CHONDROREGENERATIVE POTENTIAL ON SUBCUTANEOUS IMPLANTATION OF PLATELET-RICH FIBRIN (PRF)-IMPREGNATED DECELLULARIZED BOVINECARTILAGE SCAFFOLD

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ABSTRACT

The discovery of alternative implants with regenerative potential comparable to autologous cartilage continues to be encouraged because of the high donor site morbidity rate. This research tries to make an alternative implant that uses the concept of tissue engineering techniques in the form of endogenous regeneration by combining Decellularized Bovine Cartilage scaffold with Platelet-Rich Fibrin (BCPRF) which is implanted subcutaneously. The aim of this study was to compare the potential for chondroregeneration between BCPRF and autologous cartilage as assessed by chondrocyte cell formation, type 2 collagen thickness, and implant resorption rate in subcutaneous implantation. Using the research design method is a pretest-posttest control group design using New Zealand white rabbits. Forty eight experimental samples were divided into 2 groups which were treated with BCPRF and autologous cartilage implantation. Results were evaluated after 6 weeks. Evaluation was carried out on 39 samples. Microscopy showed better potential for autologous cartilage chondroregeneration than BCPRF with significant differences in the number of chondrocytes formed, the thickness of type 2 collagen (p=0.000), and the rate of implant resorption (p=0.000). In conclusion, the potential for chondroregeneration of autologous cartilage and BCPRF is significantly different in terms of the number of chondrocytes formed, the thickness of type 2 collagen, and the rate of implant resorption.

Keywords: Cartilage graft; endogenous regeneration; cartilage implant

INTRODUCTION

The number of needs for corrective surgery using a cartilage graft is quite high for cases of reconstruction of craniofacial congenital abnormalities, trauma, aesthetics, to cases after tumor resection. Since being popularized in 1899 by Konig and Goodale in 1901 (Goodale, 1901), cartilage graft is still an effective modality in plastic surgery (Peer, 1954).

The preferred modality is reconstruction using autologous cartilage. However, because of its weaknesses in terms of harvesting, morbidity of the donor area, the possibility of large dislocation, grated donor areas, and the risk of pneumothorax making autologous cartilage slowly its use begins to be limited (Araco, et al. 2006; Revell & Athanasasiou, 2009; Mischkowski, et al. 2008).

The new paradigm in tissue engineering has provided opportunities for xenografts to be re-developed in regenerating a tissue, especially cartilage. The concept involving 3 main factors, namely cells, scaffold, and signal (growth factors) (Chung, et al. 2008) can be applied to produce alternative implants. The xenograft material that is often used for making implants is bovine cartilage because of its abundant amount in nature. Meanwhile, Platelet-Rich Fibrin (PRF) is a source of growth factors that has been widely studied recently.

Based on this, based on the concept of tissue engineering – endogenous regeneration, researchers want to examine whether the implant is a combination of bovine cartilage with PRF (which in this study is called Decellularized Bovine Cartilage Scaffold – PRF Implant (BCPRF)).

METHODS

This type of research is true experimental research using the Pretest-Posttest Control Group Design with experimental animals in the form of rabbits. In this study the sample was divided into 2 different treatment groups. In each group, measurements were taken before and after treatment for the variable implant thickness assessment (resorption), and only post-treatment measurements for other variables, with an observation period of 6 weeks.

In this study, the subjects studied were the subcutaneous tissue on the back of the New Zealand white rabbit which was implanted with a BPRRF Implant and Autologous cartilage. Meanwhile, the sample for this study was the New Zealand white rabbit (Oryctolagus cuniculus) which received subcutaneous BCPRF implantation and autologous cartilage.

RESULTS

In this study, an experiment was conducted using 24 rabbits which were divided into 2 groups, 12 of which received autologous cartilage implantation and 12 of which received BPRRF implants.

The result of freeze-drying (lyophilization) of the combination of decellularized bovine cartilage scaffold and PRF produces a porous structure. On histological evaluation, a large porosity structure was found consisting of chondroid matrices accompanied by inter-matrix fibrin fibers with pore sizes between 30-400 µm.

The results of the immunohistochemical evaluation of the chondroid matrices revealed collagen type II expression with weak to strong intensities (figure 1).

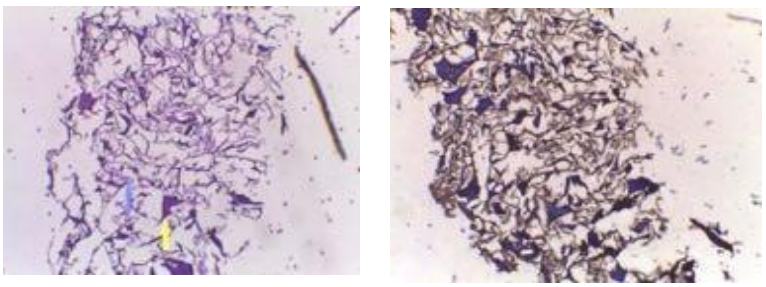

Figure 1. Histological view of the BCPRF implant microarchitecture. Evaluation with H-E staining shows chondroid matrix (blue arrow) and fibrin fibers (yellow arrow) (Left), Histological picture with immunohistochemistry (right)

The results of the evaluation of the number of chondrocytes showed that there was the formation of new chondrocytes in the autologous cartilage group. The new chondrocytes are formed in the peripheral area of the implant (periimplant) and mature towards the center. Meanwhile, in the BCPRF implant group, there was no chondrocytic cell growth. However, there is growth of fibrocollagen tissue between the chondroid matrices on BCPRF implants with a more hypertrophic chondroid matrix (figure 2).

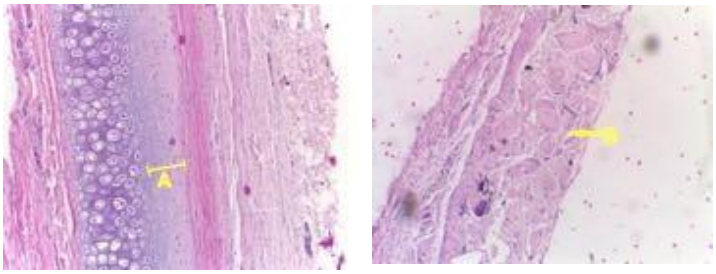

Figure 2. Comparison of histopathological results with HE staining. Growth of new chondrocyte cells in the autologous cartilage group (A) and growth of fibrocollagen tissue in BCPRF (B)

Table 1. Shows the Number of Newly Generated Chondrocytes in the Autologous Cartilage Group

Kelompok	n	Jumlah kondrosit			
		Mean	SD	Min	Max
Autologous	16	16,8438	4,46527	8,3	23,3
BCPRF	23	0	0	0	0

On histopathological examination, type II collagen growth was found in the autologous cartilage group evenly on the edge of the implant, while in the BCPRF group the thickness of collagen in the periimplant area varied greatly. However, the BCPRF group found chondroid matrix hypertrophy with a matrix size that was larger than the pre-implantation matrix size (figure 4).

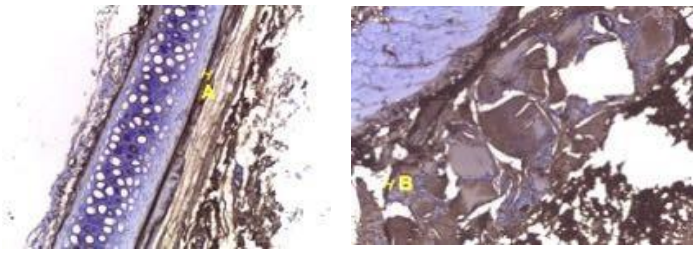

Figure 4. Measurement of collagen type II thickness in the peri-implant area. A, Collagen thickness in the autologous cartilage group. B. Collagen thickness in the BCPRF group

The average implant resorption rate in the BCPRF group was higher than that in the autologous cartilage group. In the BCPRF group the implant resorption rate reached -76.2522% compared to the autologous group which only reached -6.9413%.

DISCUSSION

In this study autologous cartilage was implanted in a distant region and did not have the same tissue characteristics as its native environment. The subcutaneous tissue on the back of the rabbit is mostly formed from fibrocollagen connective tissue in which no cartilage tissue is found in that area.

In this therapy, implantation of chondrocyte cells either with or without matrix/scaffold assistance is performed on cartilage defects that occur as a result of a pathological process. In this condition, the regenerative capacity will be quite high due to the strong regenerative signal from the native environment.

The results of this study prove that autologous cartilage has better regenerative potential than BCPRF implants in terms of chondrocyte cell growth, matrix type II collagen production, and its resorption rate. The high porosity of the implant tends to increase the hydrolysis reaction and implant degradation. Conversely, large pore size, porosity, and network will be a good medium for cell regeneration and flow of nutrients into the scaffold (Gariboldi, 2015; Loh, 2013).

The concept of Triad Tissue Engineering (Vinatier, 2009) combines the roles of cells, scaffolds, and morphogenic factors in regenerating a tissue. With the paradigm of endogenous regeneration (Gulati & Poluri, 2015), BCPRF implants are designed to be able to regenerate cartilage tissue by optimizing the recruitment of endogenous MSCs to the area of injury (implantation) to differentiate into the chondrogenic lineage through the active role of morphogenic proteins in the form of CDMP-1, CDMP-2, BMP-2, 4, 6 and 9 which are contained in the scaffold in the form of decellularized bovine cartilage matrix (Utomo, 2013).

This study found complications in the form of implant exposure (extrusion) and implant infection in the autologous cartilage group, although in several studies it was explained that the use of autologous cartilage grafts is relatively safer with a risk of infection, low extrusion due to its autologous nature will cause minimal immune reactions (Wee, 2015). The implant extrusion that occurred in this study could be caused by the creation of a skin pocket that is relatively the same size as the implant size, so that with a rigid autologous cartilage implant structure, the skin pocket created cannot accommodate implant migration.

The results of this study indicate that the regenerative capacity of implanted BCPRF in terms of chondrocyte cell growth has not been commensurate with that of autologous cartilage implantation. The absence of chondrocyte cell growth after implantation of BPRRF implants may occur due to inadequate active signaling of morphogenic factors in directing MSC differentiation towards chondrogenesis. Nakayama (2003) explained that BMP-4 as a morphogenic protein in the process of chondrogenesis works in a dose-dependent manner. In his study explained that a larger dose of BMP-4 (50ng/mL) will stimulate the formation of more cartilage. In a certain minimal dose (5ng/mL) otherwise BMP-4 is not able to stimulate the formation of cartilage. This suggests that the inability of implanted BCPRF to stimulate chondrocyte formation may occur due to an inadequate amount of morphogenic protein, so that the MSC differentiation signal is not optimal.

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