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PROGRAM & ABSTRACTS
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Antimicrobial Activity of Platelet Gel against *Staphylococcus Aureus*

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Introduction:

The use of platelet gel (PG), a mixture of platelet rich plasma (PRP) and thrombin, to stimulate bone formation and wound healing by the release of platelet growth factors has been investigated extensively. However, it has been suggested that PG might also have antimicrobial properties. In addition to several growth factors also platelets antimicrobial peptides are present in the platelet α -granules, which released their content upon activation with thrombin. PG also contains a high concentration of leukocytes, which play an important role in the normal host defense against infections. It has been shown that myeloperoxidase (MPO), mainly present in the monocytes and neutrophilic granulocytes, is the most important mediator in this process. In the current study, we investigated the *in vitro* antibacterial activity of PG against *Staphylococcus aureus*.

Materials and Methods

Production of PRP: Blood was obtained from 6 healthy donors (3 male, 3 female; age 22-45). Two 60 ml syringes were pre-filled with 7 ml of anticoagulant citrate dextrose A solution and 53 ml of whole blood was slowly drawn via an intravenous catheter. PRP was prepared, using the Angel Whole Blood Processing System™ (AWBPS; Sorin Group, Mirandola, Italy). The AWBPS is a semi-automated tabletop centrifuge system using a flat-disc, with a variable blood volume ranging from 60 to 180 ml. Following a 19 min spin at 3.200 rpm, platelet-poor plasma (PPP) was removed and platelet rich plasma (PRP) was collected. 12 ml of PPP was isolated to produce autologous thrombin (AT), using activAT (Sorin Group, Mirandola, Italy). The red blood cells were collected separately, but were discarded. PRP was mixed with AT in a 10:1 ratio to create PG. As an alternative to AT, PRP was also activated with bovine thrombin (500 U/ml; Jones Pharma Inc, St Louis, MO, USA) in a ratio 10:1. Platelet and leukocyte counts were measured in samples of whole blood, PRP and PPP as a quality control of the PRP separation process.

Bacterial kill assay: Phosphate buffered saline (control), 20% v/v PG activated with autologous thrombin, PG activated with bovine thrombin, PRP or PPP were added to sterile tubes containing *Staphylococcus aureus* Wood 46 (ATCC 10832) at a final concentration of 1×10^6 colony-forming units (CFU)/ml in Müller-Hinton broth. After 1, 4, 8, 12 and 24 hours, a 50 μ l sample was taken from each tube and antibacterial activity of MPO was inactivated by adding excess catalase. Serial 10-fold dilutions were made and plated on blood agar plates. After an overnight incubation at 37°C the number of viable bacteria were counted ($^{10}\log$ CFU/ml).

Statistical analysis: Analysis was performed using repeated measures analysis and Tukey-HSD post hoc-testing. $P < 0.05$ was considered significant.

Results:

Quality control of PRP: An average of 4.5 ml concentrated PRP was obtained from the whole blood samples. This volume was diluted with PPP to obtain a total PRP volume of 10 ml. Analysis of the samples showed that the leukocyte content increased from 5.7 ± 0.4 ($10^9/l$) in whole blood to 18.0 ± 1.2 in PRP. The platelet concentration increased from 262 ± 11 ($10^9/l$) in whole blood to 1688 ± 130 in PRP. In PPP only a few platelets were present, as expected.

Bacterial kill: Cultures showed a rapid decrease in the number of bacteria for both PG activated with autologous thrombin and with bovine thrombin. The maximum decrease was seen after 4 hours when $0.35 \pm 0.07\%$ and $1.03 \pm 0.44\%$ of the number of bacteria present in the control group were left for PG autologous and bovine, respectively. Because the bacterial kill was not complete in any of the groups, the number of bacteria increased at later time points, when growth rates exceeded bacterial kill. After 24 hours, bacterial growth had reached the stationary phase in all groups. Although PG, PRP and PPP all induced a significant decrease in the number of bacteria compared to the control ($p < 0.001$), the effect of PG activated by autologous thrombin appeared to be largest ($p = 0.093$ vs. PG bovine activated; $p = 0.004$ vs. PRP and $p < 0.001$ vs. PPP).

Discussion:

This experiment showed that platelet gel has a significant antimicrobial activity against *S. aureus*. Although it did not result in a total kill using the current set-up, it did reduce the absolute number of bacteria to less than 1% of the control up to 8 hours. Strikingly, also non-activated PRP and even PPP decreased bacterial growth. This may be explained by the presence of lower amounts of antimicrobial agents in these groups as well.

As platelet gel is a safe to use autologous blood product, which can be easily prepared during surgery, it appears to be a potentially useful prophylactic strategy against postoperative infections, for example as a coating on uncemented implants. However, further research should prove its efficacy in combination with implants and in the in vivo situation.