Chondrogenic Differentiation Potential of Human Adipose-derived Mesenchymal Stem Cells in a Type I Collagen-based Meniscus Scaffold with Activated Platelet-rich Plasma Stimulation *in vitro*

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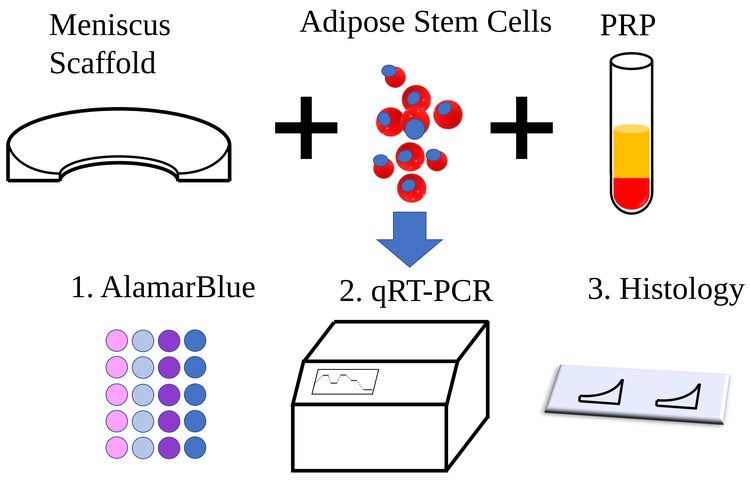
Visual Abstract



Introduction

Despite numerous attempts made in the past using resorbable and non-resorbable scaffolds to treat meniscus tears, minimal progress has been made in guiding and supporting meniscus regeneration and return of function, due primarily to the limited healing capability of the meniscal tissue [1]. In our initial report, we presented design and engineering of a new improved type I collagen-based scaffold [2]. Here we reported our first *in vitro* results, using platelet-rich plasma (PRP) as a source of growth factors to induce chondrogenic differentiation of human adipose derived mesenchymal stem cells (hASC) in this 3D type I collagen-based scaffold.

Materials

- Type I collagen fibers were purified from bovine deep flexile tendon following the procedure of Li and Stone [3].
- Hyaluronic acid (MW ~1.5 MDa) was obtained from LifeCore.
- hASC were obtained from ATCC.
- Human PRP was collected using a PRP kit from RegenLab.

Methods

Scaffold Preparation

Type I collagen-based scaffold was prepared as described previously [2]. Two different densities of scaffolds, high density (HD) and low density (LD) were produced for *in vitro* studies.

hASC and PRP Preparation

hASCs were cultured in the standard culture media per manufacturer's protocol. PRP was collected from human blood and activated according to the manufacturer's protocol.

Cell Culture

Effects of PRP on hASCs proliferation and differentiation into chondrogenic cells were investigated. A total of four groups were studied, LD alone, HD alone, LD+PRP and HD+PRP. 2 × 10^6 hASCs were seeded onto each scaffold (n=5). 1% of PRP was added to the experimental media. Cell viability was measured by alamarBlue assay up to 21 days. qRT-PCR analysis of gene expression was completed with Collagen I and Aggrecan on day 14 and 21. Histological images were obtained by light microscope.

Statistical Analysis The experimental data were analyzed using Analysis of Variance (ANOVA). Statistical significance was set to value ≤ 0.05.

Results

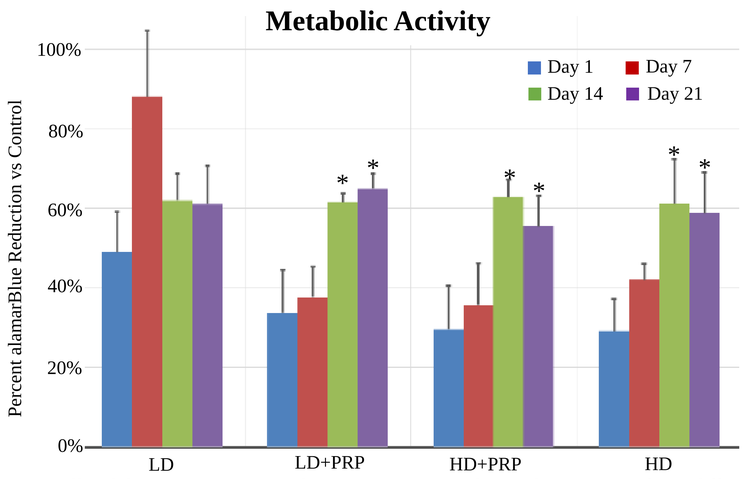


Figure 1. Cell viability. Data are presented as Mean±1 SD. * indicates there is a significant difference (P<0.05).

Fig.1 shows the cell viability for the four groups which shared similar growth kinetics. There is no statistically significant difference (p>0.05) between the groups. However, when compared within the group, day 14 and 21 were significantly higher (p<0.05) than day 1 and 7 except the LD.

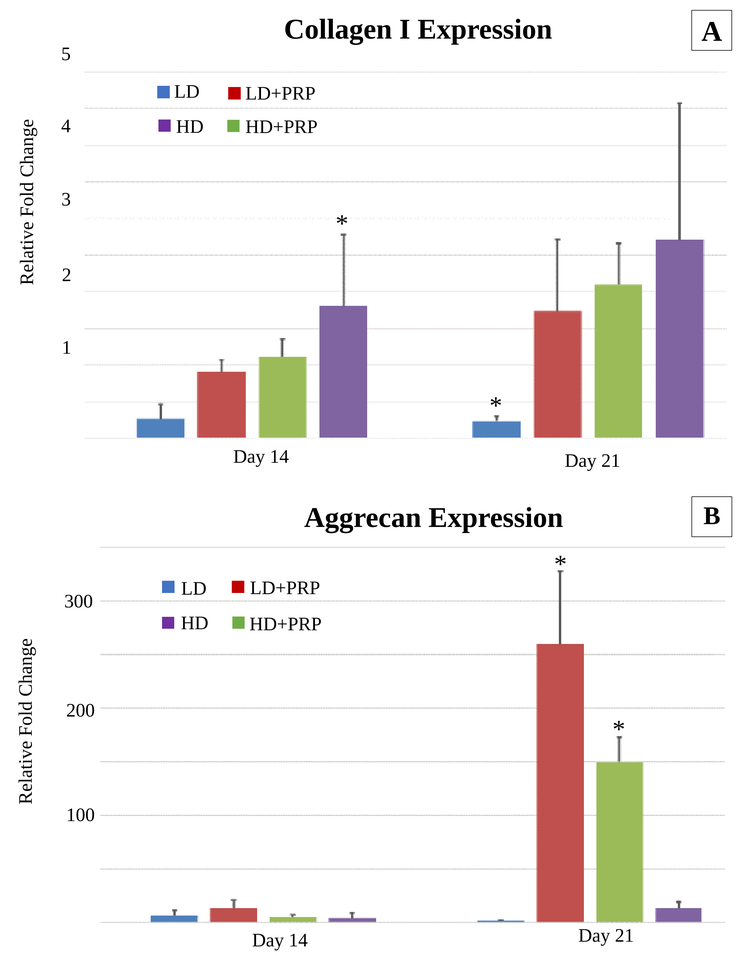


Figure 2. (A) Collagen I gene expression, and (B) Aggrecan gene expression. Data are presented as Mean±1 SD. * indicates there is a significant difference (P<0.05).

The effect of PRP on hASC can be seen in Figs. 2A and 2B. Fig. 2A shows PRP and scaffold density had a positive effect on type I collagen gene expression with time, where the highest effect was observed at day 21. Statistical analysis revealed that the HD at day 14 was significantly higher than the LD (P<0.05) and the LD at day 21 was significantly lower than the rest of the groups (P<0.05).

Fig. 2B shows the effect of PRP on gene expression of aggrecan. It had only a minimal effect at day 14. However, the amount of aggrecan expression was significantly elevated in the presence of PRP by day 21, a sign of chondrogenic differentiation of hASCs. There was a significant difference (P<0.05) when comparing the LD+PRP and the HD+PRP to the LD and the HD.

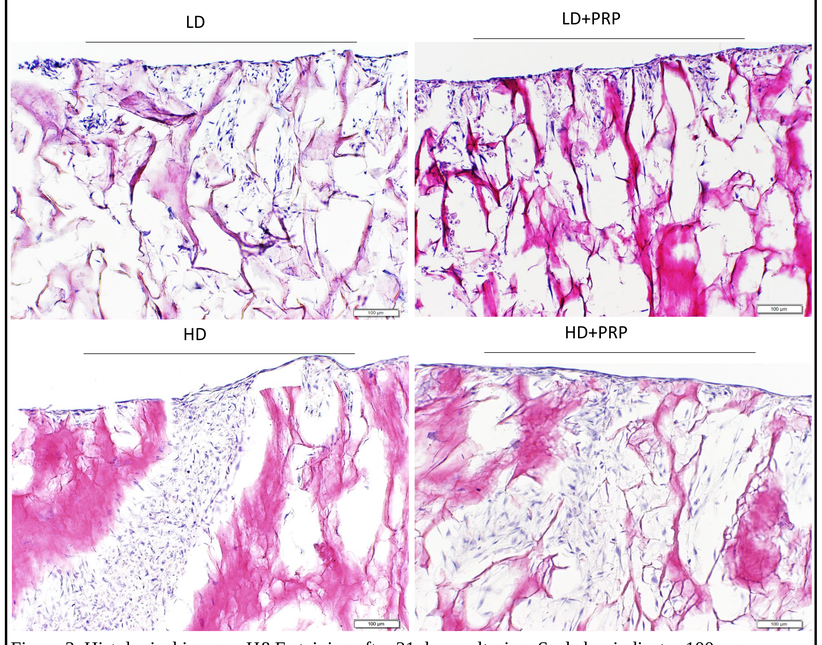


Figure 3. Histological images: H&E staining after 21 days culturing. Scale bar indicates 100 µm.

Fig. 3 shows the H&E staining of the histological slides at day 21. A uniformly dispersed cellular distribution within the interior of the scaffold can be seen in all groups.

Discussions

A properly designed scaffold is critical in tissue engineered implant development. We preferred type I collagen for the scaffold engineering as type I collagen is the major protein in the extracellular space of the meniscus. The type I collagen-based scaffold we designed has the characteristics for tissue engineered meniscus implant development [2]. The current study is to demonstrate the validity of this scaffold for cell-based implant development. We chose hASC as a cell candidate as hASC can be harvested in a large quantity from the fat tissue of the patient without additional culture manipulations. This study shows that PRP can play an important role in directing hASC towards chondrogenic differentiation in type I collagen-based scaffolds *in vitro*. We are currently investigating the effect of biomechanical stress on hASC chondrogenic differentiation in a simulated in vivo dynamic bioreactor system.

Significance

The goal of this research is to advance meniscus repair and regeneration from scaffold approach to tissue engineered implant. Tissue engineered implant can meet the needs of a broader population of meniscus repair and regeneration.

Acknowledgements

The work was supported by the Shu-Tung and Alice Li Foundation.

References

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3. Li, ST and Stone KR, 2000, U.S. Patent No. 6,042,610.