

Evaluation of Platelet-Enriched Plasma Antimicrobial Effect: In Vitro Study

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Abstract

Platelet-rich plasma (PRP) is autologous blood product, that contains platelets concentration two to three times above normal blood level. As such, PRP is a rich source of different bioactive molecules including grow factors, enzymes, cytokines, and chemokines. Beside its regenerative properties, a limited number of studies has proven that PRP can induce antimicrobial effect against single growing pathogens and biofilms. Aim of this study was to analyze PRP antimicrobial effect against three most common biofilm forming bacteria, including *S. aureus*, *E. coli* and *P. aeruginosa*. The antimicrobial property of PRP was evaluated after 24 h of incubation with selected bacteria in Brain heart infusion (BHI) media using spectrophotometer with a light source of 600 nm. To check whether PRP can inhibit bacterial biofilm formation, after 24h incubation, tube screening test (TM) was applied. Bacteria treated with PRP and platelet poor plasma (PPP) were compared with untreated control, composed of bacteria growing spontaneously in BHI media. PRP produced strong growth inhibition in all tested bacteria when compared to bacteria treated with PPP and control group. Based on the obtained results it can be concluded that PRP can induce antimicrobial effect on *S. aureus*, *P. aeruginosa* and *E. coli*. PRP also reduced biofilm formation for *P. aeruginosa* and *E. coli*. However, there was no effect on *S. aureus* biofilm formation.

Keywords: platelet-rich plasma (PRP); antimicrobial activity; biofilm

Introduction

Platelet-enriched plasma (PRP) is a plasma fraction with a concentration of platelets three to five times higher than the basic concentration of autologous human plasma. The normal number of platelets in the blood ranges from 150,000 to 350,000 platelets per 1 μ l of blood, while PRP is most often defined as a suspension of 1,000,000 platelets per 1 μ l of blood [1]. Since platelets are the main components of PRP, PRP represents a rich source of various bioactive molecules, including enzymes, growth factors, cytokines, chemokines, and molecules involved in cell adhesion. These bioactive molecules play crucial roles in tissue regeneration and healing process. For a long period of time platelets were considered to only have regenerative properties, however recent findings have revealed antimicrobial properties of platelets. Platelets can induce antimicrobial activity using different mechanism, for example platelets can directly bind and internalize pathogens, they can stimulate the production of reactive oxygen species such as superoxide and hydrogen peroxide, and most recent findings indicate that platelets can also produce small antimicrobial proteins known as platelet microbicidal

proteins/peptides (PMPs) [2]. Another advantageous characteristic of PRP is that represents an autologous product, derived from the patient own blood. As such PRP minimizes any risk of allergic and immune reactions or transmission of infectious diseases.

Today, one of the biggest challenges in fighting bacterial infections represent biofilm forming bacterial communities. Biofilm infections are difficult to treat due to their highly structured and organized multispecies communities, in which bacteria can exchange genes conferring to antibiotic resistance. Furthermore, biofilm extracellular matrix made of extracellular polymers acts as a shield protecting bacterial communities from antibacterial agents [3]. More recently antibacterial properties of PRP have been elucidated. Several studies have found that PRP has bacteriostatic and bactericidal effects on most common bacterial pathogens including methicillin-resistant and methicillin-sensitive *S. aureus* strains (MRSA and MSSA), *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. epidermis* [4-7]. However, there is a very little number of studies, studying PRP antimicrobial activity against biofilm infections. Lately, PRP activity to combat *S. aureus* formed biofilm aggregates in equine synovial fluid causing infectious osteoarthritis was studied [8]. According to the results PRP preparations containing higher number of platelets without leukocytes had increased antimicrobial activity and showed positive synergism with antibiotic amikacin. This study also showed that antibacterial effect of PRP is contributed mainly to platelets and not to leukocytes.

Aim of this study was to investigate PRP antimicrobial effect against three most common biofilm forming bacteria, including *S. aureus*, *E. coli* and *P. aeruginosa* and compare its antimicrobial activity with platelet poor plasma (PPP) and control.

Materials and Methods

PRP preparation

Institutional ethical approval was obtained before conducting this study. To prepare PRP, the same male volunteer served as blood donors (age 38). Whole blood was collected in 30ml tubes with Na-citrate as anticoagulant. PRP was prepared using two-step centrifugation. After first centrifugation the blood separates in three layers. First and second layer, known as plasma and buffy coat, are separated in new tube with thin layer of red blood cells at the bottom. After second centrifugation, the upper layer is considered platelet poor plasma (PPP) and the remaining part constitutes platelet rich plasma (PRP).

Bacterial Strains

Clinical strains used in this study were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Tested strains were collected from patients with persistent urinary and soft tissue infections. Isolated bacteria strains were stored in glycerin stocks at -20°C. Once they were ready to use, after thawing, 50µl of each bacteria was inoculated in polystyrene tubes containing 3ml Brain Heart Infusion (BHI) media and incubated overnight at 37°C. To test the ability of bacteria to form biofilm formation, tube screening test (TM) was used [9].

Evaluation of antimicrobial activity

This study included two different experimental groups and one negative control group containing bacteria in BHI media without addition of any human blood product. First group tested PRP antimicrobial activity, and second group was used to test activity of PPP. To test PRP antimicrobial activity a total of 100µl of PRP were added to tubes containing 20µl of each bacteria and 3 980µl BHI media. To test the activity of PPP, a total of 100µl of PPP was added to 20µl of each bacteria and 3 980µl BHI media. To assess bacterial proliferation OD600 was measured by a spectrophotometer with a light source of 600 nm before incubation (time 0) and after 24h of incubation at 37°C (time 24). Experiments were performed in triplicates. To check whether PRP can inhibit bacterial biofilm formation, after 24h incubation tube screening test (TM) was applied.

Results

The effect of PRP and PPP on bacterial growth inhibition were evaluated in three different bacterial strains: *P. aeruginosa*, *S. aureus* and *E. coli*. Bacteria treated with PRP and PPP were compared with untreated control, composed of bacteria growing spontaneously in

BHI media. According to the obtained results, PRP produced strong growth inhibition in all tested bacteria when compared to bacteria treated with PPP and control group (Figure 1, 2 and 3). Bacterial growth was assessed using absorbances (OD600), obtained results are presented in table 1, 2 and 3.

Experiment	OD600 (0h)	OD600 (24h)	Bacterial growth	Biofilm 24h
Control	0.04	2.50	2.46	+
PRP + <i>P. aeruginosa</i>	1.21	2.50	1.29	-
PPP + <i>P. aeruginosa</i>	0.40	2.32	1.92	-

Table 1: *P. aeruginosa* OD and tube test results for biofilm formation after 0h and 24h. Bacteria was treated with 100µl of PRP or PPP and compared with control group.

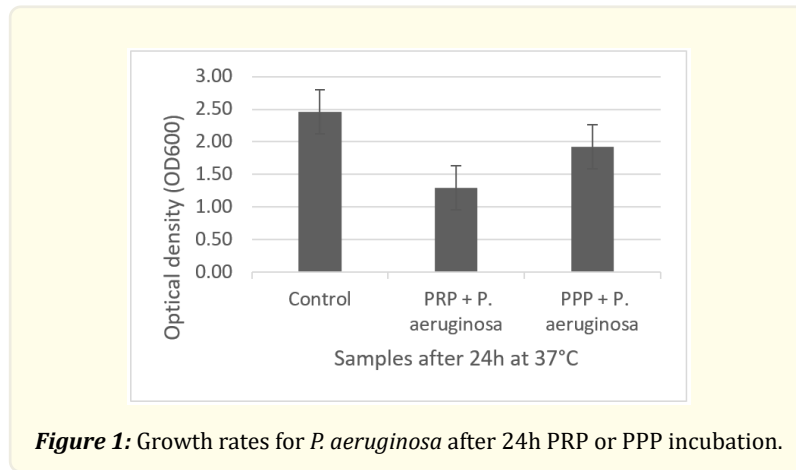


Figure 1: Growth rates for *P. aeruginosa* after 24h PRP or PPP incubation.

Experiment	OD600 (0h)	OD600 (24h)	Bacterial growth	Biofilm 24h
Control	0.19	2.36	2.17	+
PRP + <i>S. aureus</i>	1.27	2.38	1.11	+
PPP + <i>S. aureus</i>	0.55	2.50	1.95	+

Table 2: *S. aureus* OD and tube test results for biofilm formation after 0h and 24h. Bacteria was treated with 100µl of PRP or PPP and compared with control group.

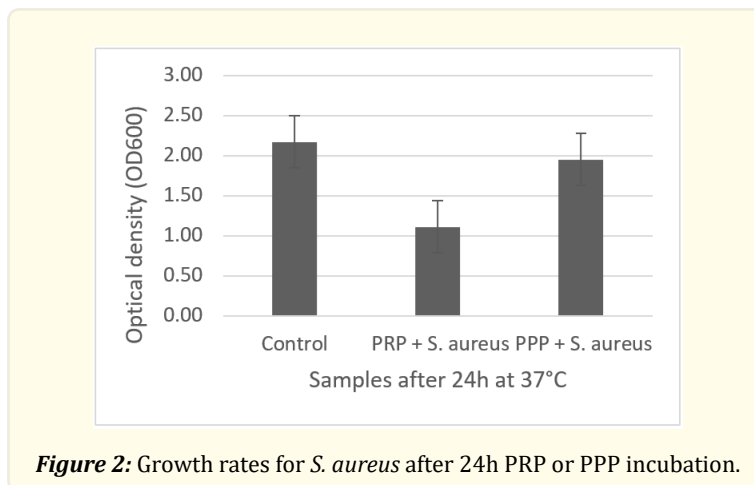


Figure 2: Growth rates for *S. aureus* after 24h PRP or PPP incubation.

Experiment	OD600 (0h)	OD600 (24h)	Bacterial growth	Biofilm 24h
Control	0.23	2.20	1.97	++
PRP + <i>E. coli</i>	1.31	2.50	1.19	+
PPP + <i>E. coli</i>	0.30	2.48	2.18	++

Table 3: *E. coli* OD and tube test results for biofilm formation after 0h and 24h. Bacteria was treated with 100µl PRP or PPP and compared with control group.

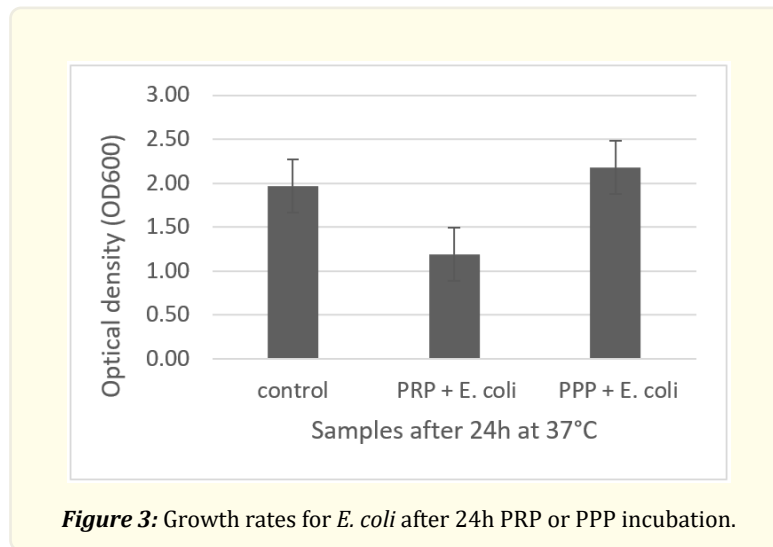


Figure 3: Growth rates for *E. coli* after 24h PRP or PPP incubation.

Discussion

In the last decade PRP regenerative and therapeutical properties have been widely studied and PRP have been used in different medical fields including orthopedics, sports medicine, dentistry, plastic surgery, gynecology, neurosurgery, ophthalmology, urology, wound care and aesthetic medicine [10]. However, antimicrobial properties of PRP preparations are beginning to emerge and very few studies have studied PRP antimicrobial mechanism. Today, we don't know all mechanisms by which platelets induce antimicrobial properties and it is in a near past that platelets have been discovered to induce antimicrobial effect. However, one thing is clear, PRP due to its rich compositions made of different bioactive molecules, most of them secreted by platelets represents a promising agent in fighting bacterial infections. This aspect of PRP is particularly important to elucidate since we are witnessing tremendous increase in antimicrobial resistance causing one of the major urgent threats to public health. In addition, antibiotic resistance is making a burden for healthcare system and increases a healthcare cost due to its prolonged hospital admission and treatment failures. In Europe it is estimated that antibiotic resistance correlates with more than nine billion euros per year [11].

S. aureus and *P. aeruginosa* are most common bacteria found in persistent infections and patients suffering from chronic wound [12]. Furthermore, *S. aureus* is most common bacteria isolated in infectious arthritis and periprosthetic joint infection (PJI) and is also associated with the highest treatment failure rates [13]. In this study we have proven that PRP can decrease the growth rates of three selected bacteria *S. aureus*, *P. aeruginosa* and *E. coli*. We have demonstrated the beneficial effect of PRP against selected bacteria, however in regards of treatment, adequate amount, and timing of PRP admissions need to be determined. It has been shown that peak PRP antimicrobial activity against MRSA and carbapenem-resistant *P. aeruginosa* after in vitro incubation are at the fifth and it continued until tenth hour [4]. It is also important to emphasize that PRP antimicrobial effect also depends on PRP preparation and quality. For example, higher antimicrobial PRP effect is seen in PRP preparations prepared with two-step centrifugation when compared with

those prepared with one-step centrifugation [6]. However, for better understanding of PRP antimicrobial properties we need to look for molecules that affect microbial metabolism and understand their method of action. Up to our knowledge there is just one study exploring PRP bioactive molecules that induce antimicrobial effect and no studies correlating its effect with bacteria metabolites [14].

Conclusion

Based on the obtained results it can be concluded that PRP can induce antimicrobial effect on *S. aureus*, *P. aeruginosa* and *E. coli*. PRP also reduced biofilm formation for *P. aeruginosa* and *E. coli*. However, there was no effect on *S. aureus* biofilm formation. Further research identifying PRP antimicrobial mechanism of action is needed to provide better understanding of PRP antimicrobial potential in fighting antibiotic resistance bacteria.

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