Concentrated Plasma as a Carrier for Stem Cell Delivery

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Introduction: Delivery of mesenchymal stem cells (MSCs) for tissue repair is limited by the permeability of the carrier. Without nutrient transfer and rapid revascularization, implanted stem cells will not survive. Non-autologous fibrin-based hydrogels have been used in animal studies for stem cell delivery [1]. The purpose of this study was to evaluate if an autologous concentrated plasma product could be used to successfully deliver and maintain the viability of the mesenchymal stem cells.

Platelet-poor plasma (PPP) that contains native levels of fibrin is a by-product of the preparation of platelet-rich plasma (PRP). PRP is used clinically to augment healing due to the presence of growth factors contained in the sequestered platelets [2]. PPP has also gained clinical use to supplement closure of surgical wounds [3] as PPP has demonstrated hemostatic potential in a skin wound model [4].

The handling and implant residence time of the PPP can be improved by the removal of excess fluid, thereby concentrating the proteins within the plasma. This desiccation can be achieved by mixing the PPP with a set volume of polyacrylamide beads. This concentrated plasma was analyzed as a potential carrier for stem cells for tissue engineering applications.

Methods:

In vitro analysis: Protein characterization was performed comparing the baseline platelet-poor plasma (PPP) and concentrated plasma. Ten healthy human volunteers donated 70 ml of blood anticoagulated 10% with anticoagulant citrate dextrose solution A (ACD-A, Citra Solutions). Ten milliliters were centrifuged and the plasma was reserved as the PPP sample. The remaining 60 mls of blood were processed using a platelet concentrate system (GPS®II, Biomet Biologics, Inc., Warsaw, IN). Twenty-five milliliters of PPP was then removed from the platelet concentration device and processed with a plasma concentrator (Plasmax® Plus, Biomet Biologics, Inc., Warsaw, IN). This device concentrates plasma by desiccation with polyacrylamide beads. An output volume of approximately 10 ml is removed from the device.

The concentration of several plasma proteins was quantified by various techniques. Total protein was measured using the Bradford Total protein assay (B6916, Sigma). Albumin, fibrinogen, complement C3, and transferrrin were detected using the ELISA technique (all AssayPro Kits). Additionally, equivalent clotting proteins were measured against a fibrinogen standard using a clotting time assay (Start4, Diagnostica-Stago).

In vivo analysis: Concentrated plasma was derived from bovine blood using the Plasmax® Plus Plasma Concentrator (Biomet Biologics, Inc., Warsaw, IN). Two milliliters of concentrated plasma were combined with 200 µl of fluorescently labeled rat-derived mesenchymal stem cells ($8x10^6$ cells)(n=2 per time point). The plasma was then coagulated with a solution of thrombin (1000 units/ml) and 10% calcium chloride and was implanted subcutaneously in an athymic rat. Explants were retrieved after 2 days and 5 days of implantation and were prepared for histological evaluation.

Statistics: Data is presented as mean ± standard deviation. Statistical significance was determined using a Student's t-test (α=0.05).

Results: A starting volume of 25 mls of PPP was concentrated to an average volume of 9.7±1.5 mls of concentrated plasma, representing a 2.6 fold increase in concentration of proteins based on volume. Protein characterization (Table 1) averaged a 2.4 ± 0.9 fold increase in the concentrated plasma compared to the baseline PPP. Each protein was concentrated a statistically significant amount by processing in the Plasmax® Plus disposable.

Table 1. Protein characterization (n=10)

Protein	PPP	Plasma Conc.	Fold Inc.
Total protein (mg/ml)	67.3 ± 5.5	286 ± 81	4.2 ± 1.1
Clotting protein (µg/ml)	372 ± 75	806 ± 178	2.2 ± 0.3
Albumin (mg/ml)	56.1 ± 7.3	109 ± 12	2.0 ± 0.3
Fibrinogen (mg/ml)	2.9 ± 0.46	4.9 ± 0.52	1.7 ± 0.3
Comp. C3 (mg/ml)	1.4 ± 0.23	3.5 ± 0.56	2.5 ± 0.4
Transferrin (mg/ml)	2.6 ± 0.51	5.3 ± 1.7	2.0 ± 0.5
		Average	2.4 ± 0.9

MSCs loaded into the concentrated plasma were visible within the matrix at both the 2 day and 5 day implantation sites (Fig.1). The cells can be visualized as green dots within the matrix. Additionally, the matrix can be evaluated due to the auto-fluorescence of the fibrin. The concentrated plasma is still present after 5 days of implantation, but the resorption process has begun to occur. Analysis of the surrounding tissue indicated the presence of positively labeled MSCs at both the 2 day and the 5 day time points. Gross analysis of the explants at day 5 revealed infiltration of blood vessels into and around the concentrated plasma (Fig 2.).

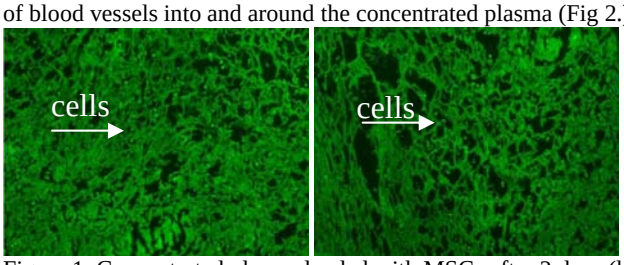

Figure 1. Concentrated plasma loaded with MSCs after 2 days (left) and 5 days (right) subcutaneous implantation.

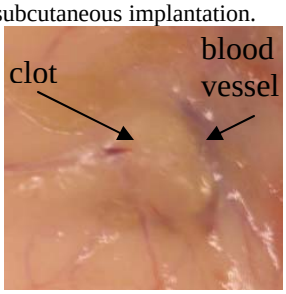

Figure 2. Concentrated plasma clot after 5 days implantation. Blood vessel infiltration visible.

Discussion and Conclusion: The concentrated plasma did provide a matrix for the delivery of MSCs. Many of the implanted cells remained within the matrix, while some cell migration into the surrounding tissue was observed. The concentrated plasma remained intact after 5 days of subcutaneous implantation, though initial resorption was evident. Additionally, the infiltration of new blood vessels is required to maintain the viability of the implanted cells and for the remodeling of the implant. While cellular function was not assayed in this model, these results suggest that concentrated plasma can provide a useful matrix for tissue engineering applications as a delivery vehicle of cells.

References:

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