Antimicrobial properties of Platelet-rich preparations. A systematic review of the current pre-clinical evidence

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Antimicrobial properties of Platelet-rich preparations. A systematic review of the current pre-clinical evidence.

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Short title: Do platelet concentrates help fighting infections?

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Abstract

In recent years autologous platelet concentrates (APC) became popular in several medicine fields, representing a valuable adjunct to regenerative surgical procedures. Beneficial effects in the control of postsurgical discomfort and infection have also been frequently reported, suggesting that APC may possess anti-inflammatory and antimicrobial properties. Aim of the present review was to summarize the current evidence regarding antimicrobial effects of platelet concentrates, investigated by *in vitro* and animal studies. This review was conducted following a systematic approach. An electronic search was performed on MEDLINE, EMBASE and Scopus databases using appropriate search terms, without language or time restrictions. Preclinical studies assessing the antimicrobial activity of APC were included and divided according to the experimental design. **Twenty** *in vitro* studies and four animal studies, investigating APC effects on a broad range of microorganisms, were included. In *in vitro* studies APC reduced the growth of microorganisms during the first hours of incubation, while they could not completely break down the microbial load. In fact, over time a recovery of bacterial growth was always observed, suggesting that APC display a bacteriostatic rather than a microbicidal activity. All animal studies showed that APC administered by local injections were able to reduce the infection caused by different microorganisms, although to a lesser extent compared to antibiotics. In conclusion, though the exact action mechanisms of interaction with microbial pathogens need further investigation, platelet concentrates proved to have antimicrobial properties, and therefore could represent a useful natural substance for controlling postoperative infections at surgical sites.

Introduction

During the past 20 years, autologous platelet-rich preparations have achieved a great popularity in various fields of medicine such as dentistry, oral surgery, orthopedics, sports medicine, dermatology, ophthalmology, cosmetic and plastic surgery [1-3]. The rationale for their use is the release of growth factors and other bioactive molecules, stored in intracellular α -granules and released upon platelet activation that help to modulate wound healing in both hard and soft tissues. In addition, anti-inflammatory and antimicrobial properties of platelet-rich preparations have been recently pointed out [4-6].

While the regenerative potential of platelet concentrates has been extensively explored since their introduction in regenerative medicine in the oral and maxillofacial surgery field during the late '90s [7-8], only few studies have investigated their antimicrobial effect, although an increase of publications in this field of research occurred in recent years.

The mechanisms underlying the antimicrobial effect of platelet concentrates are not yet clear. Platelet concentrates consist of a complex mixture of variable concentrations of platelets and plasma and, depending upon the protocol used for their production, variable content of leukocytes, embedded in a more or less dense fibrin matrix [2,9-10]. Nevertheless, the respective impact and mechanism of action of each component, as well as possible synergistic effects among these components, in the fight against infection are poorly known.

The first studies hypothesizing the antimicrobial activity of platelets date back several decades [11-12]. Platelets act as sentinels of vascular system, express a wide range of potential bacterial receptors, may have the ability to internalize bacteria and are able to release a broad variety of molecules that provide an array of host defense functions [13]. Subsequently, several attempts took place in order to isolate and characterize specific antimicrobial molecules from platelets of human and animal origin [14-19]. *In vitro* studies demonstrated that platelets, following stimulation with thrombin, are able to release proteins with antimicrobial activity against bacteria and fungi [17-18]. In addition to the release of antimicrobial peptides, platelets are also able to generate reactive

oxygen species, bind and internalize microorganisms and participate in antibody-dependent cellular cytotoxicity [20]. Recent studies underlined the direct role of platelets in recognize, sequester and neutralize invading pathogens, as well as their indirect contribution to recruit leukocytes to infection and inflammation sites, and to modulate their behavior, enhancing their ability to phagocytose and kill microorganisms by triggering different types of signaling pathways [21].

The role of leukocytes in platelet concentrates is subject of an intense debate. According to some authors, an increase in the concentration of white blood cells into platelet concentrates could help to improve the stability of the scaffold, increase the antimicrobial potential and regulate the inflammatory response [10,22]. On the contrary, other authors recommend excluding leukocytes from platelet concentrates, as these cells might lead to an increase of the inflammatory response due to the secretion of acid hydrolases, metalloproteases and other pro-inflammatory proteases [1,9].

The aim of this review was to collect and summarize the available preclinical evidence regarding the antimicrobial efficacy of platelet concentrates, in order to shed light upon their underlying mechanisms and potential benefits.

Materials and methods

Literature search

A search was performed by two authors (MDF, MB) on electronic databases (MEDLINE, EMBASE, Scopus), using the following search terms, alone and in combination, by means of Boolean operators: “platelet-rich plasma”, “platelet concentrate”, “platelet-rich fibrin”, “platelet growth factors”, “microorganisms”, “bacteria”, “antibacterial”, “antimicrobial” and “infection”. No language nor time restrictions were applied. The last electronic search was performed on **October** 2015. Reference lists of the reviews and of the identified articles were also checked for possible additional studies. Finally, the manufacturing companies producing devices for concentrating platelets were contacted to identify ongoing or unpublished studies pertinent to this review.

Inclusion and exclusion criteria

Preclinical studies (including animal and *in vitro* studies) assessing the antimicrobial activity of platelet concentrates were included. All other types of study design were excluded.

Data extraction

The titles and abstracts of the retrieved articles were independently screened by two reviewers (MB, MDF) and studies meeting the inclusion criteria were identified. When the title and abstract of an article did not provide sufficient information to make a decision, the full text was obtained and examined. Publications that did not meet the inclusion criteria were excluded. The full text of the included studies was obtained for data extraction and quality assessment. The characteristics of the selected studies were examined and the articles were sorted into two groups: (a) *in vitro* studies; (b) animal studies.

Assessment of the risk of bias

The risk of bias of the included studies was evaluated independently and in duplicate by two reviewers (MB, CC) as part of the data extraction process. *In vitro* included manuscripts were assessed considering the CRIS Guidelines, specifically developed for improving the quality of reporting of *in vitro* studies in experimental research [23]. The following criteria were considered: sample size calculation; meaningful difference between groups (comparability of control and treatment groups at entry); sample preparation and handling; randomization method; concealment of sequence allocation; blinding of operator(s) and evaluator(s); conflict of interest statement; statistical analysis. The assessment of animal studies was conducted considering criteria adapted from the ARRIVE guidelines [24] specifically developed for rating the quality of animal studies. The criteria considered were: experimental animals (species, age, weight); sample size calculation; ethical statement; randomization method; concealed allocation of animals to experimental group; blinding of operator(s) and evaluator(s); conflict of interest statement. All these criteria were judged as adequate (yes), unclear or non adequate (no). In order to summarize the validity of studies, they were grouped into the following categories: A) Low risk of bias if none of the quality criteria was judged as inadequate and no more than two of them were judged as unclear; B) Moderate risk of

bias if 1 to 3 criteria was judged as inadequate; C) High risk of bias if four or more criteria were judged as inadequate. In case of discrepancy between the two reviewers, an agreement had to be obtained by discussion. Otherwise, a third reviewer was consulted (ST) until consensus was achieved by discussion. The risk of bias assessment results were synthesized using the software RevMan (Review Manager Version 5.3, 2014; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark).

Results

The initial electronic search provided 188 articles. Figure 1 is a flowchart that summarizes the article selection process. After screening of the titles and abstracts, 34 studies evaluating the antimicrobial effect of platelet concentrates were identified. No further study was identified by other search methods. After review of the full text, ten publications were excluded for the following reasons. In three studies, antimicrobial peptides extracted from platelets were used instead of platelet concentrates [17-19]; four articles reported on clinical studies [25-28]. One publication was a review and the other two were letters to the editor [5,29,58]. Twenty-four articles fulfilled inclusion criteria and were finally included in the present analysis [6,30-52]. Twenty were *in vitro* studies and four were animal studies. The included articles were published in a period ranging from 2007 to 2015. The articles adopted a broad range of variable outcomes and evaluated a number of microorganisms for assessing the antimicrobial potential of platelet concentrates.

In vitro studies

In vitro studies evaluating the antimicrobial activity of platelet concentrates are listed in Table I. The experimental design varied considerably among studies. Several types of bacteria were tested, both Gram-positive (e.g. Streptococci and Staphylococci) and Gram-negative (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*) strains, obtained from the American Type Culture Collection (ATCC) or isolated from clinical samples, and characterized by a different susceptibility to antibiotics (e.g. MRSA and MSSA, that is methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*,

respectively). In one study, a yeast strain (*Candida albicans*) was also tested [39]. Different tests were performed in order to assess the antimicrobial activity of autologous platelet concentrates (APC), such as killing curves, Kirby-Bauer disc-diffusion method or determination of the minimum inhibitory concentration. Furthermore, various methods were used for the preparation of platelet concentrates themselves, then platelet and leukocyte content was variable among studies (Table 1). According to the classification proposed by Dohan Ehrenfest *et al.* [10], nine studies used leukocyte- and platelet-rich plasma (L-PRP), while eight studies used pure platelet-rich plasma (P-PRP) and three studies used both formulations. Platelets were activated by adding calcium chloride, thrombin (either bovine or autologous) or a mixture of the two. Moreover, in one study a comparison between activated and non-activated P-PRP was made [46]. In some studies, platelet concentrates were subjected to procedures different from the standard protocols recommended by the manufacturers of the devices for obtaining autologous platelet concentrates, in order to better understand which component in APC is the responsible for the antimicrobial activity. In the study of Edelbute *et al.* PRP was centrifuged to separate the clot from the supernatant [43]. In the study of Rozalski *et al.* platelets were lysed by repeated freezing/thawing cycles and stimulated with ADP [37], while in the study of Burnouf *et al.* PRP was subjected to a treatment based on the use of specific solvents and detergents [41]. Mariani *et al.* [52] froze PRP to assess whether the preparation maintained *in vitro* characteristics, observing that PRP may be cryopreserved without important changes to its effectiveness. The blood source from which platelet concentrates were prepared varied among studies (human or animal origin).

As regards study findings, in general it was observed a tendency of platelet concentrates to inhibit the growth of microorganisms during the first hours of incubation, while they did not seem to be able to break down completely the microbial load: after an initial reduction of the inoculum, in fact, a recovery of bacterial growth was always observed, indicating that platelet concentrates displayed an inhibitory (bacteriostatic) rather than a microbicidal activity. Concerning single bacterial species, *S. aureus* was the most tested microorganism and always showed susceptibility to platelet

concentrates, while *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Klebsiella pneumoniae* were less frequently adopted and produced contradictory results: in some studies antimicrobial activity was observed, but in others no inhibitory effect against these species was obtained. In addition, some species were tested only once (e.g. *Bacillus megaterium*, *Propionibacterium acnes*, *Proteus mirabilis*, *Fusobacterium nucleatum*, etc.) showing an antibacterial activity. Regarding quality assessment, all *in vitro* studies were classified at high risk of bias as in all cases more than four criteria were judged as inadequate. In general the sample preparation and handling was always well described, the statistical analysis was mostly appropriate, but no details were provided regarding sample size calculation, randomization procedures, allocation concealment and blinding, which may rise some concern on the validity of the results.

Animal studies

Four preclinical studies were carried out using the rabbit as animal model, the L-PRP as platelet concentrate and the *S. aureus* as tested microorganism (Table II). In particular, one study used a model of spinal implant-associated infection [50], while the other studies adopted a model of osteomyelitis [48-49,51].

The study by Li *et al.* on implant-associated infection, which also included an *in vitro* evaluation, interestingly found that the antimicrobial properties of PRP are positively related to the concentration of thrombin used for PRP activation [50]. While surgical non-infected sites were clear of infection at all time points studied (1, 2 and 3 weeks), the surgical sites with bacterial challenge displayed elevated bumps, that were relatively smaller for sites treated with PRP respect to infected control sites. Significant amount of pus was present in all experimentally infected sites [50]. Postmortem quantification of bacteria in surgical sites showed that PRP treatment sites had significantly fewer bacterial colonies in bone samples than infected control sites at all time points studied, suggesting that PRP may be a useful adjunct strategy against post-operative implant-associated infections [50].

In the other three *in vivo* studies osteomyelitis was induced in rabbits according to a standard protocol: a hole was drilled in the cortex of the right proximal tibia metaphysis using a 2.0 mm Kirschner wire and an 18-gauge needle was inserted into the medullary cavity, which was subsequently irrigated with saline after bone marrow extraction. Then, a solution containing a bacterial suspension was injected into the medullary cavity. In the test group, additional injection of platelet concentrate into the medullary cavity was performed. The effect of platelet concentrates was evaluated radiographically and histologically [48-49,51]. All the studies regarding osteomyelitis showed that L-PRP administered to animals by local injections was able to reduce the infection caused by both MRSA and MSSA, although to a lesser extent compared to antibiotics, as well as to accelerate bone regeneration [48-49,51].

Regarding quality assessment, two studies were classified at high risk of bias as four or more criteria were judged as inadequate [48,50] and two studies were judged at moderate risk of bias [49,51].

Discussion

The regenerative properties of platelet concentrates have been considerably investigated during the last two decades. On the contrary, in literature only few reports were found about their antimicrobial potential.

The aim of this work was to collect the available pre-clinical evidence, consisting of *in vitro* and animal studies evaluating the antibacterial efficacy of autologous platelet concentrates. The results of *in vitro* studies suggest that platelet concentrates may effectively inhibit the growth of a wide variety of microorganisms. However, when considering specific bacterial species, results were sometimes contradictory. For example, Bielecki *et al.* [30] reported no activity of PRP against *E. faecalis*, while four other studies found an inhibitory effect against the same microorganism [17,33,39,46]. Similarly, some authors reported the efficacy of platelet concentrates against *P. aeruginosa* [6,8,13,15,17] while other researchers indicated no antimicrobial activity [29,31,38].

Such discrepancies could be due to several reasons such as the intrinsic characteristics of the bacterial strains used, which may display a different susceptibility to platelet concentrates per se or to the different sensitivity of the test used for assessing the antibacterial activity. Other important reasons for explaining the observed variability in outcomes could be ascribed to the types of platelet concentrates used that, as also explained later, may differ in form (gel or liquid), as well as in the concentration of platelets, content of leukocytes, density of the fibrin network, the activation mode that may occur naturally by contact with tissues or may be induced by thrombin or calcium chloride adjunct, and the administration mode *in vivo* that may be topical or by injection. Though the results provided by the *in vitro* studies analyzed in the present review are certainly suggestive for a significant antimicrobial potential of platelet-rich preparations, when analyzing the quality of these studies some concern emerged. The advantages of *in vitro* studies as compared to a clinical trial include the possibility of control over independent variables and unforeseen bias and the precision of all operative steps. This should improve the internal validity of the results. However, one of the major drawbacks is that *in vitro* studies lack external validity and generalizability to clinical reality. Though *in vitro* studies are relatively simpler to perform, they may lack certain methodological rigor that characterize well-conducted clinical trials. In the present review the quality of *in vitro* studies was assessed according to established guidelines specifically developed for experimental lab research [23]. The results, however, indicate that only a few studies demonstrated a sufficient methodological rigor and the overall quality is to be considered poor. Therefore, the findings of the review should be interpreted cautiously and need to be confirmed by further studies, which should be conducted and reported in conformity to the above mentioned guidelines. Existing shortcomings among *in vitro* studies, that need to be addressed to promote quality and transparency of evidence, could include the reporting of all methodological details enabling the reader/researcher to analyze and understand the variability affecting the outcome measure of experimental procedures, materials, techniques under investigation. In particular, considering the results of the quality assessment, more details should be provided regarding sample size calculation, randomization methods, allocation

concealment, blinding of operators and evaluators, and clear definition of a meaningful difference among groups, as may be evinced from Fig. 2a.

The evidence of the *in vivo* antimicrobial effectiveness of platelet concentrates is still scarce. As regards animal studies considered in the present review, very few papers have been found investigating the antimicrobial efficacy of platelet concentrates. However, in two different experimental models of infection in rabbits a positive effect of PRP was evidenced. The experimental observations suggested that PRP might represent a novel strategy to prevent bone infections, thanks to its ability to stimulating bone regeneration together with the ability of controlling microbial growth and spreading. The quality assessment of the *in vivo* studies yielded better results as compared to the *in vitro* studies as only two *in vivo* studies were judged at high risk of bias and two at moderate risk of bias. The interesting results emerged from the animal studies need to be validated by clinical evidence. As regards human studies, some retrospective comparative investigations showed that the incidence of both superficial and deep sternal wound infection in patients undergoing cardiac surgery was significantly lower for the wounds treated with platelet gel at the time of surgical wound incision compared with untreated controls [25-26]. These studies reported that the application of platelet gel also contributed to decreasing the postoperative blood loss and the length of hospital stay and costs for the treated patients. In addition, platelet gel proved to be effective in the treatment of an infected deep cutaneous wound [27], a deep sternal wound infection developed after cardiac surgery [28], and in a case of chronic femoral osteomyelitis [53].

Though the role of platelets as effective modulators and effectors of the immune response emerged in the recent years, to date, the components responsible for the antimicrobial activity of platelet concentrates remain poorly understood, in particular because these biomaterials are a complex mixture of variable proportions of platelets, white blood cells and plasma. The observation that blood plasma contains substances acting against microbes dated back more than 100 years ago [54]. At the base of the antimicrobial properties of plasma is the presence of the complement, an element

of the immune system essential for the mechanisms of humoral defense against infectious agents. The activation of the complement cascade determines bacterial cell lysis and leukocyte recruitment. A recent *in vitro* study showed that, after heat inactivation, PRP lost its inhibitory effect against *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa* [41]. Another study showed that platelets isolated from plasmatic components were not able to inhibit the growth of *Enterococcus faecalis*, *Streptococcus agalactiae*, *Streptococcus oralis* and *S. aureus* [46]. Taken together, these data seem to suggest that some plasmatic components (such as the complement) are mainly responsible for the antimicrobial activity of platelet concentrates, and that a cooperation between platelets and plasmatic elements is necessary. The respective impact of the plasma and cellular components has not yet been studied in detail, though, as discussed below, some studies reported that in the absence of plasma the antimicrobial potential of platelets alone is negligible, suggesting that there is a synergistic effect among different components of platelet concentrates [46]. Several platelet receptors have been demonstrated to interact with a number of pathogens either directly or indirectly, suggesting a plasma protein interplay that may be crucial in the early immune defense [55-56]. On the other hand, many studies have shown that different bacterial species may interact with platelets, modulating their function and activation, in a strain- and subject-dependent manner, through different mechanisms [57]. Hence, there seems to be a two-way interaction between platelets and bacterial pathogens, that may result in complete or incomplete activation, or even inhibition of platelet activation, in function of the bacterial species and bacterial secreted product involved, which may ultimately affect the cellular soluble immune response [55-56].

Several techniques are available for the production of platelet concentrates, leading to products with different biological characteristics. According to Dohan Ehrenfest *et al.*, the various platelet concentrates can be classified into four main categories, depending on their leukocyte and fibrin content: P-PRP, L-PRP, pure platelet-rich fibrin (P-PRF) and leukocyte- and platelet-rich fibrin (L-PRF) [10]. L-PRP and L-PRF might contain substantial amount of white blood cells. The contribution of leukocytes to the observed overall effect remains unclear. As concerns the

antimicrobial effect, white blood cells play undoubtedly a fundamental role *in vivo*: leukocytes, particularly neutrophils, represent the first line of defense against invading microorganisms. White blood cells possess a phagocytic activity and constitute a rich source of antimicrobial molecules (e.g. defensins, cathelicidins, lysozyme, myeloperoxidase). However, it is unknown how they can perform their function after they are removed from the circulation for the preparation of platelet concentrates and applied directly to the surgical site, bypassing the physiological stage of migration. What emerged from this review is that, to date, only a few studies compared platelet concentrates poor and rich in leukocytes [6,44,52] and all seem to suggest that there are no substantial differences between the antimicrobial activity of the two formulations. However, no definitive conclusions can be drawn on the base of only two studies, and further comparative studies are needed in order to clarify the role of leukocytes within APC.

Among the limitations of the present review there is the heterogeneity among the study aims, materials and protocols, the overall limited number of microbial species investigated, the limited characterization of platelet concentrates used (the cell composition, the concentration of the various growth factors and molecules possibly involved in the antimicrobial action was not routinely assessed). Though all the studies considered in the present review showed to a various extent that platelet concentrates have antimicrobial potential, the great diversity in the experimental design makes difficult to perform comparisons among the different studies. A greater standardization is needed in order to draw conclusions with a sufficient level of evidence.

Conclusions and future research

In addition to the well-established regenerative properties, platelet concentrates seem to possess also antimicrobial properties, and therefore could represent a useful substance for fighting postoperative infections of surgical sites. Infections are among the most serious complications that can affect the healing of a wound. Even when applying a strict disinfection protocol, bacteria can find a pathway to infiltrate and colonize the underlying tissues. The subsequent release of

1 proteolytic enzymes and bacterial toxins-rich exudates, and the consequent chronic inflammation,
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3 can seriously affect healing. The increase of antibiotic-resistant bacteria has led the medical
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5 community to look for new treatments for infections. The development of alternative strategies,
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7 such as autologous platelet concentrates, which could reduce the incidence of post-operative
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9 infections and consequently the length of hospitalization, could have a considerable socio-economic
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11 impact. Future research should be focused on the determination of the exact antimicrobial spectrum,
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13 of the role played by the various components of platelet concentrates, and of the influence of patient
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15 characteristics (e.g. age, sex, blood parameters, drug assumption, etc.) on the antimicrobial
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17 properties. It would be also interesting to combine platelet concentrates with conventional
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19 techniques used for the treatment of infections (e.g. antibiotics), in order to assess the presence of a
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21 possible synergistic effect, and to investigate more deeply the in vivo antimicrobial efficacy in the
22
23 various fields of medicine.
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31 **Declaration of interest**

32 The authors declare that they have no conflicts of interest.
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Figure legends

Figure 1. Flowchart summarizing the article selection process.

Figure 2. Risk-of-bias graph: judgments of review authors about each risk-of-bias item presented as percentages across all included studies for *in vitro* (a) and animal (b) studies.

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Table I. Characteristics of the *in vitro* studies assessing the antimicrobial effect of platelet concentrates.

Authors, year	Platelet concentrate features				Microorganisms tested	Results
	Type	Platelet concentration (10 ³ /μl) mean value±st.dev.	Activator(s)	Leucocytes content (10 ³ /μl)		
Mariani et al., 2015 [52]	P-PRP/ L-PRP	290/861	calcium chloride	<0.2/5450	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>E. faecalis</i> , <i>S. aureus</i> , <i>K. pneumoniae</i>	In vitro antibacterial activity of both P-PRP and L-PRP. Similar activity between the two formulations.
Yang et al., 2015 [47]	P-PRP	1690 ± 0.32	calcium chloride	No (0.08 ± 0.02)	<i>F. nucleatum</i> , <i>P. gingivalis</i> , <i>A. actinomycetemcomitans</i>	Bacterial growth inhibition
Yang et al., 2010 [32]	L-PRP	n.r.	bovine thrombin and calcium gluconate ± apocynin	Yes	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Potential bacteriostatic effect against <i>S. aureus</i> by platelet mediating; No antimicrobial activity against all tested microorganisms
Wu et al., 2013 [36]	L-PRF	150 ± 113	thrombin	Yes (1.18 ± 0.17)	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	Remarkable antimicrobial activity Gram-negative bacteria relevant to fistula colonization; Platelets and leukocytes may be important in bacterial defense
Tohidnezhad et al., 2012 [35]	P-PRP	3800	bovine thrombin and calcium chloride	No	<i>E. coli</i> , <i>B. megaterium</i> , <i>P. aeruginosa</i> , <i>E. faecalis</i> ,	Antimicrobial action against all tested microorganisms; Initial infection prevention against <i>E. coli</i> and <i>P.</i>

					<i>P. mirabilis</i>	<i>mirabilis</i>
Tohidnezhad et al., 2011 [34]	P-PRP	3800	bovine thrombin and calcium chloride	No	<i>E. coli</i> , <i>B. megaterium</i> , <i>K. pneumoniae</i> , <i>E. faecalis</i> , <i>P. mirabilis</i>	Antimicrobial action against all tested microorganisms; Identification of a possible first line defense in contaminated wounds
Rozalski et al., 2013 [37]	P-PRP	100	ADP	No	<i>S. aureus</i>	Good antimicrobial activity even after the medical expiry date; Bacterial resistance test as a predictive indicator of susceptibility to infection treatment
Moojen et al., 2008 [31]	L-PRP	1688 ± 318	bovine thrombin	Yes (17.90 ± 2.62)	<i>S. aureus</i>	Antimicrobial activity against <i>S. aureus</i>
Mariani et al., 2014 [45]	P-PRP	290	calcium chloride	No (< 0.2)	<i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	Bacterial growth inhibition; Early protection against bacterial contamination during surgical interventions
Li & Li, 2013 [38]	L-PRP	2000	bovine thrombin and calcium chloride	Yes	<i>S. aureus</i> , <i>N. gonorrhoeae</i> , <i>Group A streptococcus</i>	Strong in vitro antibacterial properties against all tested bacteria; Potential to prevent infection and to reduce the need for costly post-operative treatment of implant-associated infections
Intravia et al., 2014 [44]	P-PRP/ L-PRP	386 ± 65.5 / 867 ± 234.4	n.r.	No (0.62 ± 0.265)	<i>S. aureus</i> , <i>S. epidermidis</i> , <i>P. acnes</i> ,	Significant bacterial growth decrease for all tested microorganisms;

				Yes (11.96 ± 4.74)	<i>S. aureus</i>	No antibacterial activity difference between the two preparations
Edelbute et al., 2014 [43]	P-PRP	≥ 1200	bovine thrombin and calcium chloride and pulsed electric fields	No	<i>A. baumannii</i> , <i>P. aeruginosa</i> , <i>S. aureus</i>	Significant antibacterial effect against all tested microorganisms
Drago et al., 2014 [46]	P-PRP	565 (P-PRP) 989 (2x P-PRP) 2034 (4x P-PRP)	calcium chloride	No (< 1)	<i>E. faecalis</i> , <i>S. oralis</i> , <i>S. agalactiae</i> , <i>S. aureus</i>	Antimicrobial activity against all tested microorganisms
Drago et al., 2013 [39]	P-PRP	252	calcium chloride	No (0.34 ± 0.27)	<i>P. aeruginosa</i> , <i>E. faecalis</i> , <i>S. oralis</i> , <i>S. agalactiae</i> , <i>C. albicans</i>	Antimicrobial activity against <i>E. faecalis</i> , <i>C. albicans</i> , <i>S. agalactiae</i> and <i>S. oralis</i> ; Useful substance against postoperative infections
Chen et al., 2013 [42]	L-PRP	1968.8 ± 874.58	thrombin and calcium gluconate	Yes (5.75 ± 1.46)	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Antibacterial activity against <i>S. aureus</i> ; Synergistic antibacterial effect of platelet concentrate and antibiotic combination
Burnouf et al., 2013 [41]	L-PRP	1356	calcium chloride	Yes (4.80)	<i>E. coli</i> , <i>E. cloacae</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>S. epidermidis</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>B. subtilis</i>	Antimicrobial activity only against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> and <i>S. aureus</i>
Bielecki et al., 2007 [30]	L-PRP	228 ± 59	bovine thrombin and calcium chloride solution	Yes (6.68 ± 2.20)	<i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> ,	<i>S. aureus</i> and <i>E. coli</i> growth inhibition; No activity against <i>K.</i>

					<i>E. faecalis</i> , <i>P. aeruginosa</i>	<i>pneumoniae</i> , <i>E. faecalis</i> and <i>P. aeruginosa</i>
Anitua et al., 2012 [6]	P-PRP L-PRP	636 ± 97 467 ± 62	calcium chloride	Negligible (0.22 ± 0.13) Yes (21.42 ± 8.02)	<i>S. aureus</i> , <i>S. epidermidis</i>	Antimicrobial action against all tested microorganisms during the first few hours; Potentially useful substance against postoperative infections
Alvarez et al., 2011 [33]	L-PRP	512.9	calcium gluconate	Yes (10 ± 4.98)	<i>S. aureus</i>	Antibacterial effect against <i>S. aureus</i>
Aktan et al., 2013 [40]	L-PRP	12.5*	phorbol myristate acetate (PMA) and thrombin	Yes	<i>E. coli</i>	Activated platelets inhibited <i>E. coli</i> growth

*diluted for experimental use; n.r.= not reported

Table II. Characteristics of the animal studies assessing the antimicrobial effect of platelet concentrates.

Authors, year	Platelet concentrate				Microorganisms tested	Animal model	Results
	Type	Platelet concentration (10 ³ /μl) mean value±st.dev	Activator(s)	Leucocytes (10 ³ /μl)			
Li et al., 2013 [50]	L-PRP	2050 ± 1.32	thrombin	Yes (13.51 ± 0.43)	<i>S. aureus</i> , <i>Group A Streptococcus</i> , <i>N. gonorrhoeae</i> , <i>E. coli</i> , <i>Pseudomonas</i>	Rabbit model of implant-associated spinal infection	PRP antimicrobial properties both <i>in vitro</i> and <i>in vivo</i> depend on thrombin concentration; Useful adjunct strategy against postoperative implant-associated infections
Li et al., 2013 [51]	L-PRP	1561 ± 702	bovine thrombin	Yes (24.2 ± 24.2)	<i>S. aureus</i>	Rabbit model of osteomyelitis	Antimicrobial efficacy <i>in vivo</i> ; Favorable alternative for the treatment of osteomyelitis
Jia et al., 2010 [48]	L-PRP	n.r.	bovine thrombin	Yes	<i>S. aureus</i>	Rabbit model of osteomyelitis	Bacterial reproduction inhibition <i>in vitro</i> ; Effective <i>in vivo</i> bone infection prevention
Jia et al., 2010 [49]	L-PRP	2636 ± 356	bovine thrombin	Yes (48.3 ± 4.3)	<i>S. aureus</i>	Rabbit model of osteomyelitis	Significant reduction in the infection rate and the number of viable bacteria; Novel strategy to prevent bone infection in humans

n.r.= not reported

Figure 1. Flowchart summarizing the article selection process.
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Figure 2. Risk-of-bias graph: judgments of review authors about each risk-of-bias item presented as percentages across all included studies for in vitro (a) and animal (b) studies.
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Risk-of-bias graph: judgments of review authors about each risk-of-bias item presented as percentages across all included studies for animal (b) studies
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