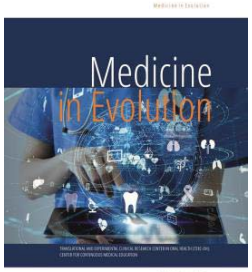
Obtaining the bicomponent tissue adhesive from blood collected preoperatively



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Abstract

The difference between primary and secondary hemostasis is that primary hemostasis is defined by the formation of the primary platelet plug whereas secondary hemostasis (coagulation) is defined by the formation of insoluble, cross-linked fibrin. Activated platelets are responsible for primary hemostasis and activated clotting factors are responsible for secondary hemostasis.

Platelets play a crucial role in hemostasis and wound healing, with platelet-derived growth factors as a source of healing cytokines. Platelet concentrates for surgical use are innovative tools of regenerative medicine and have also been tested in oral and maxillofacial surgery. These products are extracts of the blood tissue, they are tissues themselves, and not pharmaceutical preparations. Fibrin tissue adhesive has applications in several fields of medicine, can be prepared by various methods.

Keywords: Hemostasis, platelet, growth factors, tissue adhesive

INTRODUCTION

Primary and secondary hemostasis relates to clot formation. The elements of hemostasis are vascular response, platelet number and function, von Willebrand's factor (vWF) level and clotting factor levels. Primary hemostasis is a procoagulation clot-forming process associated with the initiation and formation of the platelet plug. Secondary hemostasis is associated with the propagation of the coagulation process through the intrinsic and extrinsic coagulation cascades. Secondary hemostasis depends on appropriate interactions of coagulation factors leading to fibrin clot formation.

In all surgical branches the search for adjuvant techniques is unceasing. Current research aims to develop materials capable of enhancing the healing process and regulating inflammation, focusing on solving the limitations of conventional treatments [1].

Edwin J. Cohn and his team developed in 1946 a process to extract albumin from blood plasma. The process is based on the differential solubility of albumin and other plasma proteins based on pH, ethanol concentration, temperature, ionic strength and protein concentration (Figure 1) [2].

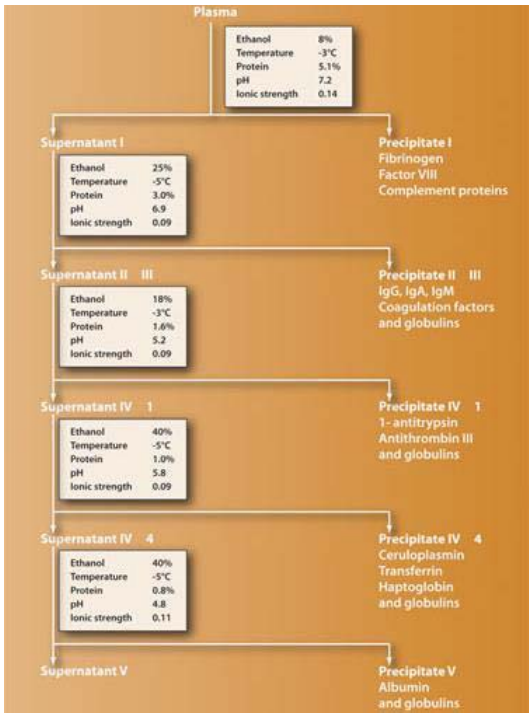

Figure 1. Basic plasma fractionation scheme using the Cohn process [3]

Studies of interactions between fibrinogen/fibrin and plasma proteins and receptors on platelets, leukocytes, and other cells have demonstrated complex functions in hemostasis, thrombosis, inflammation, infection, cancer and other pathologies [4,5].

Autologous or commercial fibrin sealants and platelet concentrates have been used alone or in association with bone substitutes to promote bone healing in oral surgery [6-8].

The autologous platelet concentrates, Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF), are used in various medical fields, for local and infiltrative use in orthopedic and sports medicine and also in oral and maxillofacial surgery [9,10]. They are obtained after different processing of a blood sample, mostly by centrifugation [11]. The purpose of the processing is to separate the blood components, remove the elements considered unusable

(red blood cells) and concentrate the elements that can be used in therapeutic applications (fibrinogen/fibrin, platelets, growth factors, leukocytes) [12].

Aim and objectives

The study is a combination of the technique of concentrating fibrinogen with ethanol originally described by Cohn and more recently by Kjaegaard and the separation of thrombin from the patient's blood with a technique derived from the studies of Thorn, Kumar and Ghassab.

MATERIAL AND METHODS

Blood collection and coarse separation

Collect 50 ml of blood in vacuum tubes with 1.4 ml of citrate phosphate-dextrose anticoagulant (Figures 2,3). Centrifuge gently at 330X g for 15 minutes. In this way most platelets remain in the superficial layer.

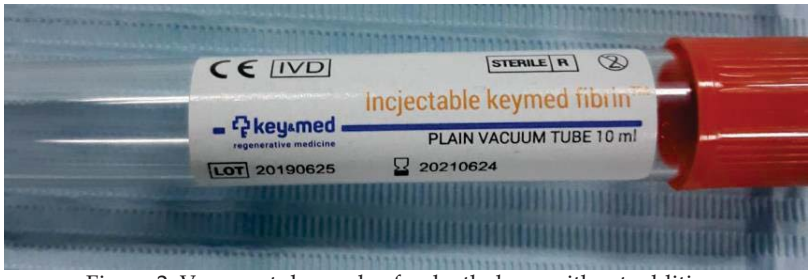

Figure 2. Vacuum tube made of polyethylene, without additives

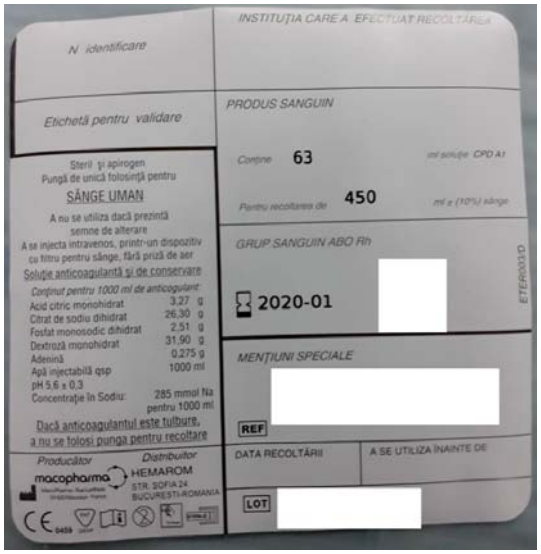

Figure 3. Transfusion bag containing 63 ml of anticoagulant citrate-phosphate-dextrose

Figure 4. Programmable centrifuge, Rotor radius 100mm

The centrifuge used is programmable and has a rotor with spaces for test tubes inclined at 45 degrees (Figure 4). So, the maximum distance between the tip of the test tube and the center of the rotor is 10 cm. To calculate the speed to which the centrifuge must be programmed, the formula was used $G=(1.118 \times 10^{-5})RS^2$, where "R" is the radius of the rotor, "S" is the speed and "G" is the relative centrifugal force.

After centrifugation for 15 min at 330xG, a separation of blood components in 3 layers is obtained, as follows: the superficial layer obtained is plasma rich in proteins plasma and the inorganic fraction of blood; the white intermediate layer contains most platelets and leukocytes; and the densest layer consists of erythrocytes (Figure 5).

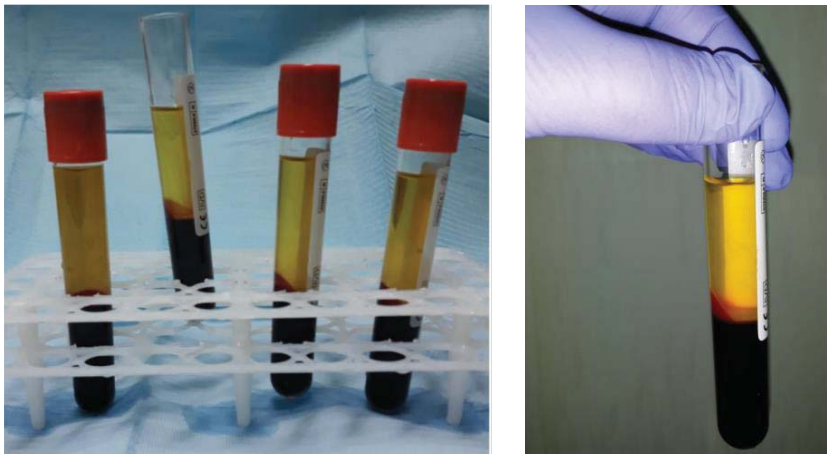

Figure 5. The blood collected in tubes with anticoagulant is centrifuged for 15 minutes at 330xG. Separation of blood elements occurs according to mass; the superficial layer is rich in platelets because the centrifugation was gentle

Separation of the euglobinic fraction and obtaining one product concentrated in thrombin

2.5ml of the plasma solution is extracted and diluted with approximately 22 ml of citric acid of concentration 2.84mM (Figure 6). The obtained solution is centrifuged for 5 minutes at 3000xG at 4°C. The supernatant is removed (Figure 7) and the precipitated fraction is dissolved in approx. 0.2 ml of calcium Ca gluconate (0.1M) (Figure 8). The pH is neutralized by adding of 0.1 ml of sodium bicarbonate of 75mM concentration (Figure 8) and thus the activation is initiated prothrombin. In the next 3-10 minutes, a fibrin clot forms. The clot is lightly pressed during formation for fibrin aggregation. After 20-30 minutes, the liquid containing thrombin is removed in a syringe. Prothrombin activation continues for another 2-4 hours, but it is important to remove the clot from the rest of the liquid because fibrin has the property of fixing thrombin.

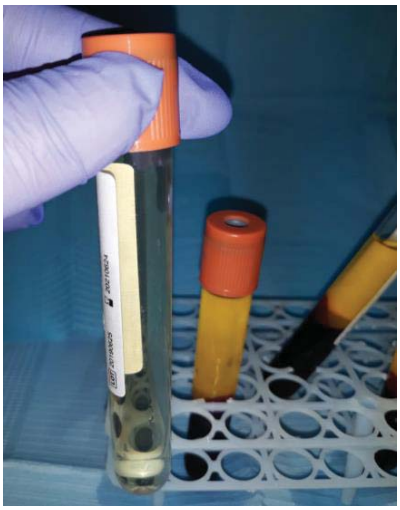

Figure 6. 1 ml of plasma diluted in 9 ml of citric acid

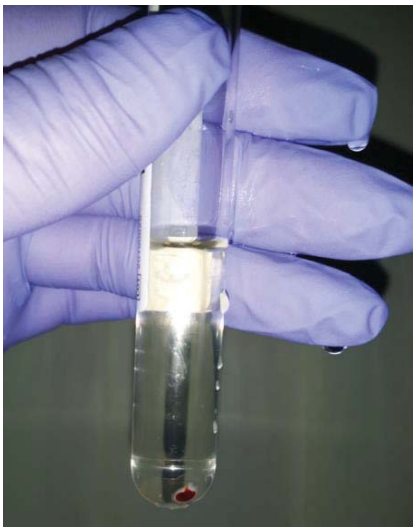

Figure 7. After centrifugation at 3000xG for 5 minutes, the supernatant is removed

Figure 8. After precipitation of the euglobulin faction, coagulation is initiated by adding calcium chloride and bicarbonate

Preparation of concentrated fibrinogen

The rest of the platelet-rich plasma is mixed with approximately 1 ml of acid tranexamic to prevent simultaneous precipitation of plasminogen with fibrinogen. Ethanol is added until a concentration of approximately 10% ethanol is obtained and the mixture is cooled in an ice water bath for 20-30 minutes to obtain a temperature close to 0°C (Figure 9). The product is centrifuged for 8 minutes at 3000xG at 0-4°C (Figure 10). The supernatant is removed and the precipitated fibrinogen is redissolved by heating to 37°C (Figure 11). Heating to 37°C this arrangement and produces a cloudy, stable liquid that coagulates very slowly when Ca is added (because it lacks the other factors present in the plasma), but it coagulates immediately in contact with thrombin.

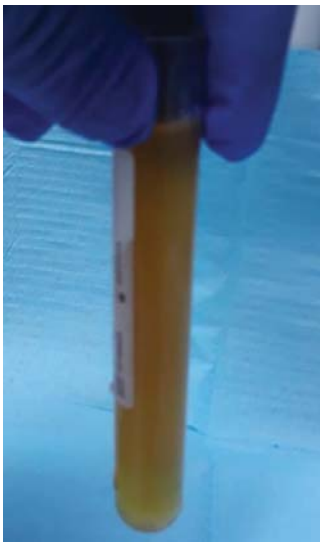

Figure 9. Plasma mixed with ethanol up to a concentration of about 10% and cooling the mixture in a bath of ice water for 20 minutes

Figure 10. Centrifuge the cooled plasma at 3000xG for 4 min and discard the supernatant

Figure 11. After centrifugation, the supernatant is separated and concentrated fibrinogen is obtained

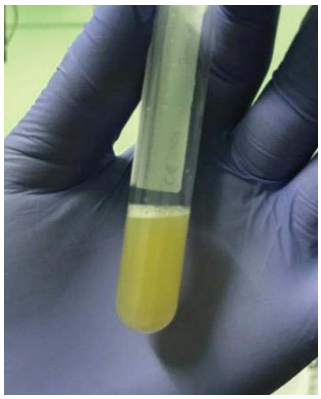

Figure 12. The precipitated fibrinogen

The precipitated fibrinogen in the 5 test tubes is mixed with 1ml of physiological serum, and after heating and mixing becomes a cloudy liquid (Figure 12).

Thrombin concentration adjustment

Concentrations range from 50 to 500 NIH u/ml, values where coagulation occurs immediately, thus diluting with calcium chloride solution 0.05M is important for a slower and more efficient polymerization of the fibrin network - three-dimensional polymerization with the inclusion of cytokines in the formed network (Figure 13).

Figure 13. Coagulated fibrinogen in contact with thrombinase product and Ca gluconate

RESULTS

Immediately before using the adhesive, the thrombin concentration is adjusted in depending on the required working time. Values of 10%, 5% or 2% are the concentrations used and should always be tried on plasma. The mixture of 150 µL of plasma with 50 µL of thrombin should coagulate in an interval of 30-60 seconds.

DISCUSSIONS

Thrombin used in surgical procedures as a hemostasis adjuvant [13-16], derived mainly from bovine sources, is associated with adverse reactions, for example, the formation of antibodies against human factor V leading to bleeding episodes [17,18], and the transmission of bovine prions that could cause a variant of Creutzfeldt-Jacob disease (vCJD) [19]. The use of autologous thrombin is an alternative in surgery because it avoids the risk of infectious diseases and immunogenicity problems.

Fibrin sealants are hemostatic agents and possess pattern characteristics for cell migration, supporting the growth of keratinocytes and fibroblasts. Research has confirmed that fibrin adhesives can be used safely at sites of infection [20].

Thorn *et al.* conducted a study with the aim of preparing autologous fibrin glue with platelet growth factors and using it together with cancellous bone particles in maxillofacial reconstructive surgery. The glue they obtained had a concentration of fibrinogen about 12 times higher, and the concentration of growth factors was about eight times higher than their value in platelet-rich plasma [4].

Kumar *et al.* conducted a study to investigate the stability of thrombin produced using the thrombin processing device (TPD; Thermogenesis Corporation) and the addition of plasma (11 ml) and reagent ($CaCl_2$ and ethanol, 3.75 ml). Their study showed that the active thrombin produced by TPD depends on both the production temperature and the storage temperature [21].

Ghassab *et al.* conducted a study to compare the coagulation efficiency of platelet-rich plasma (PRP) and platelet-poor concentrated plasma (cPPP) with citrated whole blood after activation by autologous thrombin, bovine thrombin, or calcium chloride ($CaCl_2$). Their study showed that PRP provided the best combinations for clinical use when combined with either bovine thrombin or $CaCl_2$. Autologous thrombin was suboptimal but could be an autologous alternative for clinical application, and cPPP had ineffective coagulation [22].

Fibrin glues generally contain fibrinogen and thrombin with a small amount of calcium chloride to create a clot that can be administered. Their advantage is that they do not require active bleeding and can work independently of the patient's own fibrinogen [23].

Valbonesi *et al.* confirmed the utility of fibrin-platelet glue in reducing infection and length of hospital stay in patients with skin and soft tissue loss from recent trauma or chronic pathology. The authors believe that the preparation of the glue is very easy, cheap and creates excellent and stable hemostasis [24].

CONCLUSIONS

Compared to traditional wound therapy, biologic adhesives are an attractive choice due to their ease of operation, rapid hemostasis, and wound recovery. Currently, research on adhesives is focused on improving their mechanical properties to achieve completely sutureless medical procedures.

Bicomponent tissue adhesive (platelet-rich plasma in combination with fibrin adhesive) constitutes the basic mixture for intraoral bone grafting techniques.

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