Synergistic Effects of Amnion MSCs and PRP Therapy on Osteoarthritic Rat Models

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Abstract:

Introduction: Osteoarthritis stands as the prevailing degenerative joint ailment, precipitating pain and eventual functional disability. It notably diminishes life quality and amplifies the economic strain on healthcare systems.

Objective: This investigation seeks to scrutinize the therapeutic potentials of Platelet-Rich Plasma (PRP) and Amniotic Membrane-derived Mesenchymal Stem Cells (AM-MSCs) separately and in conjunction within surgically induced osteoarthritic rat models.

Methods: Animals received intra-articular injections of PRP and AM-MSCs individually and in combination, with effects assessed via Safranin O staining, histological scoring, and immuno-histochemistry.

Results: Treatment with PRP+AM-MSCs for knee osteoarthritis yielded more pronounced outcomes compared to exclusive PRP or AM-MSC treatments. Immunostaining and histological scoring demonstrated up-regulation of proteoglycan and collagen type II following 35 days of intra-articular injection, signifying significant osteoarthritic knee improvement.

Conclusion: The combination of AM-MSCs and PRP proves more beneficial in osteoarthritis treatment.

Keywords: platelet-rich plasma, amniotic membrane mesenchymal stem cells, osteoarthritis

Introduction:

Osteoarthritis (OA) stands as the predominant form of chronically debilitating articular joint ailment, characterized by the progressive degradation of articular cartilage, leading to disability over time. Its onset typically spans a duration of 10-15 years, marked by manifestations of inflammation, pain, stiffness, gradual loss of articular cartilage, enlargement of joint capsule, degradation of ligaments and menisci, along with structural bone remodeling. As of 2020,

approximately 86.7 million individuals were afflicted by OA [1]. Owing to its avascular nature, articular cartilage exhibits limited self-repair capabilities, thereby predisposing it to osteoarthritic degeneration [2].

The utilization of Platelet Rich Plasma (PRP) and Amniotic Membrane Mesenchymal Stem Cells (AM-MSCs) for tissue regeneration presents a promising avenue for the treatment of degenerative diseases. Autologous PRP offers a swift, facile, cost-effective, and efficacious approach within regenerative medicine. Enriched with an array of growth factors such as platelet-derived growth factors (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factors (VEGF), and insulin-like growth factor type 1 (IGF-1), PRP plays a pivotal role in angiogenesis, cell migration, proliferation, anti-apoptotic functions, thereby facilitating the healing process of damaged tissues [3]. In-vitro animal studies have elucidated PRP's capacity to stimulate chondrocyte proliferation, proteoglycan synthesis, collagen type II production, and modest bone protein synthesis [4]. Furthermore, PRP has demonstrated favorable effects on cartilage repair through its myriad growth factors and cytokines [5].

The synergistic interaction between PRP and Amniotic Membrane Mesenchymal Stem Cells (AM-MSCs) holds promise as a breakthrough therapy for knee osteoarthritis. Renowned for their abundance, ease of isolation, multilineage differentiation potential, immunomodulatory activities, and lack of immunological responses due to the absence of major histocompatibility complex class II antigens, amniotic mesenchymal stem cells offer a compelling therapeutic option [6]. The secretion of various growth factors, paracrine factors, immunomodulatory cytokines, coupled with the suppression of pro-inflammatory mediators, contributes significantly to tissue repair [7]. Additionally, AM-MSCs release several anti-inflammatory cytokines including TGF-β, HGF, PGE2, and IDO [6]. Placenta-derived MSCs have garnered substantial interest among researchers owing to their non-invasive isolation techniques, minimal tumorigenesis, and diminished ethical concerns [7,8].

In this study, we postulated that the combined application of Platelet Rich Plasma and Amniotic Membrane Mesenchymal Stem Cells could offer superior therapeutic efficacy in mitigating degenerative disorders compared to individual applications.

Materials and Methods:

Animals:

All animals were housed in adequately ventilated cages under standardized conditions, maintaining a temperature of 25°C, with a 12-hour light-dark cycle, and provided access to appropriate food and water ad libitum. Animal care and experimental procedures were conducted in accordance with the guidelines established by the Committee of Animal Care, SZABMU, Pakistan (F.1-1/2015/ERB/SZABMU/998).

Platelet Rich Plasma (PRP) Preparation: PRP was obtained from autologous blood using a centrifugation method. Briefly, 1.5ml of blood was collected from the lateral tail vein into tubes containing ACDA (Haemonetic USP) to prevent coagulation. The blood was then centrifuged at 3000 rpm for 10 minutes to separate its components based on gravity, resulting in the formation of three layers: red blood cells, buffy coat layer, and plasma. The plasma and buffy coat layer

were transferred to a new tube and centrifuged at 4000 rpm for 8 minutes to obtain PRP [9]. Subsequently, 200µl of PRP was injected into each osteoarthritic knee.

Isolation and Culture of Amniotic Membrane Mesenchymal Stem Cells (AM-MSCs):

Amniotic Membrane (AM) was retrieved from the human placenta of a healthy donor and rinsed thoroughly with Phosphate Buffer Saline (PBS) containing antibiotics (penicillin 100 UL/ml and streptomycin 100mg/ml) to remove any blood clots and debris [10]. The blood-free membrane was then cut into small pieces (1-2 cm2) and treated with an enzymatic solution consisting of 0.25% trypsin (Gibco) + 0.1% collagenase IV (Gibco) to isolate mesenchymal stem cells. Following enzymatic treatment, the membrane pieces were incubated at 37°C for 5 minutes with gentle shaking. The trypsin effect was neutralized using fetal serum, and the process was repeated 3-4 times. The membrane was then centrifuged at 1000 rpm for 10 minutes, and the resulting cell pellet was suspended in a medium. Cell counting was performed using the CountessTM Automated Cell Counter from Invitrogen, and the cell pellet was seeded in Dulbecco Modified Eagle Media (DMEM). The culture medium was replaced every 2-3 days to support cell growth and nourishment. Sub-culturing of AM-MSCs was carried out using 0.5% Trypsin-EDTA (Sigma-Aldrich Corp., St. Louis, MO, USA). Cells at passage 2 (P2) were used for the experiments. Intra-articular injection of 1×10^6 AM-MSCs per 200µl was administered into each rat in the AM-MSCs treated group.

Intra-articular Injection of PRP + AM-MSCs:

Following the establishment of osteoarthritis, treatments were administered to the respective groups of rats. The control group, along with the osteoarthritis group, remained untreated. The PRP group received only PRP injections, and the AM-MSCs group received only AM-MSCs injections. The final group received an injection comprising 200µl of the combined mixture of PRP+AM-MSCs (100µl PRP and 1×10^6 cells per 100µl). On the 35th day post-treatment, all animals were euthanized to assess the effects of each treatment.

Safranin-O/Fast Green Staining:

Articular cartilage sections were fixed and stained with 2% Safranin-O (ICN Biomedicals) solution and counter stain with 0.02 % fast green stain. Tissue observation was carried out using an Optika microscope (Optika B-190TB, Italy) [11].

Immunofluorescence of Col2a1 and Aggrecan:

Immunofluorescence analysis was conducted by incubating sections with primary antibodies against Col2a1 (collagen type II alpha 1) and Aggrecan (Santa-Cruz Biotechnology, USA). Twenty images from each group were collected, and highly fluorescent cells expressing the specific markers were assessed.

Results:

Proteoglycan Content Estimation:

The proteoglycan content was evaluated through Safranin-O/Fast Green staining of articular cartilage sections, assessing the percentage of positively stained cells in each section. The normal/control group exhibited smooth cartilage with normal proteoglycan content, evenly distributed throughout the articular cartilage. Chondrocytes appeared normal in shape and distribution, with no discernible loss. Treatment with PRP, AM-MSCs, and the combination of PRP + AM-MSCs resulted in improvements in proteoglycan content, with folds increases of 1.19, 1.47, and 1.79, respectively, compared to the untreated OA group. The combined treatment showed significant results, while individual treatments with PRP and AM-MSCs demonstrated non-significant improvements in proteoglycan content (Fig. 1).

Observations of osteoarthritic knee joints revealed loss of proteoglycans, osteophyte formation, and severe fibrillation. However, post-treatment examination showed a significantly higher percentage of Safranin-O positive stained cells compared to osteoarthritic knees. Knee joints treated with PRP and Amniotic Membrane Stem Cells exhibited reduced effects of OA, with some loss of proteoglycans and minor fibrillation. In the combined therapy group of PRP+AM-MSCs, proteoglycan content was significantly higher compared to PRP alone and non-significant compared to AM-MSCs treatment. Moreover, the combined therapy showed minimal cartilage loss, no fibrillation, and chondrocyte distribution similar to normal knee joints.

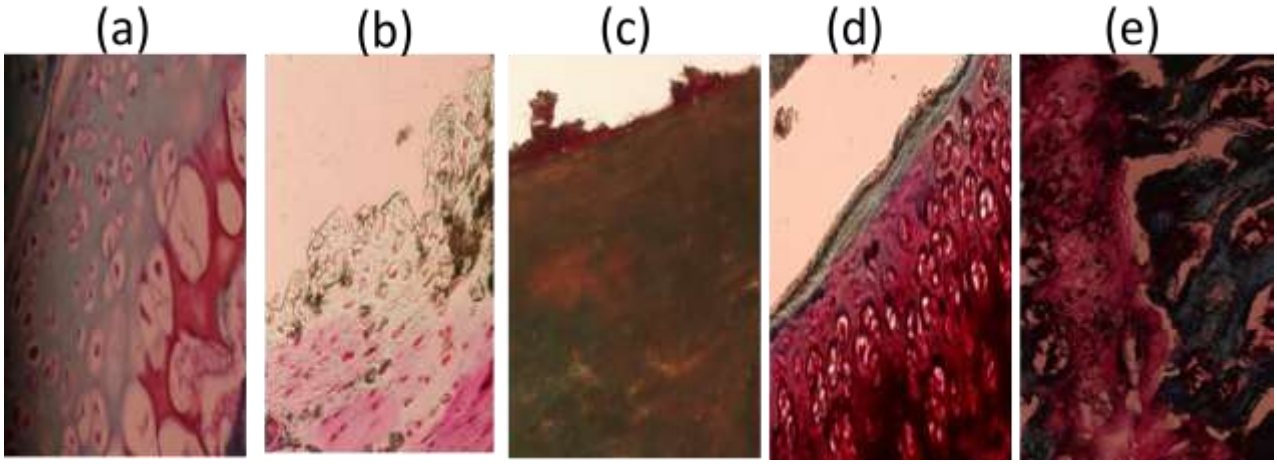

Fig. 1: Safranin-O/Fast Green staining was utilized to determine the proteoglycan content of all groups 35 days post-treatment (a) the control or normal group, (b) the untreated OA group, (c) the PRP treated group, (d) the AM-MSCs group, and (e) the combined PRP + AM-MSCs treated group.

Immunofluorescence of Knee Joints for Aggrecan (Acan) and Collagen type II alpha 1 (Col2a1):

Immunofluorescence analysis was conducted for Aggrecan (Acan) and Collagen type II alpha 1 (Col2a1) (Fig. 2). A reduction in Acan expression was observed in osteoarthritic knee joints compared to normal knee joints.

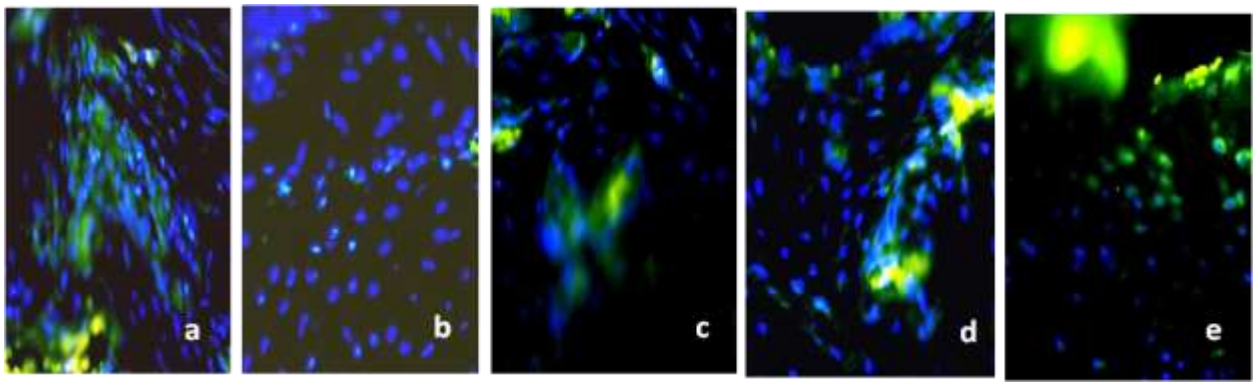

Fig. 2: Immunofluorescence was performed to determine the percentage of Acan-positive cells in all groups 35 days post-treatment. The figure displays (a) the control or normal group, (b) the untreated OA group, (c) the PRP treated group, (d) the AM-MSCs transplanted group, and (e) the combined PRP + AM-MSCs treated group.

The expression of Acan in PRP and AM-MSCs individually transplanted groups was elevated by 1.01 and 1.41 folds, respectively, compared to the osteoarthritic knee joint. The combined therapy of PRP + AM-MSCs exhibited a 1.64-fold higher expression compared to the OA group (Fig. 2).

Similarly, a reduction in Col2a1 expression was observed in the osteoarthritic joint compared to the normal joint.

The expression of Col2a1 in PRP and AM-MSCs separately transplanted groups was elevated by 1.07 and 1.33 folds, respectively, compared to the osteoarthritic joint. The combined therapy showed a significant increase by 1.72 folds compared to the OA group (Fig. 3). Immunofluorescence expression in the combined treatment group surpassed that of the individually treated and untreated groups.

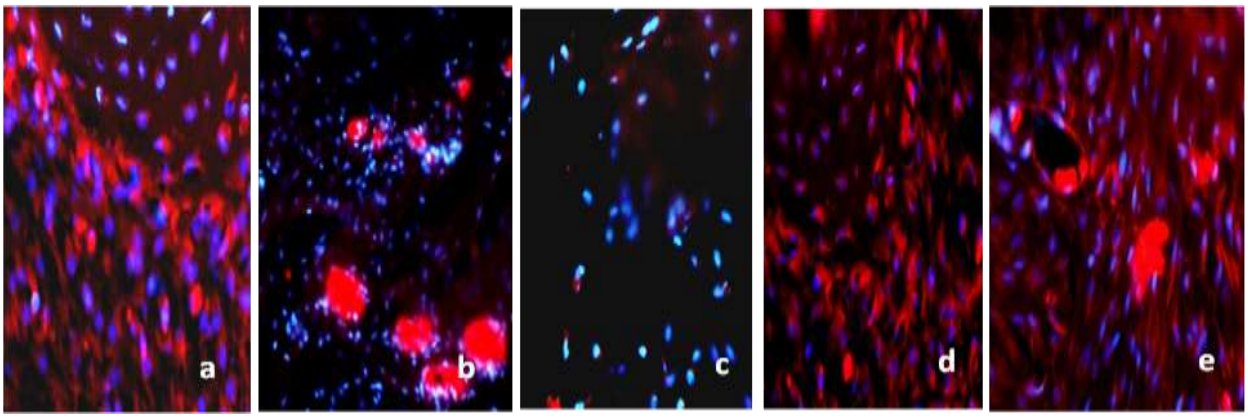

Fig. 3: Immunofluorescence analysis of Col2a1: (a) the control or normal group, (b) the untreated OA group, (c) the PRP treated group, (d) the Amniotic Membrane-Mesenchymal Stem Cells treated group, and (e) the combined PRP + AM-MSCs treated group.

Discussion:

This study aimed to evaluate the regenerative potential of Platelet Rich Plasma (PRP) and Amniotic Membrane Stem Cells (AM-MSCs), both individually and in combination, for induced osteoarthritis in rat models. Our findings unequivocally demonstrate that combined therapy is superior to individual treatments with PRP or AM-MSCs alone. After 35 days of injection, a notable enhancement in histological scores, as well as in the expression of Col2a1 and Aggrecan, was observed in the combined therapy group compared to the untreated OA group.

Our investigation revealed that treatment with PRP and AM-MSCs individually led to an increase in the expression of chondrogenic markers (Acan and collagen type 2 alpha1) compared to the untreated group. Nevertheless, the combined therapy of PRP+AM-MSCs exhibited a significantly augmented level of chondrogenesis when compared with the individual treatments. Topoluk et al. (2018) also observed a favorable chondroprotective effect of human Amniotic Membrane Stem Cells (HAMSCs), enhancing the viability of OA chondrocytes and promoting a shift in synovial macrophage phenotype from M1 to M2, surpassing that of human Adipose-derived Stem Cells (hADSCs) [12, 13].

Martinez and colleagues illustrated a significant increase in collagen (COL2a1) and Aggrecan marker (ACAN) expression in the AM-MSCs-treated group, highlighting enhanced chondrogenesis and extracellular matrix deposition [14]. Consistently, our study demonstrated improved histological features of OA cartilage, inducing chondrogenesis and a notable increase in Col2a1 and ACAN expression levels. Immunostaining revealed higher percentages of ACAN and Col2a1 in the AM-MSCs + PRP treatment group compared to the AM-MSCs and PRP-treated groups.

Moreover, previous research on intra-articular injection of Human Amniotic Membrane Mesenchymal Stem Cells (hAMSCs) showcased improved histological features of osteoarthritic cartilage and a reduction in disease progression attributed to the significant inhibition of pro-inflammatory cytokine production, such as tumor necrosis factor-α (TNF-α) and interferon- γ (INF- γ) [15]. Furthermore, another study showed a significant reduction in the level of IL-6 with the combined treatment of Adipose-derived Mesenchymal stem cells (ADMSs) and platelet rich plasma (PRP) [16]. Similarly, our study demonstrated considerable improvement in anti-inflammatory effect, histological features of osteoarthritic cartilage and decline in disease development.

Conclusion:

The findings of the present study suggest that the combination of Platelet Rich Plasma (PRP) with Amniotic Membrane Mesenchymal Stem Cells (AM-MSCs) holds greater potential for cartilage regeneration. A notable increase in proteoglycan, Col2a1, and Aggrecan expression was observed following combined therapy with PRP and AM-MSCs. Thus, it can be concluded that the concurrent administration of PRP and AM-MSCs offers greater therapeutic efficacy

compared to their individual use in the management of osteoarthritis. Given that this study was conducted on animal models, further research is warranted to assess the long-term benefits of combined therapy for clinical treatment of osteoarthritis and to establish its applicability in human subjects.

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