Rigshospitalet

Bispebjerg Hospital

Effects of Platelet-Rich Fibrin on Fibroblast Activities *In Vitro* and *In Vivo*

Rasmus Lundquist[1], Patricia Danielsen[2] Lars N. Jorgensen[2] and Magnus S. Ågren[2]

[1]Bloodbank, Rigshospitalet, [2]Department of Surgery K, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark

Aim

The overall aim of these studies is to evaluate the potential of platelet-rich fibrin (PRF®) to promote wound healing. Specifically, the effect of PRF®-derived factors on fibroblast activities are tested *in vitro* on cultured human dermal fibroblasts and *in vivo* using expanded polytetrafluoroethylene (ePTFE) tubes implanted subcutaneously in patients.

Conclusions

Platelets in an autologous fibrin preparation (PRF®) enhanced fibroblast growth *in vitro* compared to both autologous fibrin and platelet-derived growth factor-BB. A commercial tissue fibrin sealant did not stimulate fibroblast proliferation. A stimulatory effect of PRF® on fibroblasts *in vivo* was also indicated in initial tests. Our results indicate that PRF® may be a useful adjuvant in the management of problematic wounds.

Introduction

Platelets contain potent growth factors and have been used topically in various carrier systems in the treatment of acute and chronic wounds. The Vivostat® system (Vivolution A/S, Birkeroed, Denmark), initially developed for the easy generation of autologous fibrin in a closed system, has been modified to concentrate platelets in the final fibrin formulation. Results from initial feasibility tests on chronic wounds (Fig. 1) are promising but the cellular basis is unknown.

Previous *in vitro* studies using either growth factors or fibrin/fibrinogen have shown both increased cell proliferation and migration by fibroblasts[1,2]. Furthermore, fibrin binds and protects several growth factors from proteolytic degradation[3,4].

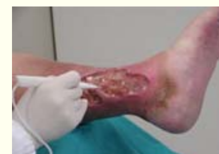

Fig. 1. Treatment of a venous leg ulcer using Vivostat® PRF®.

Materials

In vitro studies

DNA synthesis. Adult human dermal fibroblasts (Cambrex) were used for these studies. DNA synthesis was monitored in confluent, quiescent fibroblasts grown for 24 hours in 100 µl fibroblast basal medium (FBM) containing 2% FCS (fetal calf serum) in 96-well plates before being treated with 35 µl of FBM+2% FCS (control), human albumin 5% (ZLB), fibrin (Tisseel®, Baxter; Vivostat®), PRF® (Vivostat®) or PDGF-BB (Sigma). A final well volume of 200 µl was achieved by adding 65 µl FBM+2% FCS. Fibrin (Vivostat®) and PRF® (Vivostat®) were obtained from two healthy male donors with low and high basal platelet counts, respectively (Table 1). PRF®, fibrin (Vivostat®) and Tisseel® were molded in specially designed molds (Fig. 2). Cells were treated for 24 hours and BrdU incorporation was measured during the final two-hour period using a cell proliferation ELISA kit (Roche).

Cell growth. Fibroblasts (2,000/well) were seeded and grown in FBM+2% FCS for 5 hours before being treated once for up to 4 days with the substances described above. Fibroblasts were quantified on days 1, 3 and 4 indirectly by an ATP detection kit (ViaLight Plus, Cambrex).

Table 1. Platelet counts in donor blood and PRF®, and fibrin concentrations in Vivostat® fibrin and PRF®

	Donor 1	Donor 2
Platelets in full blood ($\times 10^9$/l)	141	331
Platelets in Vivostat PRF ($\times 10^9$/l)	1242	1782
Fibrin in Vivostat fibrin (mg/ml)	17.8	23.2
Fibrin in Vivostat PRF (mg/ml)	20.1	22.5

Fig. 2. Molding of fibrin clots.

In vivo study (IMPRA study)

ePTFE tubes are used to assess the effect of treatment on the formation of connective repair tissue. The ePTFE tube is 0.25 mm in diameter, a 0.12 mm lumen, and wall with pores of 90-120 µm. The tubes are implanted subcutaneously via cannulation (Fig. 3). One tube is treated with PRF® and the other with control (human albumin). The ePTFE tubes are withdrawn after 14 days (Fig. 4) and the wound healing response is measured.

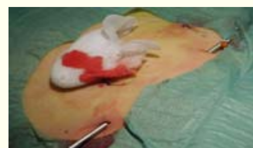

Fig. 3. Implantation of ePTFE tubes.

Fig. 4. Extirpated PTFE tubes day 14.

Results

Platelet-rich fibrin (PRF®) and fibrin were produced from 2 donors by the Vivostat® system. Despite the differences in blood platelet counts the PRF® stimulated fibroblast DNA synthesis to a similar degree (Fig. 5). Vivostat® autologous fibrin without the platelet fraction showed a lower but detectable stimulatory effect. In contrast, the tissue sealant Tisseel® with a high fibrin concentration (45 mg/ml) showed no effect. In the fibroblast growth assay (Fig. 6) PRF® promoted fibroblast growth over an extended time period exceeding the effect of a single application with PDGF-BB (10 ng/ml) or Vivostat® fibrin day 3 onward.

Our initial *in vivo* results support the *in vitro* data in that PRF® promoted fibroblast infiltration of ePTFE tubes implanted subcutaneously in patients (Fig. 7).

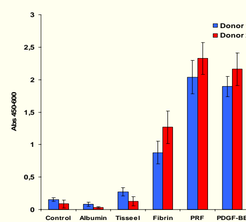

Fig. 5. Effect of treatments on DNA synthesis in human skin fibroblasts (mean ± 95% confidence intervals, n = 8).

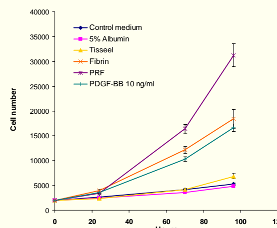

Fig. 6. Effect of treatments on the growth of human skin fibroblasts. Vivostat® fibrin and PRF® were derived from donor 2 (mean ± 95% confidence intervals, n = 8).

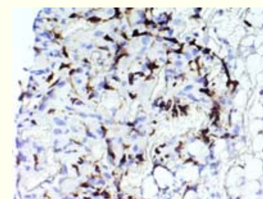

Fig. 7. Fibroblast infiltration in ePTFE treated with PRF® determined by immunohistochemistry using a monoclonal antibody against type I procollagen.

Discussion

The inclusion of platelets in autologous fibrin was shown to enhance the fibroblast growth-promoting properties of autologous fibrin. The growth-promoting properties of autologous fibrin could be due to growth factors bound to fibrin/fibrinogen during the production process, or it could be the effect of fibrin/fibrinogen and its biologically active fragments[2]. The use of autologous PRF® in wound management is largely dependent on the effect and stability of the PRF®-derived growth factors. Other studies have shown that fibrin can bind and protect known growth factors from degradation[3,4] and thereby preserving their biological activity.

References

1. Ågren MS et al. Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent. J Invest Dermatol 1999;112: 463-469.
2. Rybarczyk BJ et al. Matrix-fibrinogen enhances wound closure by increasing both cell proliferation and migration. Blood 2003;102: 4035-4043.
3. Sahni A et al. Fibrinogen and fibrin protect fibroblast growth factor-2 from proteolytic degradation. Thromb Haemost 2000;83: 736-741.
4. Sahni A et al. Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. Blood 2000;96: 3772-3778.