Journal of the Taiwan Institute of Chemical Engineers 000 (2018) 1–8

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Intraarticular injection for rabbit knee osteoarthritis: effectiveness among hyaluronic acid, platelet-rich plasma, and mesenchymal stem cells

Yu-Chun Chen[a,b,1], Yuan-Ming Hsu[a,1], Kinn Poay Tan[a], Hsu-Wei Fang[c,d], Chih-Hung Chang[a,e,*]

[a] Department of Orthopaedics, Far Eastern Memorial Hospital, No. 21, Sec. 2, Nanya S. Rd., Banciao Dist., New Taipei City 22060, Taiwan
[b] College of General Studies, Yuan Ze University, 135 Yuan-Tung Road, Zhongli Dist., Taoyuan City 32003, Taiwan
[c] Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, 1, Sec. 3, Zhongxiao E. Rd., Taipei 10608, Taiwan
[d] Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 35053, Taiwan
[e] Graduate School of Biotechnology and Bioengineering, Yuan Ze University, 135 Yuan-Tung Road, Zhongli Dist., Taoyuan City 32003, Taiwan

ARTICLE INFO

Article history:
Received 6 February 2018
Revised 11 May 2018
Accepted 12 May 2018
Available online xxx

Keywords:
Platelet-rich plasma
Mesenchymal stem cells
Hyaluronic acid
Osteoarthritis
Cell therapy

ABSTRACT

Both hyaluronic acid (HA) and platelet-rich plasma (PRP) injection are the common prescribed treatments for osteoarthritis (OA) patients. HA can serve as a good lubricant to reduce the fraction force of cartilage surface while PRP provide several growth factors to assist tissue repair. Considering the efficiency of tissue regeneration, mesenchymal stem cells (MSCs) injections becomes an attractive method because of their self-renewal and multi-differentiation potential. Thus, in the study, we compared the treatment effect of PRP and HA with/without MSCs. Eighteen rabbits underwent anterior cruciate ligament transection for OA knee induction, and then injected with saline, PRP, HA, HA+PRP, PRP+MSCs or HA+MSCs intraarticularly for treatment effect evaluation. Compared with the results of HA group, the cartilage treated with HA+PRP and PRP+MSCs showed better repair results in micromorphology and glycosaminoglycan synthesis. We also found that the Osteoarthritis Research Society International (OASRI) scores in HA+PRP and PRP+MSC groups were significant lower than that of the others at month 1 and month 3, separately. This indicates that the combination of HA and PRP could provide short-term tissue protection effect while PRP and MSC could provide a long-term tissue regeneration effect. PRP+MSC could be considered as a promising biological method for OA treatment.

1. Introduction

Osteoarthritis (OA) is a progressive and irreversible disease that is primarily exhibited as the degeneration of hyaline articular cartilage. It affects about 14% of the adult population, and becomes the second common cause of permanent disability in people over the age of 50 [1]. A variety of noninvasive alternative treatments, such as analgesic and nonsteroidal anti-inflammatory drugs, have been used for pain reduction, knee function and disability improvement [2]. However, there is some variation in individual responses to each drug as well as potential side effects of their administration [3]. Importantly, such treatment can only relieve the symptoms and do not repair the degenerated tissue.

Intra-articular hyaluronic acid (HA) injection is a widely utilized clinical therapy for OA treatment because of its unique lubricating properties. HA administration can enhance the viscosity and elastic properties of synovial fluid [4]. Furthermore, HA is a naturally occurring glycosaminoglycan, and serves as the backbone for proteoglycan formation in the cartilage matrix. In the synovial fluid, HA can bind to chondrocytes via the CD44 receptor and mediate chondrocyte proliferation and function [5]. In a randomized, controlled, double-blind, multicenter trial study in an Asian population (n = 200), five weekly intra-articular injections of sodium hyaluronate (Hyalgan, 20 mg/2 mL) has been shown to greatly improve VAS and WOMAC pain and function scores from baseline to week 25 compared with the placebo group [6].

Platelet-rich plasma (PRP) intra-articular injection is a new type of growth factor treatment. PRP is obtained by centrifugation of

[1] Contributed equally to the work.

* Corresponding author at: Department of Orthopaedics, Far Eastern Memorial Hospital, No. 21, Sec. 2, Nanya S. Rd., Banciao Dist., New Taipei City 22060, Taiwan.
E-mail address: orthocch@mail.femh.org.tw (C.-H. Chang).

https://doi.org/10.1016/j.jtice.2018.05.051
1876-1070/

autologous blood samples, which increases the platelet concentration 3- to 5-fold compared to that in whole blood. When platelets are activated by thrombin, calcium, or collagen, they degranulate and release growth factors to begin the cell growth cascade and repairing/remodeling of damaged tissues [7,8]. At least 7 major growth factors (platelet-derived growth factor, transforming growth factor, epidermal growth factor, insulin-like growth factor, basic fibroblast growth factor, and vascular endothelial growth factor) have been identified in platelets; these factors are known to orchestrate a series of molecular events that lead to collagen synthesis, angiogenesis, mesenchymal stem cell mitogenesis, and growth and differentiation of chondrocytes [9,10]. In vivo studies in rabbits demonstrated that the optimal concentration of platelets for bone regeneration is between 503,000 to 1,729,000 platelets/μL. Low platelet concentrations such as 164,000–373,000 platelets/μL have a sub-optimal effect, and high concentrations (1,845,000–3,200,000 platelets/μL) may have a inhibitory effect [11]. Another study also showed that subcutaneous PRP injection in the presence of chondrocytes can lead to cartilage regeneration in rabbits. These results indicate that chondrocytes/PRP may be a novel injectable cartilage substitute and may provide a valuable alternative to reconstructing articular cartilage in the knee and temporal mandibular joint.

Considering the efficiency of tissue regeneration, mesenchymal stem cells (MSCs) injections becomes an attractive method because of their self-renewal and multi-differentiation potential. It can be obtained from many sources, such as bone marrow aspirate concentrate, adipose tissue, and muscle tissue. We speculated MSC injection in conjunction with PRP should produce a longer therapeutic effect than that of PRP within a hydrogel such as HA.

Thus, in the study, we compared the treatment effect among PRP, HA and MSC in vivo, and anterior cruciate ligament transection (ACLT) model was used for rabbit OA knee induction. Cartilage morphology, glycosaminoglycan staining result, and Osteoarthritis Research Society International (OASRI) scores were used for treatment effect evaluation.

2. Materials and methods

2.1. Isolation of bone marrow MSCs

Bone marrow MSCs were isolated from the iliac crests of rabbits. All the MSCs is used for autologous injection. In brief, 3–5 mL bone marrow was harvested, layered onto 3 mL of Ficoll-Paque PREMIUM (GE Healthcare, Uppsala, Sweden), and then centrifuged at 1550 rpm for 30 min at room temperature. After centrifugation, the supernatant was discarded, and the opaque interface was carefully transferred to another clean tube. The total volume was then adjusted to 10 mL by Minimum Essential Medium Alpha Medium addition (α-MEM) (Thermo Scientific, Waltham, MA, USA), centrifuged again at 1000 rpm for 5 min, and then discarded the supernatant. MSCs were cultured in α-MEM containing 10% fetal bovine serum (FBS) (SAFC Biosciences, Lenexa, Kansas, USA) and 1% antibiotic-antimycotic solution (100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of Gibco Amphotericin B) (Thermo Scientific, Waltham, MA, USA). Medium was subsequently changed twice a week.

2.2. Differentiation potential of MSCs

MSCs should exhibit the potential to differentiate into various mesenchymal lineage cell types, including osteoblasts, chondrocytes, and adipocytes. We confirmed their differentiation potential according to the following protocols.

For chondrogenesis, a number of 4×10^5 MSC in 2 mL of culture medium were centrifuged at $500 \times g$ for 10 min. After overnight cultivation, the cell pellets became aggregated; the culture medium was replaced by chondrogenic medium. It is composed of serum-free DMEM-high glucose (DMEM-HG; Thermo Scientific) with ITS (BD Biosciences, USA), 10^{-3} M sodium pyruvate, 1.7×10^{-4} M L-ascorbic acid-2-phosphate, 3.5×10^{-4} M proline, 10^{-7} M dexamethasone (Sigma-Aldrich, St. Louis, MO, USA), and 10 ng/mL TGF-β1 (R&D Systems, Minneapolis, MN, USA). Medium was changed every 2–3 days. Chondrogenic induction was then further confirmed by Alcian blue staining.

For osteogenesis, the MSCs were exposed to osteogenic differentiation medium consisting of DMEM-HG supplemented with 10% FBS, 10^{-7} M dexamethasone, and 25 μg/mL L-ascorbic acid (Sigma-Aldrich) for 2 weeks. After induction, the osteogenic phenotype was assessed by staining with Alizarin red, which stains calcium-rich mineral deposits.

For adipogenic differentiation, cells were exposed to medium supplemented with 10^{-6} M dexamethasone, 6×10^{-5} M indomethacin (Sigma-Aldrich), and 5×10^{-4} M 3-isobutyl- 1-methylxanthin (Sigma-Aldrich). After induction for 2 weeks, the cells were stained with Nile red (Sigma-Aldrich) for intracellular accumulations of lipid droplets staining.

2.3. PRP preparation

We collected about 10 mL of peripheral blood from the veins of rabbit ear and prepared PRP by two centrifugation steps methods. The first spin was set at 1300 rpm for 20 min to separate erythrocytes and the second spin was set at 2000 rpm for 15 minutes to concentrate the platelets. The platelet number was counted before and after the centrifugations, and the total number of platelets per milliliter of PRP was compared with whole blood to calculate the fold increase.

2.4. OA knee induction in rabbits

Eighteen adult male New Zealand white rabbits weighing from 1.8 to 2.2 kg were used. Rabbits were anesthetized with intramuscular injection of xylazine (8 mg/kg), and then the surgical site was shaved. ACLT was performed with a surgical blade for knee OA induction, and the joint capsule and skin were closed in sterile fashion. The Animal Care and Use Protocol was approved by Far Eastern Memorial Hospital IACUC (Permit Number: 101-02-09-B).

Eight weeks after ACLT surgery, rabbits were randomly divided into five groups for different injection treatments, including (1) HA (n = 3) (ARTZ/ARTZDispo, Seikagaku, Tokyo, Japan), (2) PRP (n = 3) (from rabbit peripheral vein blood), (3) HA combined with PRP (n = 3), (4) HA combined with MSCs (n = 3) (from rabbit bone marrow), and (5) PRP combined with MSCs (n = 3). Complete details of the injection components are listed in Table 1, and the timeline is shown in Fig. 3. All these treatments were intra-articularly injected under local analgesic administration. The injection site was shaved and draped in a sterile fashion in advance. In addition, the PRP was isolated one day before the experiment and stored at 4 °C, and then activated by 10 μL thrombin solution (1000 U/mL in 100 mM $CaCl_2$) before use.

Half of the animals were sacrificed after 1 month, and the remaining animals received second injections of the above treatments, then sacrificed after 3 months. Groups four and five received second injections of HA and PRP-ONLY, separately. All the results were compared with a SHAM group (without ACLT surgery).

2.5. Histological analysis

Gross appearance of the knee cartilage surface was recorded by digital camera to evaluate osteophyte formation and cartilage degeneration. The knee specimens were fixed in a 4% buffered

Please cite this article as: Y.-C. Chen et al., Intraarticular injection for rabbit knee osteoarthritis: effectiveness among hyaluronic acid, platelet-rich plasma, and mesenchymal stem cells, Journal of the Taiwan Institute of Chemical Engineers (2018), https://doi.org/10.1016/j.jtice.2018.05.051