The Effect of Allogenic Bone Marrow Mesenchymal Stem Cell - Platelet Rich Plasma (Bmscs - Prp) Intra-Articular Injection Effect on the Regeneration of Full-Thickness Joint Cartilage Defect on Rabbit

Ahmad Taufik.S[1], Dwikora Novembri Utomo[2], Hari Basuki Notobroto[3]

[1]Faculty of Medicine, Universitas Airlangga (60131), Surabaya, Indonesia, [2]Departement of Orthopaedy and Traumatology, Faculty of Medicine, Universitas Airlangga (60131), Surabaya, Indonesia, [3]Faculty of Medicine, Universitas Airlangga (60131), Surabaya, Indonesia

Abstract

Introduction: Full thickness cartilage defects are still a problem until present since the handling has not give any satisfactory results. Current handling is performed through cartilage enginering using mesenchymal stem cells alone and or in combination with growth factor. The purpose of this study was to investigate the effect of injection of intra-articular injection of Allogenic bone marrow mesenchymal stem cell - Platelet rich plasma (BMSCs-PRP) on regeneration of full thickness cartilage defect in rabbits.

Method: The design of this study is a post-test only control group design using 36 New Zealand white rabbits divided into three groups treated with PRP, BMSCs and BMSCs-PRP. Results are evaluated after 10 weeks.

Results: In the evaluation, macroscopic images showed the best healing in the BMSCs-PRP group. Histopathologic examination showed that in the MSCs-PRP group there was a significant increase in the amount of chondrocytes (p = 0.000), cartilage area (p = 0.000), as well as the number of Agecoprogenitor expec- tion cells (p = 0.000) and type 2 collagen (p = 0.000).

Conclusions: Intra-articular injections Allogenic bone marrow mesenchymal stem cell (BMSCs-PRP) is able to regenerate and cure full-thickness joint cartilage defects through differentiation of MSCs into condroblasts.

Keywords*: Allogenic, Bone marrow Mesenchymal stem cell, Cartilage, Platelet rich plasma, Full-thickness*

Introduction

Cartilage of joints is an important part of the joint component. Damage to joints often occurs in sports activities, where some studies get nearly 49% of joint damage occurring due to sports injuries. Its high ability to hold and absorb weight is helpful in resisting the mechanical forces acting on joints during sports activities [1]. In the destruction of joint cartilage, the healing of the formed tissue is very fragile because it consists of fibrocartilage so that even small defects will cause degeneration over time, eventually leading to osteoarthritis [2].

The affected cartilage of the affected joints shows limited healing ability. Damage to joint cartilage that affects the subcondral bone shows signs of healing due to the release of bone marrow mesenchymal stem cells (BMSCs). This is the principle of microfracture. Current treatments such as arthroscopic management, autologous

Corresponding author:
Dwikora Novembri Utomo
Departement of Orthopaedy and Traumatology, Faculty of Medicine, Universitas Airlangga (60131), Surabaya, Indonesia, E-mail novembridwikora@gmail.com, (+6231) 5020251

osteochondral transfer, autologous chondrocyte implantation (ACI) all showed better results although a systematic review comparing the three methods found no consistent therapy method showed better results than other techniques [3].

Im et al. (2001) examined the ability of bone marrow mesenchymal stem cells (BMSCs) to treat defects in cartilage. BMSCs suspension is given on Ham F-12 medium before being injected into full thickness cartilage defect in the rabbit patella basin area. Evaluation after 14 weeks showed the BMSCs group resulted in full cure in the subcondral bone tissue layer. Meanwhile in the control group the healing tissue is thinner, irregular and undifferentiated with a little bit of collagen type 2 matrix. The use of BMSCs with this method improves cartilage repair although the cure is not the same as the natural content [4].

The previous researcher examined the ability of bone marrow mesenchymal stem cells (BMSCs) to treat defects in cartilage. BMSCs suspension was given on Ham F-12 medium before being injected into full thickness cartilage defect in the rabbit patella basin area. Evaluation after 14 weeks showed the BMSCs group resulted in full cure in the subcondral bone tissue layer [5]. In general, mesenchymal stem cells to be implanted in joint cartilage defects are included in the three-dimensional scaffold. Some issues relating to the use of scaffolds are material selection, the ability to support cell viability and differentiation, retention problems and research have found that PRP administration has a regenerative effect because it proves to improve the healing of osteochondral defects in rabbits [6].

The administration of PRP to MSCs cultures can increase in vitro proliferation and there is a tendency to increase MSCs differentiation into condroblasts and osteoblasts Allogenic MSCs can increase cartilage regeneration and do not cause autoimmune rejection reactions. MSCs have immunosuppressive syphles that allow them to be used allogenically.

Method

This is a true-experimental study with post-test only control group design. This study protocol has been approved by the Ethics Committee of Faculty of Veterinary Medicine Universitas Airlangga Surabaya. The inclusion criteria of this study were male rabbits, adulthood 6-8 months, body weight 2.5 - 3.5 kg. Exclusion criteria were infection, knee injuries and rabbits died before 8 weeks. The initial stage in this research is to prepare the manufacture of mesenchymal stem cells-platelet rich plasma (BMSCs - PRP) as follows; bone marrow aspirations for making Bone marrow mesenchymal stem cells (BMSCs); culture and expansion of bone marrow-mesenchymal stem cells (BMSCs) from the bone marrow; characterization of Bone marrow-mesenchymal stem cells (BMSCs); making platelet rich plasma (PRP); making cartilage defects in the knee joint; intraarticular injection of cartilage defect in the knee joint; Sacrifize rabbits at 12 weeks; evaluation of histological and immunohistochemical examinations and evaluation of data [7].

All the collected data were tabulated and statistically processed using SPSS (SPSS. Inc. Chicago IL). This research were conducted through data descriptive analysis. Normality data test was conducted to know the normally distributed data in all data of the study result. Homogeneity test of variance was done for the normally distributed data [8]. The quantitative data comparative test result of eosin hematoxylin (HE) examination of the amount of chondrocytes and cartilage area was performed using one way analysis of variance (ANOVA) test on the data with homogeneous variance to know the difference of the examination result data in the three treatment groups [9]. The quantitative data comparative test result of immunohistochemical examination (IHC) number of agrecan expression condenser and collagen type 2 using one way analysis of variance (ANOVA) test on data with homogeneous variant to know difference of examination result data in all three treatment groups. In the abnormally distributed data, statistic test was performed with Kruskal Wallis test [10].

Results

To obtain sufficient amount of BMSCs for $2x10^7$ injections, culture was carried out for 2 weeks. This amount was achieved at passage 3. The optimal number of BMSCs for mobilization to the defect at the rabbit knee was $1x10^7$ per mL (Figures 1a and 1b). Platelet rich plasma (PRP) was made with two rounds of 3000 rpm for 13 min at 1st and 3000 rpm for 15 minutes at stage 2. The final result of 20 ml of peripheral blood taken and processed was 2 ml PRP. In PRP an increase in platelet count was 5x more than the platelet count in peripheral blood (Figure 1c and d).

In groups that received BMSCs-PRP the defect was almost invisible. The defect area has been filled with the same whitish color cartilage tissue as the surrounding cartilage color. The boundary of the healing area with its surroundings is not clear with the same surface height as the surroundings. This shows the best healing occurs in the BMSCs-PRP group

(Figure.2). The area of cartilage in each treatment group. The results of analysis with Brown-Forsythe Statistic obtained p value = 0.000 indicating a difference of cartilage area in PRP, BMSCs and BMSCs-PRP groups. The width of cartilage in each treatment group. The result of analysis with Brown-Forsythe Statistic obtained p value = 0.000 indicating a difference of cartilage area in PRP, BMSCs and BMSCs-PRP group (Figure.3).

The difference in numbers was seen in the agrecan expression of different cells between tissue matrices in each treatment group using x100 magnification. Based on the number of Agrecan expression cells it is found more in the BMSCs-PRP group (Figure 3E) than in the PRP and BMSCs groups. As for the difference of Agrecan expressor cells with core and were not shown with positive or negative explanation at x400 magnification. BMSCs-PRP group (Figure 3F) had the most positive cells compared to PRP group (Figure 3B).

Immuno histochemical examination of collagen type 2 in the figure above shows the difference in the number of collagen type 2 expression cells in each group. The amount difference can be seen in the network matrix in each treatment group using x100 magnification. The number of collagen type 2 expression cells was found more in the BMSCs-PRP group (Figure 3E) than in the PRP group (Figure 3A) and BMSCs (Figure 3C). At x400 magnification, each treatment group expressed a condroprogenitor cell. (Figure 3B, D and F).

Figure 1A. Bone marrow BMSCs has reached 40% confluent, neutral filter without coloring, 1B. BMSCs have reached 70% confluent, neutral microscope filter without coloring, 1C. Results of the stage 1 process of centrifuse making, 1D. The results of the stage 2 process of centrifuse making.

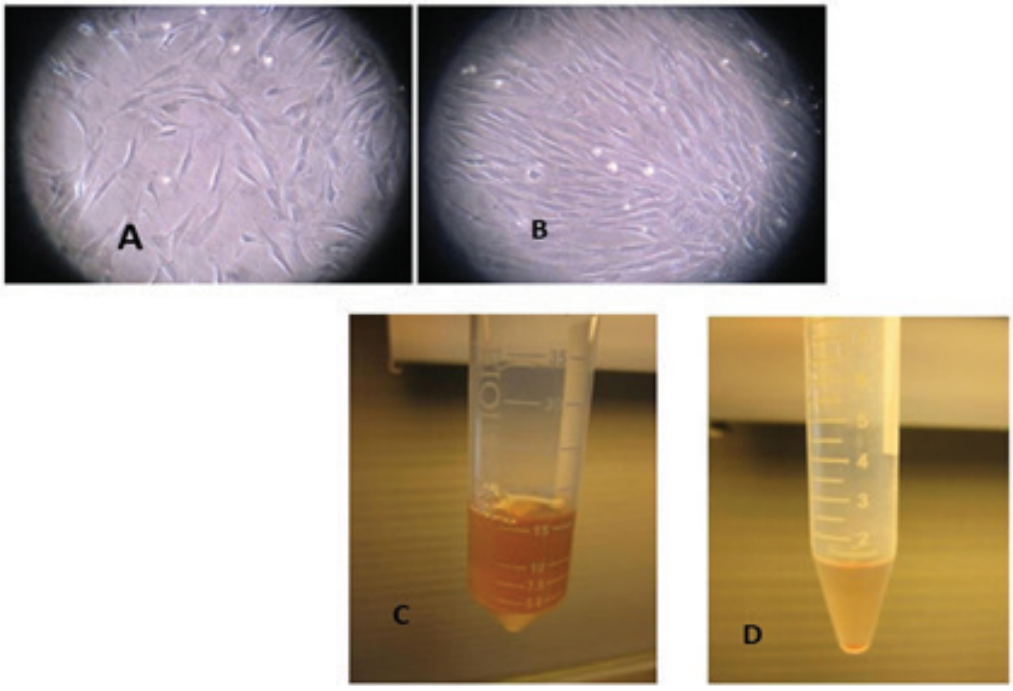
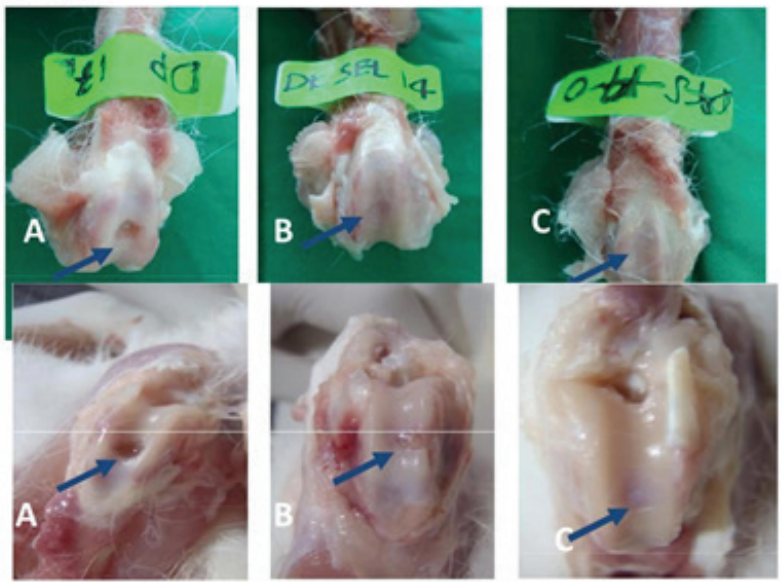


Figure 2. macroscopic defects cartilage joints after week 10. A. Injection with PRP. B. Injection with BMSCs. C. Injection with BMSCs- PRP



Discussion

The procedure of this study was carried out according to the previous research results where the process of centrifugation done 2 times with speed 3000 rpm for 13 minutes at 1st and 3000 rpm for 15 minutes in the second round. In another study another commercial system was used where leukocytes-PRP were classified according to Dohan Ehrenfest. In this system PRP was produced with platelet concentration of about 220,000 platelets/ µl. Preparation of PRP consists of a stage where the poor platelet plasma (PPP) was not disposed.

The advantage of this process is to avoid excessive manipulation that can lead to platelet stress in the second centrifuge and avoid removing growth factors that depend on PPP. Another advantage is the closed circuit system in its purification process which causes the procedure to be safer. In this process usually after centrifugation of 8 ml of peripheral blood, platelet recovery> 95% and recovery of leukocyte> 58% (mononuclear cell recovery 93%) in 4 ml PRP [11].

The platelet count levels in this study may provide optimal benefits. The concentration of platelets in PRP in this study did not differ greatly with the Anitua study which obtained platelet concentration results of about 2.5 times normal. Other studies have shown that an excessively high platelet count can decrease the expected effect (paradoxsal effect). The dose-response relationship between platelet concentration and the stimulated biological process remains unclear. Once the growth factor reaches the targeted targeted receptor surface, the additional growth factor concentration has no effect. Growth factor will decrease the effect if the required upper (high) concentration limit is reached[12]. In an intra-articular injection study of BMSCs on osteoarthritis raised by bone debris and cartilage from osteochondral fragments, there was a significant decrease in postaglandin levels in the synovium fluid [13].

This effect is not found when an injection of MSCs is sourced from fat. In this study there was an increase of tumor necrosis factor (TNF) levels in the synovium fluid. The effects of BMSCs on cartilage improvement are derived from inhibition of catabolic effects and stimulation of anabolic effects through cytokine mediators [14].

A recent study on horse experimental animals for 2 to 5 years found that BMSCs produce protein core and

chondroitin sulfate with longer chain and few shorter protein molecules than chondrocytes. This shows that BMSCs produce aggrecan with younger phenotype characteristics than chondrocytes produced. These results are in accordance with the results of this study where there is an increase in aggrecan content in defects given BMSCs [15].

Allogenic BMSCs are a new hope for healing cell-based cartilage. Allogenic BMSCs allow patients to be injected only once, no longer needing 2 actions for harvesting cells and implantation. This allogenic technique makes the treatment of defects in cartilage to be reliable, easy to apply and not invasive [16].

Conclusion

Intra-articular injection of allogenic bone marrow mesenchymal stem cell - rich plasma platelet (BMSCs-PRP) was able to increase the differentiation of BMSCs into condroblasts. It creates formation of more aggressive and collagen type 2 expression cells than those injected only with BMSCs or PRP alone in fullthickness joint cartilage defects. PRP is able to form an environment suitable for MSCs so that MSCs proliferate and differentiate into more condroblasts.

Ethical Clearance

This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest : The authors report no conflict of interest related to this paper so far

Source of Funding : All of expenses of this research are paid by authors only without external sources

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