical durability. Throughout the world search for ways of improving mechanical durability of fibrin matrixes is going on; in this case hybrid scaffold technology or fibrin reinforcement may become one of the options for solving this problem. Moreover, creation of a fully autologous fibrin-based TE vascular prosthesis may be possible.

The authors declare no conflict of interest.

REFERENCES

1. *Best C, Strouse R, Hor K et al.* Toward a patient-specific tissue engineered vascular graft. *J Tissue Eng*. 2018; 9: 2041731418764709. doi: 10.1177/2041731418764709.
2. *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; Article ID 685690: 10 p. doi: 10.1155/2015/685690.
3. *Park CH, Woo KM.* Fibrin-Based Biomaterial Applications in Tissue Engineering and Regenerative Medicine. *Adv Exp Med Biol*. 2018; 1064: 253–261. doi: 10.1007/978-981-13-0445-3_16.
4. *Scalcione C, Ortiz-Vaquerizas D, Said DG, Dua HS.* Fibrin glue as agent for sealing corneal and conjunctival wound leaks. *Eye (Lond)*. 2018; 32 (2): 463–466. doi: 10.1038/eye.2017.227.
5. *Mohan S, John B, Rajan M et al.* Glued intraocular lens implantation for eyes with inadequate capsular support: Analysis of the postoperative visual outcome. *Indian J Ophthalmol.* 2017; 65 (6): 472–476. doi: 10.4103/ijo.IJO_375_16.
6. *Bhatnagar D, Bushman JS, Murthy NS et al.* Fibrin glue as a stabilization strategy in peripheral nerve repair when using porous nerve guidance conduits. *J Mater Sci Mater Med.* 2017; 28 (5): 79. doi: 10.1007/s10856-017-5889-4.
7. *de Barros CN, Miluzzi Yamada AL et al.* A new heterologous fibrin sealant as a scaffold to cartilage repair – Experimental study and preliminary results. *Exp Biol Med (Maywood)*. 2015; 241 (13): 1410–1415. doi: 10.1177/1535370215597192.
8. *Reddy KS, Chittoria RK, Babu P et al.* Effectiveness of Fibrin Glue in Adherence of Skin Graft. *J Cutan Aesthet Surg*. 2017; 10 (2): 72–75. doi: 10.4103/JCAS.JCAS_100_16.
9. *Morin KT, Tranquillo RT. In vitro* models of angiogenesis and vasculogenesis in fibrin gel. *Exp Cell Res.* 2013; 319 (16): 2409–2417. doi: 10.1016/j.yexcr.2013.06.006.
10. *Podolnikova NP, Yakovlev S, Yakubenko VP et al.* The interaction of integrin αIIbβ3 with fibrin occurs through multiple binding sites in the αIIb β-propeller domain. *J Biol Chem*. 2014; 289 (4): 2371–2383. doi: 10.1074/jbc.M113.518126.
11. *Aper T, Teebken OE, Steinhoff G, Haverich A.* Use of a fibrin preparation in the engineering of a vascular graft model. *Eur J Vasc Endovasc Surg.* 2004; 28: 296–302. doi: 10.1016/j.ejvs.2004.05.016.
12. *Collet JP, Moen JL, Veklich YI et al.* The alphaC domains of fibrinogen affect the structure of the fibrin clot, its physical properties, and its susceptibility to fibrinolysis. *Blood.* 2005; 106 (12): 3824–3830. doi: 10.1182/blood-2005-05-2150.
13. *Lim BB, Lee EH, Sotomayor M, Schulten K.* Molecular basis of fibrin clot elasticity. *Structure.* 2008; 16: 449–459. doi: 10.1016/j.str. 2007.12.019.
14. *Liu W, Carlisle CR, Sparks EA, Guthold M.* The mechanical properties of single fibrin fibers. *J Thromb Haemost*. 2010; 8: 1030–1036. doi: 10.1111/j.1538-7836.2010.03745.x.
15. *Aper T, Wilhelmi M, Gebhardt C et al.* Novel method for the generation of tissue-engineered vascular grafts based on a highly compacted fibrin matrix. *Acta Biomater.* 2016; 29: 21–32. doi: 10.1016/j.actbio.2015.10.012.
16. *Dickneite G, Metzner HJ, Kroez M et al.* The importance of factor XIII as a component of fibrin sealants. *J Surg Res.* 2002; 107 (2): 186–195.
17. *Mosesson MW.* Fibrinogen and fibrin structure and functions. *J Thromb Haemost.* 2005; 3 (8): 1894–1904. doi: 10.1111/j.1538-7836.2005.01365.x.
18. *Siedentop KH, Harris DM, Sanchez B.* Autologous fibrin tissue adhesive. *Laryngoscope.* 1985 Sep; 95 (9 Pt 1): 1074–1076.
19. *Ayman E Ismail.* Purification of fibrinogen from human plasma. *Thromb Res.* 1987; 46 (1): 19–27. doi: 10.1016/0049-3848(87)90203-9.
20. *Weis-Fogh US.* Fibrinogen prepared from small blood samples for autologous use in a tissue adhesive system. *Eur Surg Res. 1988*; 20 (5–6): 381–389. doi: 10.1159/000128789.
21. *Aper T, Kolster M, Hilfiker A et al.* Fibrinogen Preparations for Tissue Engineering Approaches. *J Bioengineer & Biomedical Sci.* 2012, 2: 3. doi: 10.4172/2155-9538.1000115.

22. *Almelkar SI, Patwardhan AM, Divate SA et al.* Fibrin matrix supports endothelial cell adhesion and migration in culture. *OA Biology.* 2014; 2 (1): 5.
23. *Chlupáč J, Filová E, Riedel T et al.* Attachment of human endothelial cells to polyester vascular grafts: pre-coating with adhesive protein assemblies and resistance to short-term shear stress. *Physiol Res.* 2014; 63 (2): 167–177.
24. *Hasegawa T, Okada K, Takano Y et al.* Thrombin-free fibrin coating on small caliber vascular prostheses has high antithrombogenicity in rabbit model. *Artif Organs.* 2005; 29 (11): 880–886. doi: 10.1111/j.1525-1594.2005.00151.x.
25. *Hasegawa T, Okada K, Takano Y et al.* Autologous fibrin-coated small-caliber vascular prostheses improve antithrombogenicity by reducing immunologic response. *J Thorac Cardiovasc Surg.* 2007; 133 (5): 1268–1276, 1276.e1. doi: 10.1016/j.jtcvs.2006.12.049.
26. *Chapin JC, Hajjar KA.* Fibrinolysis and the control of blood coagulation. *Blood Reviews.* 2015; 29 (1): 17–24. doi: 10.1016/j.blre.2014.09.003.
27. *Eyrich D, Brandl F, Appel B et al.* Long-term stable fibrin gels for cartilage engineering. *Biomaterials.* 2007; 28: 55–65. doi: 10.1016/j.biomaterials.2006.08.027.
28. *Hotary KB, Yana I, Sabeh F et al.* Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. *J Exp Med.* 2002; 195 (3): 295–308. doi: 10.1084/jem.20010815.
29. *Linnes MP, Ratner BD, Giachelli CM.* A fibrinogen-based precision microporous scaffold for tissue engineering. *Biomaterials.* 2007; 28 (35): 5298–5306. doi: 10.1016/j.biomaterials.2007.08.020.
30. *Mol A, van Lieshout MI, Dam-de Veen CG et al.* Fibrin as a cell carrier in cardiovascular tissue engineering applications. *Biomaterials.* 2005; 26 (16): 3113–3121. doi: 10.1016/j.biomaterials.2004.08.007.
31. *Schneider-Barthold C, Baganz S, Wilhelmi M et al.* Hydrogels based on collagen and fibrin – frontiers and applications. *BioNanoMaterials.* 2016; 17 (1–2); 3–12. doi: 10.1515/bnm-2015-0025.
32. *Ritchie H, Lawrie LC, Crombie PW et al.* Crosslinking of plasminogen activator inhibitor 2 and α2-antiplasmin to fibrin(ogen). *J Biol Chem.* 2000; 275: 24915–24920. doi: 10.1074/jbc.M002901200.
33. *Valnickova Z, Enghild JJ.* Human procarboxypeptidase U, or thrombinactivable fibrinolysis inhibitor, is a substrate for transglutaminases: evidence for transglutaminase-catalyzed cross-linking to fibrin. *J Biol Chem*. 1998; 273: 27220 –27224.
34. *Ye Q, Zund G, Benedikt P et al.* Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 2000; 17: 587–591. doi: 10.1016/s1010-7940(00)00373-0.
35. *Grassl ED, Oegema TR, Tranquillo RT.* A fibrin-based arterial media equivalent. *J Biomed Mater Res.* 2003; A 66: 550–561. doi: 10.1002/jbm.a.10589.
36. *Mühleder S, Pill K, Schaupper M et al.* The role of fibrinolysis inhibition in engineered vascular networks derived from endothelial cells and adipose-derived stem cells. *Stem Cell Res Ther.* 2018; 12; 9 (1): 35. doi: 10.1186/s13287-017-0764-2.
37. *Koutsioumpa M, Hatziapostolou M, Mikelis C et al.* Aprotinin stimulates angiogenesis and human endothelial cell migration through the growth factor pleiotrophin and its receptor protein tyrosine phosphatase beta/zeta. *Eur J Pharmacol*. 2009; 602: 245–249. doi: 10.1016/j.ejphar.2008.11.046.
38. *Swartz DD, Russell JA, Andreadis ST.* Engineering of fibrin-based functional and implantable small-diameter blood vessels. *Am J Physiol Heart Circ Physiol.* 2005; 288: H1451–H146070. doi: 10.1152/ajpheart.00479.2004.
39. *Barsotti MC, Magera A, Armani C et al.* Fibrin acts as biomimetic niche inducing both differentiation and stem cell marker expression of early human endothelial progenitor cells. *Cell Prolif.* 2011; 44: 33–48. doi: 10.1111/j.1365-2184.2010.00715.x.
40. *Almelkar SI, Patwardhan AM, Divate SA et al.* Fibrin matrix supports endothelial cell adhesion and migration in culture. *OA Biology.* 2014; 14: 2 (1): 5.
41. *Pajorova J, Bacakova M, Musilkova J et al.* Morphology of a fibrin nanocoating influences dermal fibroblast behavior. *Int J Nanomedicine*. 2018; 13: 3367–3380. doi: 10.2147/IJN.S162644.
42. *Voss A, McCarthy MB, Allen D et al.* Fibrin Scaffold as a Carrier for Mesenchymal Stem Cells and Growth Factors in Shoulder Rotator Cuff Repair. *Arthrosc Tech.* 2016; 5 (3): e447–e451. doi: 10.1016/j.eats.2016.01.029.
43. *Chiu CL, Hecht V, Duong H et al.* Permeability of three-dimensional fibrin constructs corresponds to fibrinogen and thrombin concentrations. *Biores Open Access*. 2012; 1 (1): 34–40.
44. *Kurniawan NA, van Kempen TH, Sonneveld S et al.* Buffers Strongly Modulate Fibrin Self-Assembly into Fibrous Networks. *Langmuir.* 2017; 27; 33 (25): 6342–6352.
45. *Jockenhoevel S, Chalabi K, Sachweh JS et al.* Tissue engineering: complete autologous valve conduit – a new moulding technique. *Thorac Cardiovasc Surg.* 2001a; 49: 287–290.
46. *Discher DE, Janmey P, Wang YL*. Tissue cells feel and respond to the stiffness of their substrate. *Science.* 2005; 310: 1139–1143.
47. *Liu W, Jawerth LM, Sparks EA et al.* Fibrin fibers have extraordinary extensibility and elasticity. *Science.* 2006; 313: 634.
48. *Guthold M, Liu W, Sparks EA et al.* A comparison of the mechanical and structural properties of fibrin fibers with other protein fibers. *Cell Biochem Biophys*. 2007; 49: 165–181.
49. *Feng X, Tonnesen MG, Mousa SA, Clark RA*. Fibrin and collagen differentially but synergistically regulate sprout angiogenesis of human dermal microvascular endothelial cells in 3-dimensional matrix. *Int J Cell Biol.* 2013; 2013: 231279. doi: 10.1155/2013/231279 PMID: 23737792.
50. *Reinke JM, Sorg H.* Wound repair and regeneration. *Eur Surg Res.* 2012; 49 (1): 35–43. doi: 10.1159/000339613 PMID: 22797712.
51. *Collen A, Hanemaaijer R, Lupu F et al.* Membrane-type matrix metalloproteinase-mediated angiogenesis in a fibrin-collagen matrix. *Blood.* 2003; 101: 1810–1817.

52. *Van Hinsbergh VW, Collen A, Koolwijk P.* Role of fibrin matrix in angiogenesis. *Ann NY Acad Sci.* 2001; 936: 426–437.
53. *Monroe DM, Hoffman M.* The clotting system – a major player in wound healing. *Haemophilia.* 2012; 18 (5): 11–16.
54. *Hadjipanayi E, Kuhn PH, Moog P et al.* The Fibrin Matrix Regulates Angiogenic Responses within the Hemostatic Microenvironment through Biochemical Control. *PLoS One.* 2015; 10 (8): e0135618.
55. *Martino MM, Briquez PS, Ranga A, Lutolf MP, Hubbell JA.* Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix. *Proc NatlAcad Sci USA.* 2013; 110 (12): 4563–4568.
56. *Schultz GS, Wysocki A*. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen*. 2009; 17 (2): 153–162.
57. *Martino MM, Tortelli F, Mochizuki M et al.* Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. *Sci Transl Med.* 2011; 3 (100): 100ra189. doi: 10.1126/scitranslmed.3002614.
58. *Martino MM, Hubbell JA.* The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain. *FASEB J.* 2010; 24 (12): 4711–4721. doi: 10.1096/fj.09-151282.
59. *Patel SD, Waltham M, Wadoodi A et al.* The role of endothelial cells and their progenitors in intimal hyperplasia. *Ther Adv Cardiovasc Dis.* 2010; 4 (2): 129–141. doi: 10.1177/1753944710362903.
60. *Wong CS, Sgarioto M, Owida AA et al.* Polyethyleneterephthalate provides superior retention of endothelial cells during shear stress compared to polytetrafluoroethylene and pericardium. *Heart Lung Circ.* 2006, 15: 371–377. doi: 10.1016/j.hlc.2006.08.002.
61. *Post A, Wang E, Cosgriff-Hernandez E.* A Review of Integrin-Mediated Endothelial Cell Phenotype in the Design of Cardiovascular Devices. *Ann Biomed Eng.* 2019; 47: 366. doi: 10.1007/s10439-018-02171-3.
62. *Schaufler V, Czichos-Medda H, Hirschfeld-Warnecken V et al.* Selective binding and lateral clustering of α5β1 and αvβ3 integrins: Unraveling the spatial requirements for cell spreading and focal adhesion assembly. *J Cell adhesion & migration.* 2016; 10 (5): 505–515. doi: 10.1080/19336918.2016.1163453.
63. *Isenberg BC, Williams C, Tranquillo RT.* Endothelialization and flow conditioning of fibrin-based media-equivalents. *Ann Biomed Eng.* 2006b; 34: 971–985.
64. *Al-Maawi S, Herrera-Vizcaino C, Dohle E et al.* Homogeneous pressure influences the growth factor release profiles in solid platelet-rich fibrin matrices and enhances vascular endothelial growth factor release in the solid platelet-rich fibrin plugs. *Int. J. Growth Factors and Stem Cells in Dentistry.* 2018; 1 (1): 8–16.
65. *Moreira R, Neusser C, Kruse M et al.* Tissue-Engineered Fibrin-Based Heart Valve with Bio-Inspired Textile Reinforcement. *Advanced Healthcare Materials.* 2016; 5 (16): 2113–2121.
66. *Munster S, Jawerth LM, Fabry B, Weitz DA.* Structure and mechanics of fibrin clots formed under mechanical perturbation. *J Thromb Haemost.* 2013; 11: 557–560.
67. *Syedain ZH, Meier LA, Bjork JW et al.* Implantable arterial grafts from human fibroblasts and fibrin using a multi-graft pulsed flowstretch bioreactor with noninvasive strength monitoring. *Biomaterials.* 2011; 32: 714–722.
68. *Flanagan TC, Cornelissen C, Koch S et al.* The *in vitro* development of autologous fibrin-based tissue-engineered heart valves through optimised dynamic conditioning. *Biomaterials.* 2007; 28 (23): 3388–3397.
69. *Stekelenburg M, Rutten M, Snoeckx CM et al.* Dynamic Straining Combined with Fibrin Gel Cell Seeding Improves Strength of Tissue-Engineered Small-Diameter Vascular Grafts. *Tissue Engineering. Part A.* 2009; 15 (5): 1081–1089.
70. *Tschoeke B, Flanagan TC, Cornelissen A et al.* Development of a composite degradable/nondegradable tissueengineered vascular graft. *Artif Organs.* 2008; 32: 800–809.
71. *Koch S, Flanagan TC, Sachweh JS et al.* Fibrin-polylactide-based tissue-engineered vascular graft in the arterial circulation. *Biomaterials.* 2010; 31 (17): 4731–4739.
72. *Morgan BE, Ginn B, Fukunishi T et al.* Regenerative and durable small-diameter graft as an arterial conduit. *Proceedings of the National Academy of Sciences.* 2019; 116 (26): 12710–12719.
73. *Zhijuan He, Xu Ma, Yan Wang et al.* Decellularized Fibrin Gel-Covered Canine Carotid Artery: A Completely Biological Composite Scaffold for Tissue-Engineered Small-Caliber Vascular Graft. *J Biomaterials and Tissue Engineering.* 2018; 8 (3): 336–346. doi: 10.1166/jbt.2018.1745.
74. *Pankajakshan D, Krishnan VK, Krishnan LK.* Functional stability of endothelial cells on a novel hybrid scaffold for vascular tissue engineering. *Biofabrication.* 2010; 2 (4): 041001. doi: 10.1088/1758-5082/2/4/041001.
75. *Sreerekha PR, Krishnan LK.* Cultivation of endothelial progenitor cells on fibrin matrix and layering on dacron/polytetrafluoroethylene vascular grafts. *Artif Organs*. 2006; 30 (4): 242–249.
76. *Matveeva V, Khanova M, Sardin E et al.* Endovascular Interventions Permit Isolation of Endothelial Colony-Forming Cells from Peripheral Blood. *Int J Mol Sci.* 2018; 2; 19 (11). pii: E3453. doi: 10.3390/ijms19113453.
77. *Paschalaki KE, Randi AM.* Recent Advances in Endothelial Colony Forming Cells Toward Their Use in Clinical Translation. *Front Med (Lausanne).* 2018; 5: 295. doi: 10.3389/fmed.2018.00295.
78. *Simper D, Stalboerger PG, Panetta CJ et al.* Smooth muscle progenitor cells in human blood. *Circulation.* 2002; 106 (10): 1199–1204. doi: 10.1161/01.cir.0000031525.61826.a8.
79. *Kolster M, Wilhelmi M, Schrimpf C, Hilfiker A, Haverich A, Aper T.* Outgrowing endothelial and smooth muscle cells for tissue engineering approaches. *J Tissue Eng.* 2017; 8: 2041731417698852. doi: 10.1177/2041731417698852.

The article was submitted to the journal on 22.08.2019