CHARACTERIZATION OF A SCAFFOLD COMPOSED OF PLATELET-RICH PLASMA FOR AUTOLOGOUS CHONDROCYTE IMPLANTATION

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INTRODUCTION: Platelet-rich plasma (PRP) belongs to one plasma division isolated by centrifugation of whole blood. From 1981 to 1991, a number of different growth factors containing platelets were reported one after another, such as platelet-derived growth factor (PDGF), transforming growth factor beta 1 (TGF-b1), transforming growth factor beta 2 (TGF-b2), insulin-like growth factor (IGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF). Since then PRP has been watched with interest as a source of autologous thromboctyic growth factors. In 1998, it was shown radiographically and histologically that both bone formation and density increased after autologous bone grafting with PRP in oral surgery. However the effects of PRP on proliferation and differentiation of various cells have not been investigated sufficiently, especially on chondrocytes. In this study we investigated the effects of PRP on proliferation and differentiation of human chondrocytes, and the clinical usefulness of auto-fibrin gel with PRP (PRP gel) as a scaffold for autologous chondrocyte implantation (ACI).

METHODS: Whole blood and knee joint cartilage were supplied from 10 adult donors after informed consent. Half of the donors supplied both whole blood and cartilage. Chondrocytes were isolated by enzyme digestion from knee cartilage and suspended nutrient mixture medium with 10% fetal bovine serum (FBS). When cultures became near confluent, cells were passed only one time. Passage 1 cells were used for all experiments. Blood was drawn into a bag containing anticoagulant and centrifuged twice to obtain PRP. By mixing calcium gluconate and thrombin to PRP, platelets released growth factors from their granules and PRP gel was obtained. PRP was filtered with a 0.2mm pore filter and stored. The quantitative determination of cellular proliferation was performed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. We investigated the effect of different PRP concentrations on chondrocyte proliferation and also performed a time course experiment to establish a growth curve of cells cultivated in control condition (30% platelet-poor plasma) or in 30% PRP. Alcian blue and anti-type II collagen antibodies were used for histochemical and immunohistochemical examinations to analyze the influence of PRP on re-differentiation. We also assessed optimal fibrinogen concentration from point of initial hardness, fibrin lysis, cell growth, and cell spreading.

RESULTS: Cell proliferation of human chondrocytes was sensitive to PRP. The increase was dose dependent, and MTT values increased as the percentage of PRP increased. Thirty percent PRP induced a marked increase of MTT values compared to control culture. Time course experiments performed with chondrocytes cultivated in 30% platelet-poor plasma (PPP) or in 30% PRP showed that PRP promoted an increase of MTT values that was observed for the entire 12 days testing period (Figure 1). In PRP gel, cell bodies and pericellular matrix were stained more strongly with alcian blue compared with that in PPP gel. Likewise, immunoreactivity to the anti-type II collagen antibodies was shown more certainly in PRP gel. Histochemical and immunohistochemical examinations demonstrated that cultivated chondrocytes can easily lose their phenotype even in passage 1 cells, however PRP enhances re-differentiation of de-differentiated chondrocytes (Figure 2).

The concentration of 4 mg/ml of fibrinogen was optimal for producing PRP gel that had usefulness as a scaffold for ACI (Table 1).

DISCUSSION: The two major advantages of PRP gel for ACI are the reduction in cost of the culture and the increased safety of this cell-based protocol, since factors and cells are autologous. Originally, auto-fibrin gel exploits the final stage of the coagulation cascade. Therefore this gel could have many advantages as a scaffold for ACI in terms of biocompatibility, biodegradation and hemostasis. The results presented in this study demonstrate that PRP can increase the rate of human chondrocyte proliferation and re-differentiation. These increases would be advantageous for ACI, because it has been pointed out that the number of chondrocytes in the initial implantation and preservation of their phenotype are important for successful ACI.

CONCLUSION: 30% PRP promotes human chondrocyte proliferation. Cells expanded with 30% PRP can express chondrocyte phenotype. It is suggested that PRP gel provides an invaluable source of growth factors for human chondrocyte proliferation and re-differentiation, and PRP gel composed of 4 mg/ml of fibrinogen concentration could serve as a scaffold for successful ACI.

ACKNOWLEDGEMENT: Supported by NIH Grant AG07996

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Figure 1. A: Cell proliferation (MTT assay): human chondrocytes were cultured in 1%, 10%, 30% PRP, or control conditions for 12 days. The result presents the mean ± SD. Asterisks indicate a significantly greater absorbance compared to the other groups. *p < 0.05
B: Cell proliferation time course: Cells were exposed to 30% PRP or 30% PPP for 3, 6, 9 and 12 days. Asterisks indicate a significantly greater absorbance compared to control group. *p < 0.05

Table 1. Chondrocyte-loaded PRP gel properties expressed by fibrinogen concentration.

<table>
<thead>
<tr>
<th>PRP gel (fibrinogen concentration)</th>
<th>2mg/ml</th>
<th>4mg/ml</th>
<th>10mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial hardness (rigidity)</td>
<td>low</td>
<td>satisfactory</td>
<td>high</td>
</tr>
<tr>
<td>Fibril lysis, start at 2 weeks</td>
<td>complete</td>
<td>intermediate</td>
<td>weak</td>
</tr>
<tr>
<td>Cell growth</td>
<td>very good</td>
<td>very good</td>
<td>good</td>
</tr>
<tr>
<td>Spreading</td>
<td>high</td>
<td>intermediate</td>
<td>weak</td>
</tr>
</tbody>
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Figure 2. Alcian blue staining (A and B) and immunostaining with anti-type II collagen antibody (C and D) of human chondrocytes treated with PRP (A and C) or PPP (B and D) for 28 days.