

Study on Prp-Induced Chondrogenesis of Adipose Stem Cells

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Abstract: *Background:* In the field of repair and reconstruction surgery, the use of cartilage is large, but the source of cartilage is small; adipose stem cells come from a wide range of sources and can be induced to differentiate into a variety of cells under specific circumstances. By using platelet-rich plasma to induce differentiation of adipose stem cells into chondrocytes, tissue engineering construction of cartilage will become possible. *Objective:* The optimal culture conditions for PRP-induced differentiation of adipose stem cells into chondrocytes were screened, and REAL-TIME PCR detection of aggrecan and sox-9 were used to confirm that adipose stem cells can be induced by PRP to differentiate into chondrocytes. *Methods:* After isolating and culturing adipose stem cells, use DMEM culture medium containing 0, 5% and 10% PRP to subculture the cells to induce differentiation. The CCK8 kit detects cell proliferation; use DMEM culture medium containing 5% PRP to culture adipose stem cells. Cells were collected on days 3, 7, 14 and 21 of culture, and Real-Time PCR were performed to detect specific antigens aggrecan and sox-9. *Results:* In the DMEM culture medium containing 5% PRP, adipose stem cells grow well and are not prone to aggregation reactions. On the 3rd, 7th, 14th and 21st days of differentiation induced by 5% PRP, Real-Time PCR tests found that aggrecan and sox-9 were continuously expressed, and their expression was found to be progressively enhanced. *Conclusion:* DMEM medium containing 5% PRP is a suitable medium for inducing the differentiation of adipose stem cells into chondrocytes. Adipose stem cells can be used as seed cells to differentiate into chondrocytes after being induced by PRP and have the characteristic antigen expression of chondrocytes.

Keywords: Platelet-Rich Plasma (Prp), Adipose Stem Cells (Adsc), Chondrocytes, Aggrecan, Sox-9

1. Background

The repair of congenital deformities and traumatic defects of the ear and nose has always been a difficult problem for plastic surgery, and the most difficult part is the construction of cartilage [1]. In recent years, with the development of bioengineering technology, cartilage construction has attracted more and more attention, and there are many methods [2-4]. However, so far, there is still no simple, feasible, safe, reliable, and economical method that has been widely used [5].

We have successfully isolated, cultured and identified adipose stem cells in the past. The ability of platelet-rich plasma (PRP) to induce chondrocytes from adipose stem cells is an important factor for the application of adipose

stem cells in cartilage tissue engineering [6, 7]. In this experiment, human adipose stem cells were cultured and transformed into chondrocytes under the induction of PRP, thus providing experimental basis for clinical application in cartilage tissue engineering.

2. Materials and Methods

2.1. Materials

Adipose stem cells (ADSC) were extracted and identified from fat extracted from liposuction patients; PRP collected whole blood from volunteers and collected through PRP kit; fetal bovine serum, low-sugar DMEM, high-glucose DMEM (Gibco, American); tissue cell total RNA extraction reagent Trizol (MRC, American), RT reaction kit, PCR reaction kit

(MBI, American).

2.2. Methods

2.2.1. Isolation and Culture of ADSC

The fat of liposuction patients is fully washed with PBS under sterile conditions, and digested with 0.075% type I collagenase with shaking at 37°C for 40-50 minutes; an equal amount of low-sugar DMEM containing 10% fetal bovine serum is The culture medium was terminated for digestion, filtered through a 200-mesh filter to remove large pieces of undigested fat tissue, centrifuged at 1,300 r/min for 10 minutes, and the supernatant was removed; cells were resuspended in PBS and washed, filtered again through a 200-mesh filter, and centrifuged at 1,300 r/min. min Centrifuge for 10 minutes, remove the supernatant; resuspend the cells in DMEM medium containing 10% fetal bovine serum (containing 100 μ mL penicillin, 100 μ g/mL streptomycin), take samples for trypan blue staining, and count the number of cells. and viability, use the bottom area of 10⁶/100 mm² culture plate to plant viable cells, and culture them at 37°C, carbon dioxide, and saturated humidity. After changing the medium once every 2 to 3 days, the cells proliferate and cover about 80% of the bottom of the culture bottle. When they are close to confluence, they are digested and passaged with 0.25% trypsin.

2.2.2. Chondrogenic Induction of ADSC

Collect 3-passage ADSC, resuspend the cells in low-sugar DMEM medium containing 10% fetal bovine serum, adjust the density to 1.5 \times 10⁷/mL, and add 10 μ l per well in a 24-well culture plate. 0%, 5% and 10% PRP was used as a specific medium for induction culture. Alician Blue staining was used to observe the osteogenic differentiation status of the cells, and the CCK8 cell kit was used to observe the cell proliferation status.

2.2.3. Real-Time PCR Detection

After induction and culture of adipose stem cells for 3 days, 7 days, 14 days and 21 days, Real-Time PCR was used to

detect the expression of aggrecan and Sox9 m RNA genes of ADSCs. Total cellular RNA was extracted using Trizol kit, and cDNA was synthesized using RT kit. Primers were designed using Primer Premier 5.0 and verified by Gene Bank. The primer sequences are: internal reference 18S rRNA (upstream 5'- GACGGACCAGAGCGAAAGC- 3', downstream 5'-CGCCAGTCGGCATCGTTTATG-3'; 119bp); Aggrecan (upstream5'- TGGCTTCCACCAGTGCG -3', downstream 5'-CGGATGCCGTAGGTTCTCA-3'; 127bp); Sox9 (upstream 5'- GTACCCGCACCTGCACAAC - 3', downstream 5'- TCCCGCCTCCTCCACGAAG - 3'; 100 bp). PCR amplification was performed based on the designed primers to synthesize DNA. Reaction conditions: Aggrecan (pre-denaturation at 4°C for 5 min; denaturation at 94°C for 45 s, annealing at 54°C for 45 s, extension at 72°C for 1 min, 28 cycles; further extension at 72°C for 7 min); Sox9 (pre-denaturation at 94°C for 5 min; denaturation at 94°C for 45 s, annealing at 54°C for 45 s, extension at 72°C for 1 min, 32 cycles; then extension at 72°C for 7 min). PCR products were identified by 1.5% agarose gel electrophoresis.

3. Results

3.1. Morphological Characteristics of ADSC After Induction

The morphology of the third generation ADSC was uniform and long spindle shaped. In the DMEM culture medium containing 5% PRP, adipose stem cells grow well and are not prone to aggregation reactions (Figure 1). After 2 days of induction, the cell morphology gradually changed from spindle-shaped to triangular, polygonal, and short spindle-shaped. After 14 days, cell proliferation slowed down significantly, and they grew in aggregates, secreted matrix, and formed nodules, around 21 days, the nodules had a cartilage-like appearance and no longer enlarged.

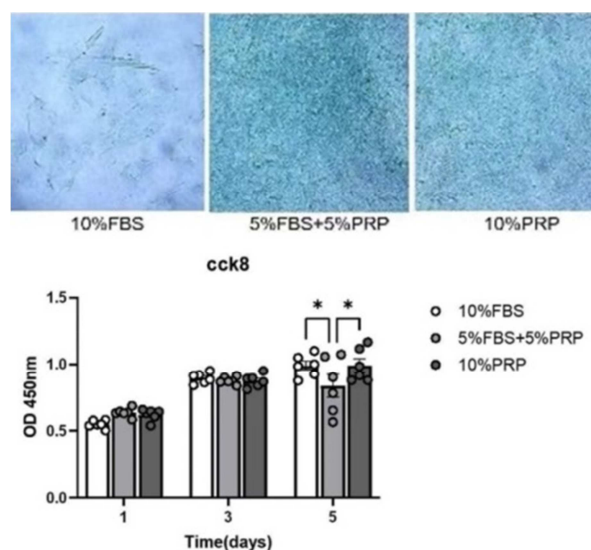


Figure 1. Microscopic view of adipose stem cells cultured under 0%, 5% and 10% PRP respectively (up). CCK8 kit detects cell proliferation on days 1, 3 and of cultured cells with 0%, 5% and 10% PRP respectively (down). It can be seen that 5% PRP can induce the differentiation of adipose stem cells, maintain cell proliferation, and minimize cell coagulation reaction during the culture process.

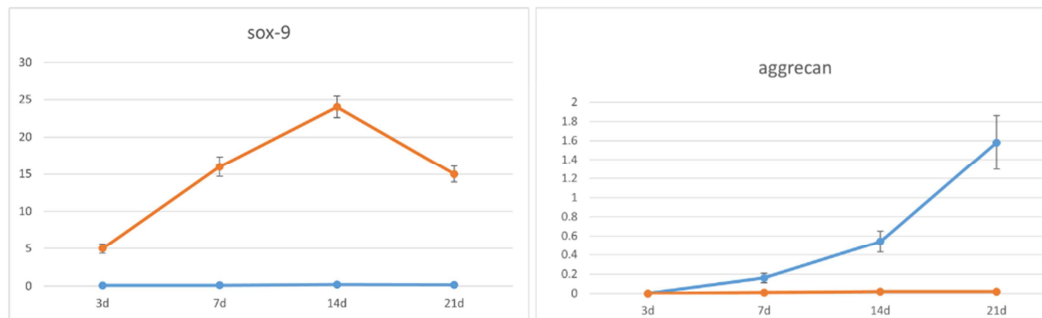


Figure 2. Real-Time PCR detection found that the expression of aggrecan continued to increase on days 3, 7, 14, and 21, and the expression of sox-9 reached a peak on day 14, and the expression began to decrease on day 21 of culture.

3.2. Real-Time PCR Detection After ADSC Induction

On days 3, 7, 14 and 21 of ADSC induction, Real-Time PCR was used to detect the mRNA expression of aggrecan and Sox9 at the gene level. It can be seen that the total RNA expression is complete, and the internal reference β -actin is expressed; when not induced, In the group, no mRNA expression bands of aggrecan and Sox9 were found. The mRNA expression of aggrecan and Sox9 in the induced group (Figure 2).

4. Discussion

Cartilage tissue engineering combines cell biology, engineering, materials science and surgery to reconstruct new functional tissues and lay the foundation for cartilage-like engineered bone reconstruction to obtain a stable source of available engineered cartilage [8]. Most research on cartilage tissue engineering seed cells focuses on chondrocytes and bone marrow mesenchymal stem cells. In recent years, with the in-depth research on ADSCs, there are more and more studies on ADSCs as cartilage tissue engineering seed cells [9]. Preliminary studies have shown that under specific culture conditions in vitro, ADSCs can express the phenotypes of osteoblasts, nerve cells, muscle cells, adipocytes, and chondrocytes, and therefore have the potential to become stable and reliable seed cells [10]. ADSCs are relatively simple to obtain and are abundant in number, making them ideal seed cells [11]. This experiment refers to recent culture conditions for the differentiation of adipose mesenchymal stem cells into chondrocytes, using fetal bovine serum containing 5% PRP as an inducer. This induction may be through various growth factors in PRP, such as TGF- β growth factor, platelet-derived growth factor, insulin-like growth factor, etc. are achieved by promoting the expression of chondrocytes [12].

Aggrecan and Sox-9 are the most specific surface markers of chondrocytes and therefore are the most important indicators for detecting chondrocyte differentiation and formation [13, 14]. By comparing the expression levels of the two markers at different time points in the ADSC group and the induction group, we can know that PRP can effectively induce the differentiation of adipose stem cells into chondrocytes, and the differentiated cells can express specific

antigens of chondrocytes. Adipose stem cells It is feasible for seed cells to be induced by PRP to differentiate into chondrocytes [15].

5. Conclusion

DMEM medium containing 5% PRP is a suitable medium for inducing the differentiation of adipose stem cells into chondrocytes. Aggrecan and Sox-9 can be expressed in adipose stem cell differentiated cells induced by PRP, and their expression gradually increases over time, indicating that adipose stem cells can be induced into chondrocytes by PRP. Adipose stem cells can be used as seed cells to differentiate into chondrocytes after being induced by PRP and have the characteristic antigen expression of chondrocytes.

Ethical Approval

The study was approved by the Yangzhong People's Hospital Institutional Review Board (202141).

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