REVIEW ARTICLE

Regenerative Potential of Platelet Rich Fibrin In Dentistry: Literature Review

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ABSTRACT

Platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane. Choukroun and his associates were amongst the pioneers for using PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. Autologous PRF is considered to be a healing biomaterial, and presently, studies have shown its application in various disciplines of dentistry.

Key words: Platelet rich fibrin (PRF), Growth factors, Regeneration, Wound healing

INTRODUCTION

Regenerative potential of platelets was intorduced in 1974, and Ross *et al.*[1] were amongst the pioneers who first described a growth factor from platelets. Growth factors released after activation from the platelets trapped within fibrin matrix, and have been shown to stimulate the mitogenic response in the periosteum for bone repair during normal wound healing.[2] Last two decades has seen the better understanding of physiologic properties of platelets in wound healing that led to increased therapeutic applications in the various forms with varying results. However, controversies owing to the complexity of the production protocols for autologous fibrin adhesives or risk of cross infection for commercial adhesives, alongwith legal restrictions on blood handling with concentrated platelet rich plasma (cPRP), a new family of platelet concentrate, an autologous cicatricial matrix, platelet rich fibrin (PRF) appeared in France.[3]

Choukroun's platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines and cells are trapped which are released after a certain time,[4] and that can serve as a resorbable membrane. More recently, Gassling *et al*.[2] have shown that PRF is a suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering applications.[5]

Autologous platelet rich fibrin (PRF), considered to be a healing biomaterial, was initially used in oral implantology by its promotors,[6] and presently, studies have shown its application in various disciplines of dentistry. This paper is intended to overview its prospective and clinical relevancy in present day dental practice.

METHOD

Dental literature was searched with Medline/ Pubmed Central/ Google for "platelet rich fibrin" term was searched in Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed) which was further filtered for "platelet rich fibrin in dentistry", "platelet rich fibrin in dental", "platelet rich fibrin in maxillofacial surgery", "platelet rich fibrin in oral implantology", "platelet rich fibrin periodontics", "Choukroun platelet rich fibrin"and abstracts of all relavent papers were scrutinised thoroughly and in the end articles pertaining to the topic (PRF) were included. Relavent literature for "platelet-rich fibrin" in common textbooks on periodontology, oral implantology, oral and maxillofacial surgery; bibliographies of papers and review articles together with appropriate peer reviewed journals were also scrutinized for additional information.

BIOLOGICAL ASPECT

Platelet-rich fibrin (PRF), classified as a leukocyte- and platelet rich fibrin (L-PRF), often named as Choukroun's PRF after its inventor, to avoid any confusion with other techniques using similar names such as Vivostat PRF (Vivolution, Alleroed, Denmark), a pure platelet rich plasma (PRP) or Fibrinet PRF (Cascade Medical, Wayne, NJ) matrix (without leukocyte),[7,8]

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belongs to the second generation platelet concentrate, collecting on a single fibrin membrane containing constituents of blood sample favorable for healing and immunity.[9,10] Although PRF belongs to a new generation of platelet concentrate, it is in the first place a fibrin technology and biologic activity of the fibrin molecule is enough in itself to account for its significant cicatricial capacity and a perfect understanding of its components and their significance will comprehend the clinical results obtained and subsequently extend the fields of therapeutic applications of this etiquette.[3]

Developed in France by Choukroun *et al.*[11] the PRF production protocol attempts to accumulate platelets and released cytokines in a fibrin clot. Granules present in platelet contain many proteins, which may be platelet specific (eg. beta-thromboglobulins) or non platelet specific (fibronectin, thrombospondin, fibrinogen, and other coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins etc) calcium and serotonin etc. Also phospholipids double layer of platelet membrane constitute many receptors for other molecules.[12] Growth factors released by alpha-granules encompass a group of cytokine polypeptides with relatively low molecular weight ranging from 6 to 45 kDa.[13] Activation and degranulation is important to initiate and support aggregation at the healing site and the release of the cytokines (IL-1 beta, IL-6, TNF-alpha)[14] and growth factors (TGF beta 1, PDGF, VEGF, EGF) that stimulates cell migration and proliferation within the fibrin matrix and thus begins the first stage of healing.[12]

Dohan *et al.*[14] suggested that PRF addition may decrease many harmful effects at inflammatory site natural to surgical act by correcting certain destructive and noxious excesses during healing process of wound tissues and thus could be an immune regulation node with inflammation retro-control abilities and explained the reduction of post operative infections.

Interlukin-1 (IL-1) is a key mediator of inflammation control and its main activity is to stimulate T-helper lymphocytes and in combination with TNF-alpha, it activates osteoclasts and inhibits bone formation.[15] Interleukin-6 (IL-6) is a differentiation factor for B-lymphocytes and an activator for T lymphocytes and indeed, within the B-lymphocytes populations, significantly stimulates the sectretion of antibodies, thus supports the reaction chains leading to inflammation, destruction and remodeling.[14] Tumor necrosis factor alpha (TNF alpha) activates monocytes and stimulates the remodelling capacities of fibroblasts and in addition increases phagocytosis and neutrophil cytotoxicity and modulates the expression of IL-1 and IL-6.[16,17] IL-4 supports proliferation and differentiation of activated B cells and during inflammation it supports healing by moderating inflammation. It increases fibrillary collagen synthesis by fibroblast and inhibits stimulation of MMP-1 and MMP-3 by IL-1Beta.[14] Li-Weber *et al.*[18] and Brown and Hural[19] suggested that it prevents the poduction of IL-1beta, TNF-alpha and prostaglandins (PGs) in response to cell activation by bacterial endotoxins or IFN-gamma.

Presence of cytokine vascular endotheilial growth factor (VEGF), the most powerful and omnipresent known vascular growth factor, functions to start angiogenesis and combination of its different isoforms will make it possible to direct and redefine the development plan of the network growth.[14]

Transforming growth factor Beta-1 (TGF beta-1), an inflammatory regulator, is the most powerful fibrosis agent amongst all cytokines,[20] and can induce a massive synthesis of collagen and fibronectin.[3] Platelet derived growth factors (PDGF) were first described by Ross *et al.*,[1] and has been shown to accelerate proliferation of monkey arterial smooth muscle cell in cell culture, and essentially regulate migration, proliferation and survival of mesenchymal cell lineages, and thus plays an essential role in physiologic cicatrisation and pathogenesis of atherosclerosis and other fibroproliferative diseases.[12]

Insulin like growth factors (IGFs) 1 and 2 are cell multiplication mediators in apoptosis by inducing survival signals protecting cells.[21] Also IGF-1 present in plasma may exert chemotactic effects towards human osteoblasts.[22]

A soluble fibrillary molecule, fibrin is an activated form of plasmatic molecule fibrinogen that is massively present both in plasma and in the platelet alpha granules that plays potential role in platelet aggregation during homeostasis.[3] Fibrinogen is the final substrate of all coagulation reactions which is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the first cicatricial marix of the injured site.[23-25] Characteristic of polymerisation naturally and slowly during centrifugation, alongwith physiologic thrombin action on collected autologus fibrinogen is crucial to determine the three dimentional organization of fibrin network that will give great elasticity and very strong PRF membrane.[3]

METHOD FOR OBTAINING PLATELET RICH FIBRIN (PRF)

Choukouran's PRF, in contrast to Platelet rich fibrin matrix (PRFM) used by Simon *et al.*[26] which uses additives such as buffered tri-sodium citrate and calcium chloride togetherwith double centrifugation, can be prepared by using a very simple technique which is nothing more than centrifuged blood without any additives, which makes it possible to avoid all blood-dervied product reimplantation related restrictions of French law and requires neither anticoagulants nor bovine thrombin.[3,11] PC-02 table centrifuge and a collection kit from Process (Nice, France)[3] containing 24 gauge butterfly needle and blood collection tubes[27] can be used for its easy

collection.[3] Anilkumar *et al.*[28] and Sunitha and Munirathnam[30] reportedly used REMI Laboratories (India) tabletop for cetrifugation. A blood sample is taken without anticoagulant in 10 ml tubes and immediately centrifuged at 2700-3000 rpm for 10-12 minutes. The resultant product consists of following three layers: (a) RBC at the bottm, (b) PRF clot in middle and (c) upper most layer consisting of platelet poor plasma (PPP).[3,11,28-30] The outcome of this technique entirely depends upon the speed of blood collection and transfer to the centrifuge. As the blood sample starts to coagulate almost immediately upon contact with the tube-glass and it takes a minimum of few minutes of centrifugation to concentrate fibrinogen in middle and upper part of the tube, rapid handling is the only way to obtain a clinical usable PRF clot and any delay to collect blood and starts centrifugation may be the cause of failure resulting in polymerisation of fibrin in a diffuse way in the tube with small blood clot without consisitency.[3] Driving out the fluids trapped in the fibrin matrix by squeezing the PRF clot between the sterile dry gauze, practitioners will obtain a highly resistant autologous PRF membrane (a highly promising biomaterial) for multiple clinical usage.[3,11,28,30]

Recent introduction of PRF box (Process, Nice, France), devised to produce homogenously thickened hydrated (for several hours) membrane[7] and an exudate rich in platelets, leukocytes, vitronectin and fibronectin expressed from the fibrin clots,[27] has improved the issues regarding the handling of the PRF clot, a living biomterial.[7] PRF clot obtained after centrifugation is removed from the test tube and attached red blood cells scraped off and discarded,[27] but it should be remembered that PRF membranes are inhomogenous and platelets and leukocytes are concentrated within one end (towards RBCs) of membrane.[7] PRF clot is then placed on the grid in the PRF box and covered with the compressor and lid, resulting in production of an economical autologous fibrin membrane in approximately one minute. Exudate collected may be used to hydrate graft materials, rinse the surgical site and store autologous grafts.[27]

The technique for obtaining PRF over its first generation counter-parts viz. PRP has certain distinct advantages. First, the technique is quite simple involving less armamentarium and less time consuming as well. PRP can be prepared by two techniques whch differ in their technical aspects and are divided into either general purpose cell separators or platelet concentrating cell separators. The first one requires large amount of blood (450 ml) and is done in hospital settings. For platelet concentrating sytems a double spin or single spin technique is utilised,[31] on other hand PRF can be procured by using a table top centrifuged in a matter of 10 minutes.

However, the most important advantage of PRF over PRP has been the deletion of any additive constitutent such as bovine thrombin which is mandatory for making PRP. It has been discovered that use of bovine thrombin may be associated with development of antbodies to the factors-V, XI, and thrombin resulting in the risk of life threatening coagulopathies.[32]

CLINICAL IMPLICATIONS

Choukroun *et al.*[11] were amongst the pioneers for using autologous PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. Since then, this emerging field of biomaterial has revolutionised the tissue engineering as the relatively new, highly promising field of reconstructive biology, which represents the recent advances in medicine and surgery, molecular and cellular biology, polymer chemistry and physiology.[30] Lack of adequate bone and proximity to anatomic structures are amongst the most common encountered problems at the implantation site and recent advancements in surgical procedures can predictably combat such difficulties.

Choukroun *et al.*[33] attempted to evaluate the potential of PRF in combination with freeze-dried bone allograft (FDBA) (Phoenix; TBF, France) to enhance bone regeneration in sinus floor elevation and nine sinus floor augmentations were performed; in 6 sites, PRF was added to FDBA particles (test group), and in 3 sites FDBA without PRF was used (control group). Four months later for the test group and 8 months later for the control group, bone specimens were harvested from the augmented region during the implant insertion procedure. After 4 months of healing time, histologic maturation of the test group appears to be identical to that of the control group which was for a period of 8 months. Moreover, the quantities of newly formed bone were equivalent between the 2 protocols.[33]

Jang *et al.*[34] determined the capability of silk fibroin powder as biomaterial template for the restoration of peri-implant defects when mixed with Chaukroun's PRF in ten New Zealand white rabbits. Histomorphometric analysis show greater bone formation and removal torque force shows significant higher values for experimental (silk fibroin and PRF) than in control (unfilled) group.[34] Lee *et al.*[35] further suggested that because silk is cheap and readily available material, acid digested silk fibroin combined with Chaukroun's PRF could provide a possible new bone substitute for the reconstruction of various bone defects. Mazor *et al.*[36] assessed the relevance of autologous leukocyte- and platelet-rich fibrin (PRF) concentrate and membranes as the sole filling material during a lateral sinus lift with immediate implantation in a case series. From a radiologic and histologic point of view at 6 months after surgery, the use of PRF as the sole filling material during a simultaneous sinus lift and implantation stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants. Further, they advocated that Choukroun's PRF is a simple and inexpensive biomaterial, and its systematic use during a sinus lift seems a relevant

option, particularly for the protection of the Schneiderian membrane.[36] Toffler *et al*.[27] advocated membrane insurance by possibly sealing an undetected perforation during lateral window osteotomy procedure using PRF membrane. In a multicenteric study by Kfir *et al*.,[37] minimally invasive antral membrane balloon elevation (MIAMBE) done using platelet-rich fibrin and bone substitutes, injected under the antral membrane and implant placement alongwith primary closure executed at the same sitting, revealed that MIAMBE can be applied to all patients in need of posterior maxilla bone augmentation with high procedural success, low complication rate, and satisfactory bone augmentation and implant survival. As it is minimally invasive and associated with minimal discomfort, MIAMBE should be an alternative to the currently employed methods of maxillary bone augmentation.[37]

Simonpieri *et al*.[38,39] reported maxillary reconstruction using FDBA, PRF membranes and 0.5% metronidazole solution in twenty patients who were treated using this new technique and followed up during 2.1 years (1-5 years). Finally, 184 dental implants were placed, and they found no implant or graft loss in a case series, thus confirming the validity of this reconstructive protocol. Small quantities of 0.5% metronidazole solution (10 mg) provide a proficient protection of the bone graft against unavoidable bacteial contamination.[38] PRF membranes protect the surgical site and promote soft tissue healing and cut few milimeters PRF fragments mixed with graft material functioned as a "biological connector" between the different graft elements, and as a matrix that supports neo-angiogenesis, capture of stem cells and migration of osteoprogenitor cells to the center of the graft.[27] Meyer *et al*.[40] advocated that the long-term reliability of betaTCP associated to growth factors (PRP or PRF) without bone graft, in massive sinus-lift procedures induces fewer complications, and the implant success as well as resorption rate is comparable to the one obtained by using autologous bone grafts.

Bone-added osteotome sinus floor elevation (BAOSFE) technique using platelet-rich fibrin (PRF) as grafting material while placing microthreaded implants in subsinus residual bone can lead to an endosinus bone gain. Despite a limited residual bone height (RBH), a healing period of 2-3 months was found to be sufficient to resist a torque of 25 N.cm applied during abutment tightening. At 1 year, formation of a new recognizable bone structure delimiting the sinus floor was identified radiologically and led to a predictable implant function.[41]

Toffler[42] advocated osteotome-mediated sinus floor elevation (OMSFE) or crestal core elevtion (CCE)[43] with simultaneous implant placement using PRF plugs, prepared by placing PRF clot into the cylinder in the PRF box and slowly compressing with piston.[27] Thick PRF plugs or small disks of 1 cm diameter can also be easily inserted into the residual extraction sockets.[27] Immediate bone augmentation after infected tooth extraction using titanium membranes applied to the socket walls followed by socket filling with autologous platelet-rich fibrin and primary closure was found to be feasible and safe and yielded adequate bone filling to support implant fixation at 8 weeks or above.[44] However, in a randomised control trial, Gürbüzer *et al*.[45] using bone scintigraphy based on technetium-99m methylene diphosphonate uptake revealed that platelet-rich fibrin (PRF) might not lead to enhanced bone healing in soft tissue impacted mandibular third molar extraction socket 4 weeks after surgery.

Platelet rich fibrin as a potential novel root coverage approach has been reported by Anilkumar *et al*.[28] for covering localised gingival recession in mandibular anterior teeth using combined laterally positioned flap technique and PRF membrane. Joint use of platelet rich fibrin and bone graft has also been reported for combined periodontic – endodontic furcation defect.[29] In a 6 month randomised clinical trial by Aroca *et al*.[6] evaluating the modified coronally advanced flap alone or in combination with a platelet rich fibrin for the treatment of adjacent Miller Class I and II multiple gingival recession, discovered inferior root coverage of about 80.7% as compared to about 91.5% achieved at control site, but an additional gain in gingival/ mucosal thickness compared to conventional therapy.

Soadoun and Touati[46] discussed tridimensional implant placement and the use of connective tissue grafting to complete the aesthetic restoration as means of limiting soft tissue recession around implants and showed that platelet-rich fibrin enables the simple, effective, and predictable management of the gap between alveolar bone and an implant.

DISCUSSION AND CONCLUDING REMARKS

Since its inception in 2001 by Chaukron *et al*.,[11] who introduced PRF in dentistry for the first time in an implant case, today PRF is an accepted and most extencively worked upon current biological material with immense regenerative potential by other discplines of clinical dentistry (Table 1). Dohan *et al*.[3,12,14] and Chaukron *et al*.[10,33] have worked extensively on Chaukroun's PRF and their series of articles published in Triple O, provides an indepth detail for understanding underlying biologic properties that is useful for the initiators, for practicing and clinical usage of this novel approach in regeneration. In one of their letter to the editor published in Journal of Periodontology,[7] they have also cautioned the beginner practioners to use this technique with expert training or supervision as PRF is a complex living biomaterial with a specific biology not a comercially available inert membrane and utmost importance should be given regarding the preparation and conservation of the material.[7]

Table 1: Publications pertaining to clinical implicatons of Platelet rich fibrin.

Publications pertaining to Platelet rich fibrin
Choukroun *et al.*[10] : PRF can be considered as healing biomaterial (Research).
Dohan *et al.*[3] : Slow polymerization during PRF preparation seems to generate a fibrin network very similar to the natural one (in vitro).
Dohan *et al.*[12] : PRF, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling (In vitro).
Dohan *et al.*[14] : PRF could be an immune regulation node with inflammation retrocontrol abilities (in vitro)
Choukroun *et al.*[33] : Sinus floor augmentation with FDBA and PRF leads to a reduction of healing time prior to implant placement. (Comparative Study/ Controlled Clinical Trial)
Soadoun and Touati[46] : Platelet-rich fibrin enables the simple, effective, and predictable management of the gap between alveolar bone and an implant. (Review)
Schwartz-Arad *et al.*[49] : There is still lack of scientific evidence to support the use of PRP and PRF in combination with bone grafts during augmentation procedures. (Review)
Kfir *et al.*[50] : Immediate bone augmentation after infected tooth extraction (Case series).
Kfir *et al.*[51] : The range of vertical gain was 2.4 to 5.1 mm, while horizontal gain measured 1.3 to 3.9 mm. Implants were successfully placed in 6 patients. (Case series)
Diss *et al.*[41] : The BAOSFE procedure with PRF as grafting material can lead to an endosinus bone gain (Prospective pilot study)
Sunitha *et al.*[30] : This article describes the evolution of novel platelet concentrate, referred to as PRF (Review).
Gassling *et al.*[52] : PRP application in cell cultures leads to higher levels of growth factors than PRF application (in vitro).
Anilkumar *et al.*[28] : Root coverage on the labial surfaces of the mandibular anterior teeth was accomplished using laterally displaced flap technique with platelet rich fibrin (PRF) membrane at the recipient site. (Case Report)
Meyer *et al.*[40]: The use of betaTCP associated to growth factors (PRP ou PRF) without bone graft, in massive sinus-lift procedures induces few complications. (Comparative Study)
Aroca *et al.*[6] : The addition of a PRF membrane positioned under the MCAF provided inferior root coverage but an additional gain in GTH at 6 months compared to conventional therapy. (Randomised Clinical Trial)
Kanakamedala *et al.*[29] : PRF along with bone graft can be used to treat combined periodontic-endodontic lesion. (Case Report)
Simonpieri *et al.*[38] : PRF membranes are particularly helpful to protect the surgical site and foster soft tissue healing. (Case Series)
Simonpieri *et al.*[39] : PRF membranes are particularly helpful for periosteum healing and maturation (Case Series)
Su *et al.*[13] : PRF membrane should be used immediately after formation to maximize release of GF to the surgical site (in vitro)
Kfir *et al.*[37]: Minimally invasive antral membrane balloon elevation (MIAMBE) should be an alternative to the currently employed methods of maxillary bone augmentation. (Multicenter study)
Mazor *et al.*[36]: PRF as the sole filling material during a simultaneous sinus lift and implantation stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants. (Case Series)
Lee *et al.*[35] : A combined application of Choukroun PRF with acid-digested silk fibroin showed more rapid bone healing than unfilled control. (Animal study)
Gassling *et al.*[2] : PRF appears to be superior to collagen (Bio-Gide) as a scaffold for human periosteal cell proliferation. PRF membranes are suitable for in vitro cultivation of periosteal cells for bone tissue engineering. (in vitro)
Gürbüzer *et al.*[45]: PRF might not lead to enhanced bone healing in soft tissue impacted mandibular third molar extraction sockets 4 weeks after surgery. (Comapartive study / Randomised Control trial)
Chang *et al.*[53] : Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. (in vitro)
Jang *et al.*[54] : A peri-implant defect can be successfully repaired by the application of Choukroun PRF and silk fibroin powder. (Research)

Further more, Su *et al.*[13] while determining in vitro, the release of growth factor in PRF, quantified PDGF-AB, TGF-BETA 1, EGF and VEGF obtained in the relesate of PRF clot at 300 minutes equivalent to $0.295x10^{-16}$, $0.998x10^{-16}$, $1.7x10^{-18}$, and $0.5x10^{-18}$ g per platelet respectively and also opined that if PRF clots are squeezed between sterile cotton gauze as advocated by Chukraun group to prepare a membrane then that membrane should be put to use immediately over surgical sites as the release of growth factor is found to be maximum for first 60 min and for this they advocate to expose surgical site first before obtaining PRF in order to save time.[13] They further recommend to use the discarded fluid after squeezing the PRF clot (PRF releasate) as another source of growth factor which can be mixed with the graft materials and used accordingly[13] or rinse the surgical site and store autologous grafts.[27]

Some points have been raised concerning the safety issue of PRF methodology. O'Connell[47] in a letter to editor pertaining to one such issue concerning the types of tubes to be used

preferentially to produce PRF and rasied doubts regarding the silica particles in glass tubes or of glass coated plastic tubes as a health hazard and remarked against its contact to human tissues. This Issue has been answered by Dohan *et al*.[48] by conducting cytotoxicity analysis of PRF on wide range of human cells and concluded that silica microparticles coating these tubes represent a quite immpossible risk of cytotoxicity and PRF produced with glass coated plastic tubes is not cytotoxic for the tested human cells, and for some even seems to improve their mitotic proliferation. Moreover, they opined that with plastic tube alone PRF cannot be obtained and the contact with silica is necessary to start the polymerisation process as silica behaves as clot activator and to produce PRF either dried glass tubes or glass coated plastic tubes must be used.

PRF, developed in France, is being widely used over there and rest of Europe for the past 10 years, and now in almost every part of world in orthopaedic and plastic surgery and has found its application in oral maxillofacial surgery, implantology as well as periodontal surgeries as well. The popularity of this material and manifold advantages along with indepth details in publications provided by the pioneers as well as in vitro, in vivo, clincal trials, and histologic evaluations from the other parts of the world after understanding of this second generation autologous Choukran's PRF has revolutionsied the field of regenerative dentistry and motivated the researchers and clinicians further to apply this procedure alongwith tissue engineerging protocol.

Authors believe that ongoing research togther with advanced information technology revealing frequent addition of publications in pubmed regarding the topic, may help the clinicians to review and re-search its evidence based clinical usage and certain issues that includes the relationships between quantity and quality of PRF with aging, systemic diseases (thrombocytopenia, afibrinogenemia, bleeding disorders, diabetes, leukocyte adhesion syndromes etc) or conditions, socio-economic status or nutrition, ethinic or racial groups, environmental, blood profile, autoimmunity and genetic presdisposition, besides standardised PRF preparation protocol, may be answered in the coming few years.

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