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OPTIMIZATION OF CHONDROGENIC POTENTIAL IN TRANSPLANTED
ADIPOSE-DERIVED STEM CELLS FOR TREATMENT OF OSTEOARTHRITIS

A Seminar Paper Submitted in Partial Fulfillment of the Requirements for the Degree of
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OPTIMIZATION OF CHONDROGENIC POTENTIAL IN TRANSPLANTED ADIPOSE-
DERIVED STEM CELLS FOR TREATMENT OF OSTEOARTHRITIS

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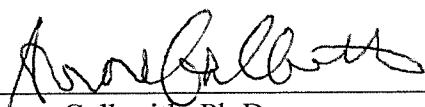
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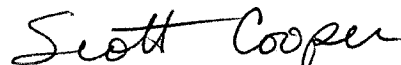
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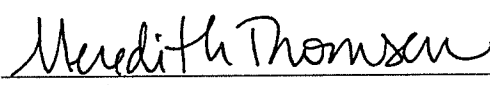
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ABSTRACT

Importance: Osteoarthritis (OA) has, and will continue to be, the leading form of musculoskeletal disease in the United States. More pragmatic and less invasive techniques for treating OA are required if physicians are to effectively manage and/or ameliorate the disorder.

Observations: Adipose-derived stem cell (ADSC) and platelet-rich plasma (PRP) dual therapy has shown much promise with regard to cartilage regeneration in animal models and is currently undergoing testing in multiple human trials. This type of regenerative therapy involves multiple intra-articular injections of autologous ADSCs and PRP over a brief period of time (approximately one month). Growth factors (GFs) and bioactive proteins, such as Soluble Vascular Endothelial Growth Factor – 1 (sFlt) and Bone Morphogenic Protein – 4 (BMP-4), may influence the chondrogenic potential of the ADSCs used in the dual therapy approach.

Conclusions and Relevance: It is reasonable to conclude that the addition of certain bioactive proteins, like sFlt and BMP-4, to the current ADSC and PRP dual-therapy approach for cartilage repair may result in heightened chondrogenic potential of the ADSCs used in the treatment.

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INTRODUCTION

Osteoarthritis (OA) is the leading form of musculoskeletal disease in the United States¹. OA is characterized by erosion of the articular cartilage and the subchondral bone due to biomechanical abnormalities causing irregular load distribution and, therefore, wear in an articulating joint^{1,2}. Currently there are few methods other than invasive surgery to effectively treat OA^{3,4}. At this time, the most effective treatment for OA is total joint replacement in patients who aren't qualified for more conservative surgical approaches. Because of the extensiveness of total joint replacement, recovery can be a long process, and much of the current research in this area is aimed at limiting this recovery time⁴.

Increased bodyweight and age, improper biomechanics, and presence of metabolic syndrome are all factors that can influence OA development. The occurrence of virtually all of these factors are rising, indicating that an increase in OA prevalence in the near future is highly probable¹. With the costs of medical procedures like total joint replacement also on the rise, it is imperative that a more feasible, cost-effective treatment for OA be developed for the benefit of the patient. The most promising types of treatments that meet these criteria are cartilage regeneration techniques using cell-based, platelet and/or growth factor-based, or combination therapies².

OBSERVATIONS

Adipose-derived Stem Cells

Human adipose tissue contains a relatively large number of mesenchymal stem cells (MSCs). MSCs derived from this tissue source are referred to as adipose-derived stem cells (ADSCs). Although ADSCs are one specific type of MSCs, there are many other types of MSCs including bone marrow-derived stem cells and muscle-derived stem cells which, due to their origin, have similar potential^{3,5,6}. However, ADSCs are a much more accessible cell type to use in cell-based therapies because of the much greater numbers of these stem cells available in the vast reservoirs of adipose tissue stored throughout the body^{5,7}. Obtaining and isolating ADSCs from these fat tissue deposits is a relatively straightforward process. It can be accomplished in a few simple steps including aspiration of the adipose tissue (lipoaspiration) and subsequent centrifugation of the obtained lipoaspirate in order to separate the much smaller ADSCs from the larger adipocytes, blood cells, and tissue fragments. After isolation ADSCs can then be passaged multiple times and expanded to an optimal cell density for use in cell-based therapies via traditional mammalian cell culture techniques⁵. ADSCs also maintain superior proliferation and differentiation capacity for longer periods of time during passage than other MSCs³.

Platelet-rich Plasma and Associated Growth Factors

Platelets play large roles in events such as growth and proliferation of cells that are targets of the bioactive proteins and other molecules they contain⁸⁻¹¹. Platelets are known to contain many bioactive molecules known commonly as growth factors (GFs) and cytokines. Most pertinent to this review are the GFs related to connective tissue repair. These GF

include Transforming Growth Factor – β (TGF- β), Platelet-derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Fibroblastic Growth Factor (FGF), Connective Tissue Growth Factor (CTGF), Epidermal Growth Factor (EGF), and Insulin-like Growth Factor – 1 (IGF-1) (though IGF-1 is not produced in platelets but is found in the plasma). These GFs are notable due to the manner in which they can influence stem cell differentiation and affect the structure and composition of cartilage tissue^{9–14}.

Of this specific group of GFs, TGF- β and PDGF are most responsible for proliferation of mesenchymal stem cells^{13,14}. With regard to chondrogenesis, TGF- β has been implicated as a powerful proliferator of MSCs when used in conjunction with chondrogenic stimuli *in vitro*¹⁵ (Figure 1). TGF- β has also been shown to regulate the production of collagen and secretion of collagenase from chondrocytes^{11,13,14}. Platelet GFs FGF and CTGF have been indicated as having positive effects on chondrocyte extracellular matrix (ECM)

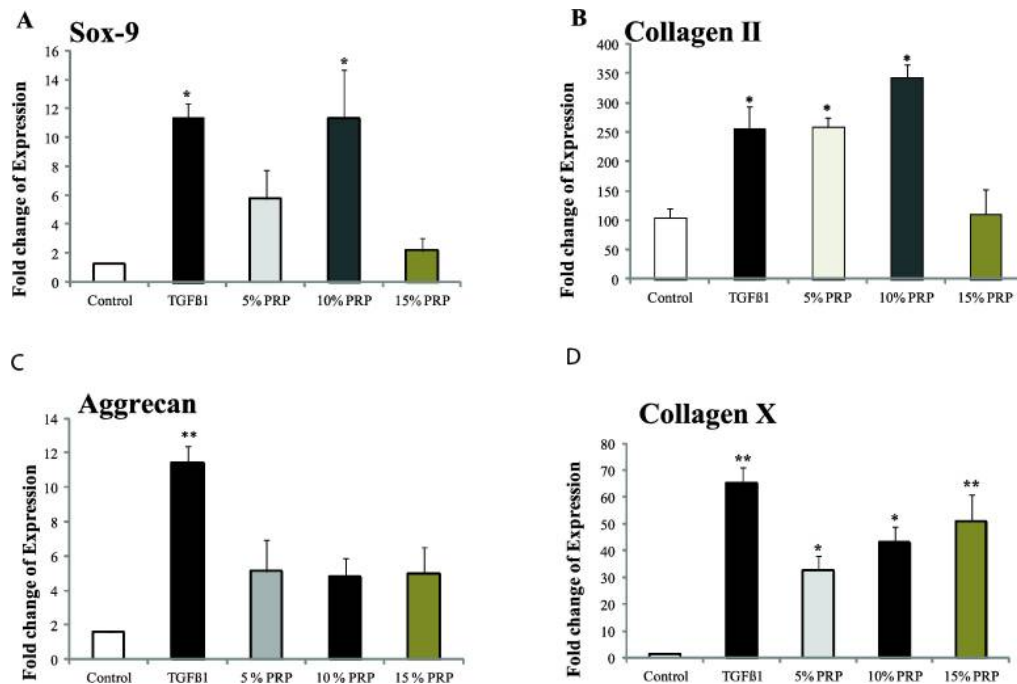


Figure 1. Graphical representation of the effects of TGF- β 1 and different PRP concentrations on expression of known chondrogenic markers in ADSCs cultured in transwells. Modified from Mardani *et al.* (2013).

protein synthesis and therefore, possibly cartilage regeneration^{13,14}. GFs responsible for angiogenesis of target tissues include, most notably, VEGF, but also EGF and TGF- β ^{10,11,13,14}. IGF-1 was included in this group of notable GFs found in platelets (though it is actually found in the plasma) because there is still a significant amount of IGF-1-containing plasma in traditional platelet-rich plasma solutions. IGF-1 is an anabolic GF that can strongly promote osteogenesis, thus providing the potential to differentiate target stem cells in the cartilage tissue to bone¹³.

PRP is created from a whole blood product with virtually all the red blood cells and a large portion of the thin plasma layer separated via centrifugation and then removed. Therefore, the concentration of platelets left and resuspended in the plasma solution is significantly greater than in normal plasma¹¹⁻¹³. This highly concentrated form of platelets provides a dense source of GFs that can be utilized in treating damaged connective tissues like cartilage, but PRP can also be utilized as a tool in the differentiation of stem cells to connective cell lines^{9,12,13,15}. Although, PRP itself is best utilized when it is fresh, certain techniques exist that can be used to activate the platelets and release the GFs in high concentration into the surrounding plasma^{13,15,16}. The PRP can be centrifuged to remove the platelets while the remaining GFs stay in the plasma solution. In this form the plasma can be aliquoted and stored at -80 °C¹⁵. This preparation is much more manageable for experiments and trials with multiple treatment events because blood only needs to be collected from the donor one time.

Cartilage Repair via Transplanted ADSCs and PRP Dual Therapy

Due to the high concentrations of specific growth factors contained in PRP solutions, PRP has shown a positive influence on chondrogenic differentiation in cell culture and animal models^{6,12,15,17-19}. Of PRP's major components, TGF- β is the most potent inducer of chondrogenesis¹⁵ (Figure 1). With regard to transcription of genes known to be associated with chondrogenesis such as SOX9 and aggrecan, TGF- β has been shown to have a greater influence on increasing transcription of these genes than optimal concentrations of the entire spectrum of PRP GFs¹⁵. However, optimal PRP concentrations have been shown to have more influence on the major chondrogenic indicator COL2A1, the gene that is responsible for type-2 collagen production¹⁵.

The other portion of the dual-therapy approach to cartilage repair, ADSCs, have also been implicated as a potential cartilage repair technique for the treatment of osteoarthritis. However, the effects of sole ADSC treatment for cartilage repair in vivo are limited²⁰. Without the use of other treatments in conjugation with ADSCs like PRP, there is an inadequate ability for the ADSCs to incorporate themselves into the damaged tissue or cause the cascade response necessary to facilitate a substantial regeneration event. Moreover, there does appear to be evidence that mesenchymal stem cells, like ADSCs, contain fair amounts of growth influencing molecules. Simply stated, the incorporation of ADSCs into the damaged target tissue might not be the only capability these cells have for assisting in tissue repair^{21,22}.

The most promising method of using any type of mesenchymal stem cell in regenerative therapy for cartilage regeneration is the use of both ADSCs and PRP

collectively to stimulate cartilage repair²⁰ (Figure 2). Moreover, the next step in creating even more efficient treatments that can be utilized in human trials is to provide more streamlined treatment approaches with faster and more substantial recovery. One avenue of attaining this goal would be to introduce molecules and bioactive elements to increase the efficacy of PRP GFs that influence chondrogenesis. Another possibility may be to inhibit certain GFs that in some tissues have major positive effects on tissue repair, but in this specific

tissue could have negative effects on repair. VEGF is a molecule found in PRP that has been implicated as a possible negative influence on chondrogenic differentiation²³. Therefore, it is reasonable that when VEGF-containing PRP is utilized with the ADSC portion of the dual therapy, slight inhibition of the cartilage regeneration may be occurring.

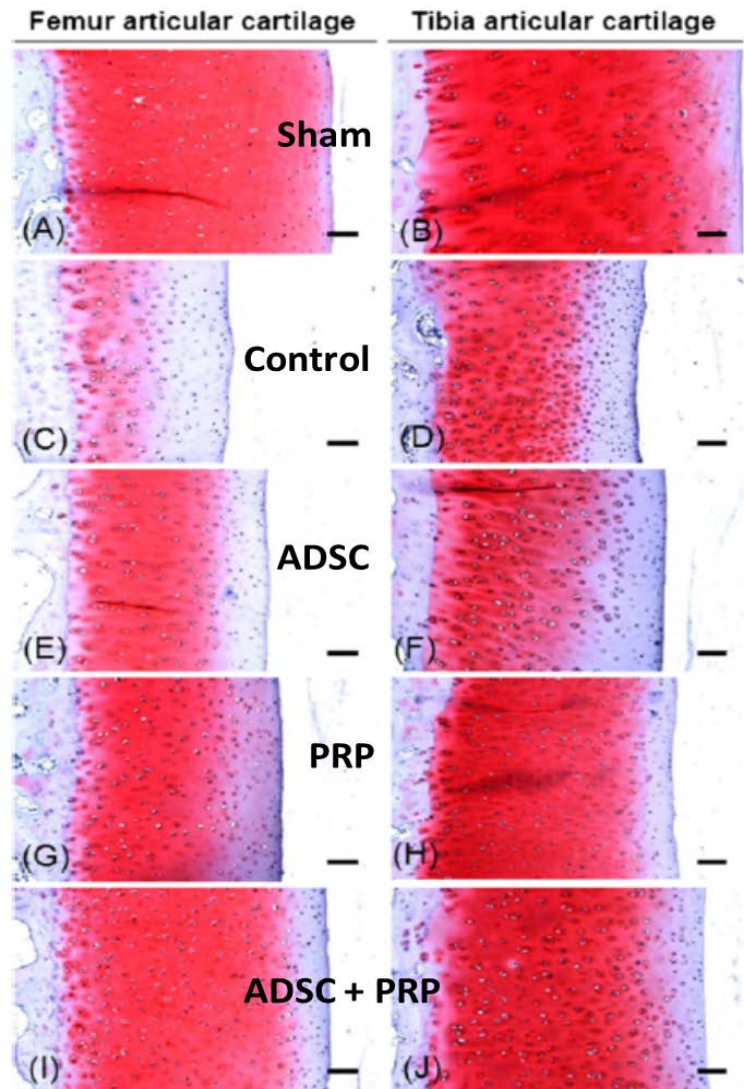


Figure 2. (A-J), Images of articular cartilage regeneration after surgical induction of osteoarthritis and subsequent treatment with specified therapy in canines. Modified from Yun *et al.* (2016).

Optimization of Chondrogenic Potential in MSCs via Growth Factors

VEGF is a potent GF, highly capable of promoting angiogenesis in target tissues^{13,14}. Although increases in vascularization would appear to be beneficial to all tissues with regard to regeneration and growth, cartilage tissue does not seem to fall under that umbrella in all circumstances. When exposed to muscle derived stem cells (MDSCs, a similar type of mesenchymal stem cell) and PRP dual treatment for cartilage regeneration, rats with monoiodoacetate chemically-induced osteoarthritis fared better when they were treated with MDSCs that carried out production of a protein that effectively sequesters VEGF, than when the VEGF-sequestering protein's production had been virtually eliminated⁶. It should also be noted that in the treatments where the MDSCs produced the VEGF-sequestering protein, they were also producing bone morphogenic protein – 4 (BMP-4), which is a known bioactive protein responsible for influencing chondrogenesis⁶.

Soluble Vascular Endothelial Growth Factor – 1 (sVEGFR-1), also referred to as soluble FMS-like Tyrosine Kinase – 1 (sFlt), is a non-membrane-bound receptor of VEGF. This receptor protein is an isoform of endogenous VEGFR-1 that has been post-transcriptionally modified via alternative splicing and does not contain the necessary transmembrane domain required for membrane implantation^{24,25}. Since sFlt is not bound to the membrane, it does not have the capacity for the downstream signaling of the membrane-bound VEGF receptors which would result in angiogenesis²⁴. Therefore, when VEGF is introduced near an sFlt-expressing cell, sFlt effectively sequesters VEGF. As alluded to previously, this sequestration of VEGF when MDSCs are exposed to a chondrogenic medium containing PRP and BMP-4 causes a heightened chondrogenic potential (Figure 3) of the

MDSCs, as evidenced by type-2 collagen production and reduction in chondrocyte apoptosis⁶.

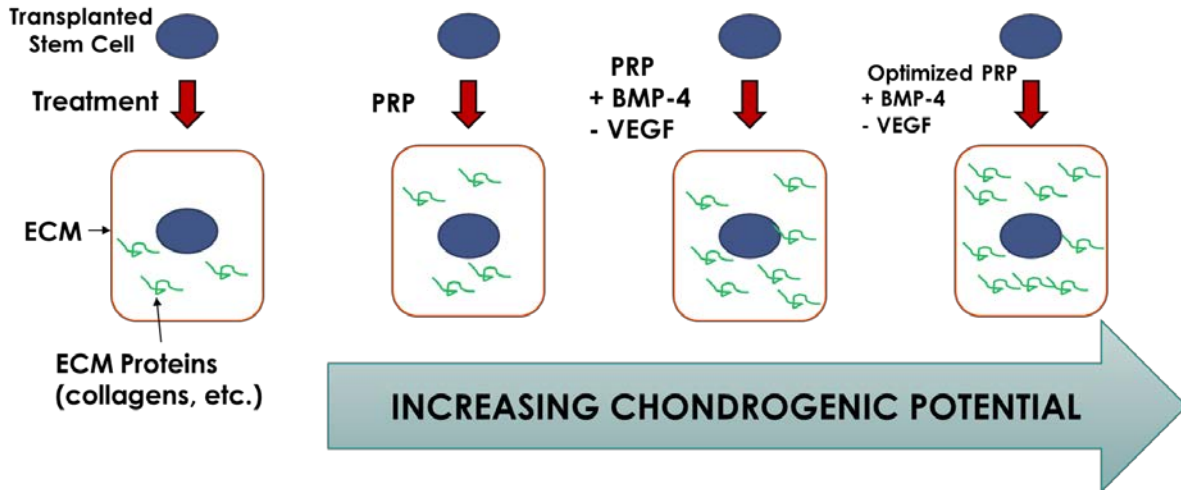


Figure 3. Illustration of different extrinsic factors' influence on chondrogenic potential of transplanted stem cells.

CONCLUSIONS

With an inevitable increase in OA patients suffering from pain and limited mobility, it is necessary to discover more practical cell-based therapy alternatives to invasive surgical techniques for OA patients. Now that current techniques exist to better isolate and preserve mesenchymal stem cells as well as PRP and its growth factors, the necessary components used in these regenerative therapies, the next step is to further optimize these treatments by using other bioactive molecules in tandem with PRP and ADSCs to further augment the dual therapy approach. Previous research has revealed that proteins, such as sFlt and BMP-4 may have the effect of increasing chondrogenic potential of muscle-derived stem cells⁶. Moreover, *in vitro* experimentation using sFlt and BMP-4 to determine the effects of each on chondrogenic potential of donated human ADSCs treated with PRP growth factors would be productive. This approach could result in an improvement of these cell-based regenerative

techniques to a more specific approach and stimulate the attention of research in this field to isolating which GF concentrations are most important for regenerating articular cartilage tissue specifically. If successful, there would be a clinical impact by reducing recovery times and increasing the quantity of cartilage tissue recovered in patients with OA.

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