Research Paper

Comparative analysis of Blood clot, Plasma rich in growth factors and Platelet rich fibrin resistance to bacteria induced fibrinolysis

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ABSTRACT

The aim of this study was to evaluate the resistance of blood clot, Plasma Rich in Growth Factors (PRGF) and Platelet Rich Fibrin (PRF) to fibrinolysis induced by five different microbes and to assess the activity levels of these micro-organisms. Blood from forty-five (45) human volunteers was used to prepare four mediums: blood clot medium as control group, PRF and PRGF 1st fraction (PRGF I) and PRGF 2nd fraction (PRGF II) as study groups. Additionally, collected blood was used for blood plasma preparation on which evaluation of initial value of D-dimer concentration was performed. A solution of five different microbes (Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Bacillus cereus and Candida albicans) was adjusted to 0.5 McFarland (1 × 10^8 CFU/ml) and then diluted to 0.25 McFarland (1 × 10^4 CFU/ml). The D-dimer concentration was evaluated after one and three hours of bacteria exposure. The resistance to fibrinolysis was not statistically distinguished between all medium groups at any time. S. pneumoniae was statistically active in PRF after three hours, while C. albicans was statistically active in PRF II after one hour, in PRF between the first and third hour and after three hours. S. aureus and B. cereus were statistically active in PRF II after three hours. S. pyogenes was statistically active after one hour between the first and third hour and after the third hour in all groups. S. pyogenes was the most active bacterium. Different blood formulations were not distinguishable based on resistance to bacteria induced fibrinolysis. Low fibrinolytic properties of the found major microbes defines the factor that bacteria-induced fibrinolysis is one of the leading causes of absence of a clot in a post-extraction socket as clinically insignificant. The initial absence of a clot or its mechanical elimination during formation or the healing period are major causes of dry-socket.

Key words: Platelet concentrate, fibrinolysis, dry-socket, D-dimer, bacteria.

INTRODUCTION

Alveolar osteitis (AO) is a common, painful post-operative complication that occurs one to three days after tooth extraction (Blum, 2002). Symptoms include severe pain in the extraction site, moderate to severe dull headache with pain radiating to the ears in some cases, halitosis and dysgeusia or altered taste and can last up to 28 days (Field et al., 1985). The prevalence varies from 1 to 5% for all dental extractions and up 30% for third molar extractions, depending on the degree of tissue trauma caused by tooth extraction (Daly et al., 2012) and predispositional risk factors such as smoking and poor oral hygiene (Haraji et al., 2012; Punia, 2016).

The etiology of alveolitis is associated with partial or total loss of the blood clot (Blum, 2002). Fibrinolytic activity in the extraction socket has been shown to be one of the etiological factors associated with the clot loss orlysis and...
exposure of the bone to the oral cavity (Blum, 2002). The increased fibrinolysis is thought to be attributed to surgery trauma, pathogenic fibrinolitics and enzymatic changes due to inflammation and the presence of high amounts of bacteria before and after surgery (Haraji et al., 2012; Punia, 2016).

The oral cavity is colonized by numerous bacteria including Staphylococcus Aureus, Streptococcus Pneumoniae, Streptococcus Pyogenes, Candida Albicans (Beaufort et al., 2008; Bottone, 2010; Cannon and Chaffin, 2016). The balance of this microflora contributes to active fibrinolysis which may induce blood clot lysis (Fulde et al., 2013; Jong et al., 2003; Kolokythas et al., 2010; Loof et al., 2014; Vijayaraghavan and Vincent, 2014). One fibrinolytic bacterium, Bacillus cereus, is abnormal to the oral cavity and may originate from B. cereus colonized food intake (Jensen, 1978; Kolokythas et al., 2010).

Numerous methods have been reported for prevention and management of alveolar osteitis (Haraji et al., 2012). One of the prevention approaches is the application of platelet concentrates such as plasma rich in growth factors (PRGF; BTI Biotechnology Institute, San Antonio, Spain) or platelet-rich fibrin (PRF). Both concentrates consist of various growth factors to boost the healing process (Anitua et al., 2012; Su et al., 2009). The difference between PRGF and PRF is the white blood cell count: PRGF expresses none of these (Sharma et al., 2017). They are listed effective in reducing the incidence of AO (Eshghpour et al., 2014; Haraji et al., 2012; Unsal and GN, 2018). Authors have shown that both the aforementioned platelet concentrates are effective for management of already present AO (King et al., 2018; Sharma et al., 2017).

Platelet concentrates have an antibacterial effect. Positive activity of leukocyte- and platelet-rich plasma against S. Aureus, Enterococcus faecalis and Pseudomonas aeruginosa was reported (Cieslik-Bielecka et al., 2018). Kour et al. (2018) found out that PRF products exhibit an antibacterial effect on Porphryromonas gingivalis and Aggregatibacter actinomyctete mcomitans. A 2016 review offered an overview of the anti-microbial effect of platelet concentrates on various microbes (Kour et al., 2018).

Even though the leading factor of alveolar osteitis is the lysis of blood clot, no present studies on platelet concentrates considering the resistance to microbe-induced fibrinolysis were found. In this study, the resistance of both PRGF and PRF to fibrinolytic bacteria (S. Aureus, S. pneumoniae, S. pyogenes, B. cereus and C. albicans) was examined and the results to the blood clot medium compared.

MATERIALS AND METHODS

The study was performed following the principles of the Declaration of Helsinki, as revised in 2008 and after approval from Kaunas Regional Biomedical Research Ethics Committee, Lithuania.

Preparation of PRF

Blood samples were collected from 15 healthy volunteers (20 to 24 years old) using 18G needles. For each individual, 1 tube of peripheral blood was collected and immediately placed in a centrifuge (Process for PRF, Nice, France). Centrifugation was performed according to the following protocol: A-PRF+, sterile glass coated plastic tube (10 ml and 1300 rpm for 8 min). The PRF was then separated from the remaining blood clot and divided into 5 parts (for each bacterium) and transported to five different tubes without additional reagents.

Preparation of PRGF

Preparation of plasma rich in growth factors was conducted according to the manufacturer’s instructions. Blood samples were collected from 15 healthy volunteers (20 to 24 years old) using 18G needles and PRGF-Endoret® Tubes (BTI Biotechnology Institute, S.L., Miñano, Spain) containing 0.2 ml of sodium citrate. For each individual, two tubes of peripheral blood were collected and immediately centrifuged at 580 g for 8 min. Fraction 1 (above Fraction 2) and Fraction 2 (the fraction 2 ml above the buffy coat) were collected and subjected to two separate tubes. Fractions 1 and 2 fibrin scaffolds were prepared by activating PRGF fractions with calcium chloride (Endoret Dentistry) at 37°C for 1 h (50 μl of calcium chloride for each ml of PRGF). Thereafter, each PRGF fraction was divided into five pieces and then transported to five different tubes for each fraction without additional reagents.

Preparation of blood clot

Blood samples were collected from 15 healthy volunteers (20 to 24 years old) using 18G needles. For each individual, five (5 ml each) tubes of peripheral blood were collected. The tubes were kept at room temperature for 10 min and exposed to 37°C for three hours. After the blood sample was fully coagulated, the blood serum was removed using a Pasteur pipette.

Preparation of blood plasma

Additional blood samples were collected from each group volunteer (45 total) using 18G needles. For each individual, two (5 ml each) tubes (containing 0.2 ml of sodium citrate) of peripheral blood were collected and immediately placed in a centrifuge for 9 min, 2500 RCF (HettichRotina 35, Germany). After three hours of incubation at room
temperature, concentration of D-dimers of blood plasma was measured (STAGO Diagnostics, STA Compact, France). Blood plasma D-dimer count was used as the initial value.

Preparation of bacteria and fungus suspension

Three to five isolated colonies on the blood medium were touched with a sterile tag and transferred into a tube of saline. The solution was adjusted to 0.5 McFarland ($1 \times 10^8$ CFU/ml) and then diluted to 0.25 McFarland ($1 \times 10^4$ CFU/ml). The solution was poured into disposable tubes of 1 ml each.

PRF, PRGF and blood clot fibrinolysis activity assay

400 µl of blood plasma was carried to every PRF, PRGF and blood clot tube using a Finn pipette batcher. Then, using the same batcher, 600 µl of prepared bacteria suspension was carried to the same tubes. In the PRF, PRGF and blood clot tubes concentration of D-dimers was evaluated after one and three hours (STAGO Diagnostics, STA Compact, France).

Statistical analysis

Results are presented as the mean, as appropriate. Due to the assumption that the outcome is approximately not normally distributed D-dimer differences were analyzed with the use of non-parametric tests. Differences among the formulations were evaluated using the one-way ANOVA (analysis of variance) test, the Wilcoxon matched-pairs test and Kruskal-Wallis test for multiple comparisons. A p-value of <0.05 was considered significant for these tests.

RESULTS

The results were structured into two categories. The first category was evaluated based on the resistance of each medium (blood clot, PRF, PRGF I and PRGF II) to bacteria-induced fibrinolysis and what the D-dimer concentration was in a specific group at the entire timescale. In the second category, bacteria and their fibrinolytic activities were evaluated by highest D-dimer concentration or its change in the presence of a microbe at a specific time.

Initially, the resistance to fibrinolysis was not statistically distinguished between all medium groups in the initial stage, after the first and third hour. D-dimer concentration was not statistically diverse ($p>0.05$). However, mean D-dimer concentration of PRF group was $1.02\pm0.21$ µg/ml and $0.66\pm0.12$ µg/ml in the blood clot group in the presence of $C.\ albicans$. The earlier mentioned values were statistically diverse ($p<0.05$). Thus, PRF showed elevated D-dimer count as compared to the blood clot group during the entire timescale (Figure 1).

Different types of bacteria expressed diverse fibrinolytic activity based on the medium and time of exposure. $S.\ pneumoniae$ did not show an elevated D-dimer count after one and three hours in any medium group except for PRF after three hours. Mean D-dimer concentration of $0.89\pm0.21$ µg/ml in the PRF group after three hours was statistically higher as compared to all other mediums ($p>0.05$) (Figure 2). $C.\ albicans$ had raised D-dimer concentration in PRGF II group after one hour and mean D-dimer concentration was held at $0.7\pm0.21$ µg/ml ($p<0.05$). In the PRF group between the first and third hour D-dimer concentration was elevated by $0.17\pm0.09$ µg/ml ($p<0.05$) (Figure 3). After three hours.
the PRFD-dimer value of 0.96±0.34 µg/ml was statistically higher as compared to other mediums and initial value (Figure 2) (p<0.05). Comparing PRGF II and PRF groups in the presence of C. albicans showed that Candida bacterium is more active in the PRF medium (p<0.05). S. aureus and B. cereus increased D-dimer concentration after three hours in PRGF II medium with values of 0.69± 0.28 and 0.7±0.32 µg/ml, respectively (p<0.05). S. pyogenes exhibited D-dimer

Figure 2: D-dimer concentration in PRF group after 3 h.

Figure 3: D-dimer concentration in PRF group between 1st and 3rd h.
DISCUSSION

As platelet concentrates became a major factor in everyday oral surgery practice it is essential to evaluate each product as an anti-bacterial approach. Blood clot lysis is mostly dependent on micro-organism fibrinolytic activity. Platelet concentrates undoubtedly provide an acceleration of tissue regeneration. Hence, it is important to assess fibrinolytic resistance of platelet products prepared by different protocols. We created and adapted a unique fibrinolysis measurement and evaluation protocol, including five fibrinolytic active pathogens in direct contact with the concentrates and blood clot as no comparative characteristics in-vitro have been described before. Clinical studies report that both PRF and PRGF are effective (Haraji et al., 2012; Sharma et al., 2017). The question remains as to how the structure and composition of these concentrates may influence fibrinolytic resistance. Therefore, our study aimed at carrying out in-vitro circumstances. Various studies have measured the level of fibrinolysis using spectrophotometry method (Bonnard et al., 2017; Cellai et al., 2010); however, this assay requires the elimination of living circulating cells.

Our objective was to create an actual in-vitro model of blood clot and platelet concentrate interaction with pathogenic microbes. The D-dimer assay was chosen for the measurement of fibrinolysis level as it is sensitive and offers predictive value describing clot dissociation (Chapin and Hajjar, 2015).

PRF and PRGF are two leading products which differ in both preparation protocol and biological composition. As the literature states, L-PRF includes leukocytes, fibrin mesh and platelets, while PRGF consists mostly of platelets and fibrin (Anitua et al., 2015; Dohan et al., 2014). Different studies describe the presence of leukocytes unevenly and pro-inflammatory or anti-inflammatory properties are observed. Both in-vitro studies and literature review studies support these discussions (Cerletti et al., 2010; Marenzi et al., 2015; Scott and Krauss, 2012). During our research no significant difference in the rate of fibrinolysis was observed in all groups indicating that the presence of leukocytes does not have any significant role in a product’s anti-fibrinolytic properties.

Furthermore, as described by Loof et al. (2014), S. pyogenes presents a thorough fibrinolytic potential in blood clot alone, processing through plasminogen-induced fibrinogen conversion to fibrin. Our results determined exactly the same properties of S. pyogenes in both platelet concentrates and blood clot alone, processing through plasminogen-induced fibrinogen conversion to fibrin. Our results determined exactly the same properties of S. pyogenes in both platelet concentrates and blood clot alone, processing through plasminogen-induced fibrinogen conversion to fibrin.
concentrates, as the bacteria stands out as the most fibrinolytically active group. This data may reveal that platelet concentrates are unable to prevent streptokinase-induced fibrinolysis and present the same anti-fibrinolytic properties as blood clot alone.

In the oral surgery field, platelet concentrates are routinely used for socket preservation and as an alveolitis prevention measure (Alzharni et al., 2017). Numerous studies display significant results in new bone formation, its quality and quantity, as well as, reduced pain and healing time (Solakoglu, 2012; Temmerman et al., 2016; Zhang et al., 2018). Considering the results obtained from our research, PRF, PRGF and blood clot present no significant difference in anti-fibrinolytic characteristics, which implies that the physical properties of fibrin mesh of different blood preparations were not distinct (Weisel and Livintov, 2008).

Bacteria or fungi-induced fibrinolysis is described as one of the leading factors causing absence of a clot in post-extraction socket. The low fibrinolytic properties of four major microbes found in the oral cavity defines this factor as clinically insignificant, implying that the initial absence of a clot or its mechanical elimination during formation or healing period are major causes of dry-sOCKET in practice. In clinical practice, it is essential to assure adequate post-extracational bleeding using anesthetics without vasoconstrictors and mechanical protection of the wound. Nevertheless, fibrinolysis is dependent on bacterial activity. Thus, pre- and post-operative oral cavity treatments with antibacterial agents such as chlorhexidine is suggested to fully minimize the incidence of alveolar osteitis (Sridhar et al., 2011).

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REFERENCES


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