Antibiotic release from leukocyte- and platelet-rich fibrin (L-PRF) – an observational study.

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ABSTRACT

Introduction

Leukocyte- and platelet-rich fibrin (L-PRF), an autologous derived platelet and leukocyte concentrate, was first introduced by Choukroun et al. in 2001 and is currently used in a wide range of medical procedures. Although various biological properties have been attributed to L-PRF, nevertheless, when tested for inherent antimicrobial activity, the biomaterial fails to demonstrate a clear and significant effect against a range of oral microbiota. Aims and objectives: To determine whether L-PRF prepared after a single oral dose of antibiotic had any significant antimicrobial effect over a 48 hour period. Methods: An in vitro laboratory study for which L-PRF was prepared from a single healthy volunteer who had previously ingested oral antibiotics. The resultant prepared L-PRF was tested for antimicrobial activity against Streptococcus mutans (ATCC 35668) using standard laboratory methods. Results: For all samples tested, measurable zones of inhibition were clearly visible after 24 hours, but were absent after 48 hours. Conclusions: L-PRF prepared after a single dose of oral antibiotic results in a measurable antimicrobial effect that is sustainable for 24 hours. Although L-PRF will remain structurally intact for a few days, it does not appear to influence the duration of the antimicrobial activity.

Introduction

Leukocyte- and platelet-rich fibrin (L-PRF), an autologous blood-derived biomaterial, was first introduced by Choukroun et al. in 2001 as a simple method of introducing autogenous growth factors to a wound site.[1] Since then, L-PRF has been utilized for a number of medical procedures, including the management of diabetic wounds, soft and hard tissue augmentation and dermatological lesions.[2-5] L-PRF is prepared using a single spin protocol that results in an easily manipulated biomaterial that can be applied directly to the site of surgery.[1] The structure of this biomatrix, which consists of a platelet and leukocyte concentrate interwoven within a fibrin mesh, is claimed to be one of the major factors that contribute to its clinical success. Previous studies indicate that the unique characteristic of fibrin make it an ideal drug delivery system that allows the distribution of an active agent directly to the site required.[6] The fact that fibrin undergoes fibrinolysis over a period of time, has the potential of prolonging the drug release and may therefore influence the clinical outcome. Previous studies have combined antibiotics with fibrin (mostly fibrin sealants) for the management of various conditions such as osteomyelitis, endocarditis, and other "difficult to treat" infections.[7-11]

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ACRONYMS

L-PRF : Leukocyte- and platelet-rich fibrin

Although various biological properties have been attributed to L-PRF, nevertheless, when tested for inherent antimicrobial activity, the biomaterial failed to demonstrate a clear and significant antimicrobial effect against a range of oral microbiota.[12] We hypothesize that incorporating antibiotics into L-PRF may enhance its antimicrobial profile and that the structure of the L-PRF would allow for a prolonged release of the drug. The aim of this in-vitro pilot study was to determine whether L-PRF prepared after a single oral dose of antibiotic had any significant antimicrobial effect over a 48 hour period.

MATERIALS AND METHODS

The study was conducted at The University of the Western Cape, Cape Town, South Africa, in 2017. Ethical clearance was obtained from the University Research Ethics Committee (reference number: BM 16/3/31). Informed consent was obtained from the blood donor.

Preparation of the L-PRF

Thirty six milliliters of blood were obtained from a 24 year old healthy female volunteer who had undergone dental implant surgery. She had ingested a single dose of antibiotics, as surgical prophylaxis (Amoxicillin, 2 g orally), one hour prior to the surgical procedure. One hour after antibiotic ingestion, blood samples were collected in blood collecting tubes that contained clot activator, i.e., Vacuette® 9ml serum tubes with Z Serum Clot Activator (Greiner BioOne International AG, Germany). These were then immediately centrifuged at 400 x g for 12 minutes in a standard benchtop centrifuge (PLC-03, HiCare International, Taiwan) as previously described.[12] Some of the resultant L-PRF clots were then used during the surgical procedure, whilst the remaining clots were used for the current study. These L-PRF clots were then compressed using the PRF Box ™ (Process for PRF, Nice, France) to obtain uniform thickness of the sample specimens, two of which were selected and designated as Sample and Sample Two respectively.

Microbial Culture

The Oral and Dental Research Laboratory (Faculty of Dentistry, University of the Western Cape) sponsored samples of Streptococcus mutans (ATCC 35668, Quantum Biotechnologies, South Africa) for the study. The authenticity of the bacteria was confirmed via Gram stain, growth morphology on blood agar and API STREP 20 system (bioMerieux). Cultures were grown for 24 hours at 37°C. Two colonies were adjusted to 0.5 McFarland's standard (DensiCHEK Plus, bioMerieux) in sterile physiological saline. One millitre (1 ml) of the adjusted colonies was then spread plated on CASO agar (Merk Life Science GmbH, Germany).

Antimicrobial activity

The samples of prepared L-PRF were of comparable size and thickness due to the standardized protocol used in preparation. Each sample was placed in the centre of a bacteria-streaked agar plate and then incubated for 37°C in a standard laboratory incubation chamber for 24 hours. At that stage the plates were removed and the clear zones surrounding each L-PRF sample (zones of microbial inhibition), were repeatedly measured using a digital vernier caliper (Mastercraft, South Africa). In order to test the antimicrobial efficacy over the subsequent 24 hours, the samples were removed and placed in a fresh bacteria-impregnated agar plate. The plate was incubated for 24 hours as before and the inhibition zones again measured.

DATA ANALYSIS

Data from the test samples were captured using Microsoft Excel 2010 (Microsoft Corporation, Washington, USA) and statistically analyzed using one-way ANOVA and Tukey's test.

Table 1: Zones of inhibition measured in millimeters

24 Hours (Sample 1)	24 Hours (Sample 2)	48 Hours (Sample 1)	48 Hours (Sample 1)
33.97	29.41	0	0
41.57	42.70	0	0
32.20	31.22	0	0
29.53	44.03	0	0
32.90	31.99	0	0
32.91	47.15	0	0
39.65	47.06	0	0
40.75	31.82	0	0
35.11	42.82	0	0
41.33	31.60	0	0

RESULTS

The 24 and 48 hour results were recorded as the observable zones of inhibition in millimeters and are represented in Table 1. Zones of inhibition were noted for both L-PRF samples after 24 hours. According to statistical analysis using one-way ANOVA and Tukey HSD Test, no significant differences were noted for the sizes of inhibition zones when the two samples were compared after 24 and 48 hours, i.e., similar results were recorded at both 24 and 48 hours. No measurable inhibition zones were seen for either sample after 48 hours (Table 2), indicating that all antimicrobial activity had been lost at that time. A statistically significant difference was seen in the data between the two time points.

DISCUSSION AND CONCLUSIONS

Repeated systemic use of antibiotics to treat or minimize localized infection has its limitations, especially in areas with constrained blood supply. Furthermore, localized drug delivery systems often require specialized carriers to allow for the delivery of therapeutically sustainable doses of the active agent. In the present study we attempted to determine whether using an antibiotic- laden autologous blood-derived concentrate had any significant antimicrobial effect over a period of time. The results

Table 2: Tukey HSD results

Samples	Tukey Q stat	Tukey p-value	Tukey inference
S1 (24hrs) vs S2 (24hrs)	1.4702	0.7058178	insignificant
S1 (24hrs) vs S1 (48hrs)	26.6181	0.0010053	p<0.01
S1 (24hrs) vs S2 (48hrs)	26.6181	0.0010053	p<0.01
S2 (24hrs) vs S1 (48hrs)	28.0883	0.0010053	p<0.01
S2 (24hrs) vs S2 (48hrs)	28.0883	0.0010053	p<0.01
S1 (48hrs) vs s2 (48hrs)	0	0.8999947	insignificant

from the current study indicates that L-PRF prepared after a single dose of antibiotics provides measurable antimicrobial activity for at least 24 hours against specific oral microbiota. After 48 hours, the antimicrobial effect is markedly reduced, with no statistically relevant antimicrobial effect seen. This may suggest that although L-PRF has a unique structure, its ability to concentrate antibiotics and release them over time, may be limited. This has been seen in previous studies in which fibrin sealants directly supplemented with antibiotics showed a rapid antibiotic release over a short period of time (85% over 72 hours).[14-16] Woolverton et al. (2001) attributed this to rapid diffusion as a result of the antimicrobial molecules being small, ionic, and designed for oral and parenteral delivery.[6] However, antibiotics having a less soluble nature, unlike the current study, have been shown to exhibit a much longer sustained release from fibrin sealants.[17] Another reason for the rapid diffusion of antibiotics from L-PRF may be the limited binding capacity of the antibiotic used in the present study. Amoxicillin, when orally ingested, is 20% protein bound in the blood and about 60% of the drug is excreted in the urine within 6-8 hours.[18] The protein binding is mostly to albumin (the most abundant plasma protein) which has a specific binding site but a relatively low affinity for the antibiotic.[18] The drug has no known affinity to fibrin and this may explain the limited antimicrobial effect observed in the present study. It is therefore assumed that the drug was concentrated in the plasma component of the L-PRF and not directly bound to the fibrin or cells associated with the L-PRF matrix.

Unlike previous studies that have directly combined the antibiotic with the fibrin matrix, we attempted to incorporate antibiotics that were already in the blood (at peak plasma concentration) during the process of preparing the L-PRF matrix. This has the advantage of not requiring any additional materials or steps in incorporating the antibiotic into the L-PRF. Interestingly, certain clinical studies involving the use of L-PRF for dental surgical procedures have made use of oral antibiotic surgical prophylaxis prior to preparing L-PRF.[19] In these studies, no mention was made of the influence of antibiotic ingestion in the healing outcomes of the site treated with the L-PRF. The present study indicates that antibiotics are presumably incorporated into L-PRF after a single oral dose and may be active for at least 24 hours. We can therefore presume that the clinical studies that have administered antibiotics prior to surgery, have indeed incorporated antibiotics into the L-PRF, if the L-PRF was prepared at least one hour after antibiotic prophylaxis. A further investigation into the influence of pre-operative antibiotics as a factor influencing the clinical behaviour of L-PRF is therefore required.

CONCLUSION

L-PRF has restricted intrinsic antimicrobial activity. Within the limitations of the present study, we have shown that L-PRF prepared after a single dose of oral antibiotic, results in a measurable antimicriobial effect that is sustainable for 24 hours. Although L-PRF will remain structurally intact for a few days, this does not appear to influence the duration of the antimicrobial

activity. We therefore assume that the antibiotic is mostly concentrated in the plasma and is not directly bound to the structural components of the L-PRF matrix. Further research is required to determine the significance of these preliminary findings.

Conflict of interest.
The authors declare no conflict of interest regarding the publication of this paper. This paper forms part of the requirements for partial fulfillment of the degree PhD.

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