INTRODUCTION

Platelet Rich Plasma (PRP) has been identified as having a potential application in articular cartilage tissue engineering via its delivery of autologous growth factors to cartilage defects[1]. Platelets must be activated to allow the release of growth factors such as transforming growth factor beta 1 (TGF-β1), basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF), all of which have been shown to have a positive effect on cartilage repair[3]. Platelet activation can be achieved either with platelet contact on collagen or the addition of thrombin[2].

The aim of this study was to ascertain whether the addition of exogenous thrombin is required to achieve activation and adequate growth factor release when PRP is applied to a fixed volume regionally specific collagen/glycosaminoglycan (CG) osteochondral scaffold (Chondromimetic, Orthomimetics Ltd, Cambridge, UK).

Secondly, we sought to establish whether the release profile of PRP on CG differed when PRP was applied to a polylactide co-glycolide (PLG) osteochondral scaffold (Trufit CB, Smith and Nephew Endoscopy, Andover, MA) without the addition of thrombin.

METHODS

PRP and thrombin preparation: Following local research ethics committee approval, 60ml of venous blood was taken from two healthy volunteers and PRP made using the SmartPrep 2 centrifuge (Harvest Pharmaceuticals Inc, Bristol, TN) was reconstituted with 10ml calcium chloride (1000units/ml).

Application of PRP +/- thrombin to CG scaffolds: 8mm diameter x 5mm deep CG scaffolds were fashioned to which equal combined volumes of test substances were added as follows (n=3): 500µl PRP; 375µl PRP + 125µl autologous thrombin (3:1); 455µl PRP + 45µl bovine thrombin (10:1).

Growth factor release: One ml of DMEM/F12 medium was added to each scaffold in 48 well plates which were incubated at 37 °C on a rocker table. The medium was changed completely at 12 and 24 hours, and 3 and 10 days following which release of TGF-β1, PDGF-AB and bFGF were measured using ELISA (Quantikine, R&D Systems).

Application of PRP to CG and PLG scaffolds: 7mm diameter x 5mm deep CG and PLG scaffolds were fashioned to which 500µl of PRP were added (n=3). Similar conditions were followed as previously except that only PDGF-AB was assayed.

Statistical analysis: Statistical significance between groups and within groups for each end point was determined using a one-way analysis of variance (ANOVA) and Bonferroni’s post hoc test. A level of P < 0.05 was accepted as significant.

RESULTS

In the first experiment all groups showed a similar cumulative release profile of all growth factors over the 10 day period (Figs. 1a-c). A burst release was found in the initial 24 hours with a sustained release up to 10 days thereafter.

DISCUSSION

This study shows that the addition of exogenous thrombin is not required when PRP is used in combination with a CG scaffold. The increase in amount of growth factor released is in part due to the volume of PRP which can be applied to a fixed volume scaffold, as no other substance needs to be added in combination to achieve activation. However, when the release is corrected for volume differences (Fig. 1d), an increase is still seen with the PRP only group suggesting that this is not just a volume-related phenomenon. In fact, the addition of thrombin in this system may be inhibitory.

In conclusion, if incorporating PRP with a CG scaffold for articular cartilage tissue engineering applications, no exogenous thrombin need be applied to achieve activation. Likewise, if using PRP with PLG scaffolds or similar, our results suggest that the PRP should be activated with thrombin to achieve optimum growth factor release.

REFERENCES