

Injectable composite of calcium alginate hydrogel and Adipose-derived Stem Cells to remediate bone defect

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Abstract. To investigate the feasibility of injectable composite of calcium alginate hydrogel and adipose-derived stem cells(ASCs) to remediate bone defect, the experiment was carried out. ASCs from New Zealand rabbit cultured in vitro were mixed with 1.5% sodium alginate and calcium gluconate, preparing the calcium alginate hydrogel with cell density $5 \times 10^9/L$. The cell growth curve was used to determine the biocompatibility of ASCs and hydrogels. The mixture was injected into the radius defect area of the New Zealand rabbits(group A); the calcium alginate hydrogel without cells was injected into the same area of other rabbits(group B). All rabbits were examined with radiodetection and bone mineral density(BMD) in the 12 postoperative weeks. Radiological assessment showed that 12 weeks after injection, the bone formation was obvious in experimental group A. In group B, a small number of new bone appeared at both ends, with the defect still visible. In group C, no bone formation was found. It also indicated that from 4 to 12 weeks post-surgery, the average BMD value of group A was higher than group B and group C($P < 0.01$). Percutaneous injection of calcium alginate hydrogel and ASCs composites can help to remediate bone defect in animals.

1. Introduction

In this era, diversified modern life brings more personal medical problems. For example, accidental injury of high speed vehicle results in a variety of bone defects[1]. In the 1990s researchers put forward the use of tissue engineering bone for complement as a new way of biological repair[2]. There are many choices of seed cells in tissue engineering. The Adipose Derived Stem Cells(ASCs) are the advantageous one. A systematic study involving more than 1,400 patients who were treated with ASCs therapy had shown a safety profile[3]. The implanted seed cells need suitable carriers. Studies have shown that injectable tissue materials can repair tissue defects through micro-trauma and have wide applicability in therapeutics[4]. This combination of ASCs with carrier has more accurate and reliable osteogenic effect and technical feasibility than the pure cell by percutaneous injection. Sodium alginate molecules crosslinked with bivalent cation can be changed from liquid to gel state, the ASCs in calcium alginate hydrogel composite could be injected percutaneously to study its osteogenesis.

2. Methods

2.1. Separation, culture and Osteogenic induction of ASCs

2-month-old male New Zealand rabbits($n=22$) were housed in standardized animal center of Changsha Medical University with free access to food and water. Adipose tissue cells were isolated from 4 rabbits. The methods of separating ASCs are mainly based on the correlational study[6,7]. The cells



were resuspended in DMEM/F12 medium containing 10% newborn bovine serum. After 24 h, the unattached cells were removed. Cell surface antigen profile was evaluated by flow cytometry. ASCs were induced using DMEM/F12 supplemented with 1 nM dexamethasone, 2 mM β -glycerolphosphate and 50 μ M ascorbate-2-phosphate 14 days. This part of the experiment had been completed in the early stage, and the data is not listed in this paper [5].

2.2. Calcium alginate hydrogel mixed with ASCs

The sodium alginate dry powder was prepared to be 1.5% solution. After trypsinization, ASCs of the 3rd generation were centrifuged and counted to adjust the density to 5×10^9 /L before fixed with the sodium alginate solution. Calcium gluconate was added to the solution with calcium ion concentration of 50 mmol/L, and the calcium alginate hydrogel with cell density 5×10^9 /L were formed.

2.3. Growth of ASCs with calcium alginate hydrogel

The cells were inoculated with the composite materials at density of 5×10^6 /ml in the CO₂ culture box in experimental group. The control group was set without calcium alginate hydrogel. The ASCs in 4 wells were rinsed with PBS, digested by pancreatic enzyme and collected respectively. The numbers of ASCs were counted after trypan-blue staining to draw the growth curve within 14d (n=4).

2.4. Radial defect model Preparation and composite injected

10mm long radial defects were created surgically in the rabbits. After surgery, the rabbits were given 20,000 U/d gentamycin for 3 days and housed separately. 3 days after the operation, the rabbits were divided into three groups as follows (n=6): the radial defect was injected with 1ml calcium alginate hydrogel fixed with ASCs (group A); the bone defect injected with 1ml calcium alginate hydrogel only as the negative control group (group B); the defect without treatment served as the blank control group (group C).

2.5. Radiological assessment

Just after surgery and 12 weeks after surgery, the rabbits were anesthetized. The front and lateral radiographs were taken to observe the repair of bone defect area on DR scan bed. The bone mineral density (BMD) of the defect was measured by GE Lunar Prodigy Primo after radiological assessment at 4, 8, 12 weeks.

2.6. Data analysis

SPSS 10.0 software was used to analyze the data. Differences between two groups were estimated by Student's t-test; differences among three groups were determined by one-way ANOVA, $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Growth curve of ASCs

Through cell counting, the number of living ASCs was counted from the beginning of subculture to the 14th day to plot the growth curve. In experimental group and control group showed similar growth trend. The number of cells in experimental group continued to grow, reaching the peak on the 10th day, and the number was slightly less than that of the control group. In the control group, the maximum was reached in the 9th day, and there was no significant decrease between 9 and 14 days. The difference between these two groups was not significant ($P > 0.05$), suggesting that calcium alginate hydrogel had no significant effect on ASCs proliferation.

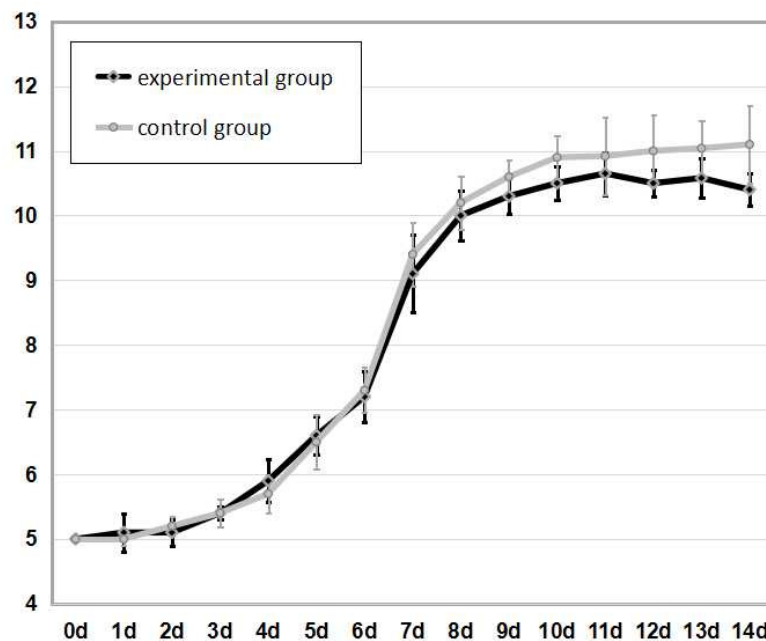


Fig.1 Growth curve of ASCs with calcium alginate hydrogel ($\times 10^6/\text{ml}$, $n=4$)

3.2. Radiological examinations

To compare which method yielded best performance in vivo, the bone repair on rabbits model was imaged by DR scan bed. The group A showed promising improvement during the healing process, the density of bone callus was increased significantly, and the bones were well connected. The bone defects were almost impossible to detect 12 weeks post-surgery. In group B, a small number of new bone appeared at both ends, with the defect still visible. In group C, there was no significant improvement in the bone outline whereas the bone defects were still obvious at 12 weeks post-surgery. (Fig.2)

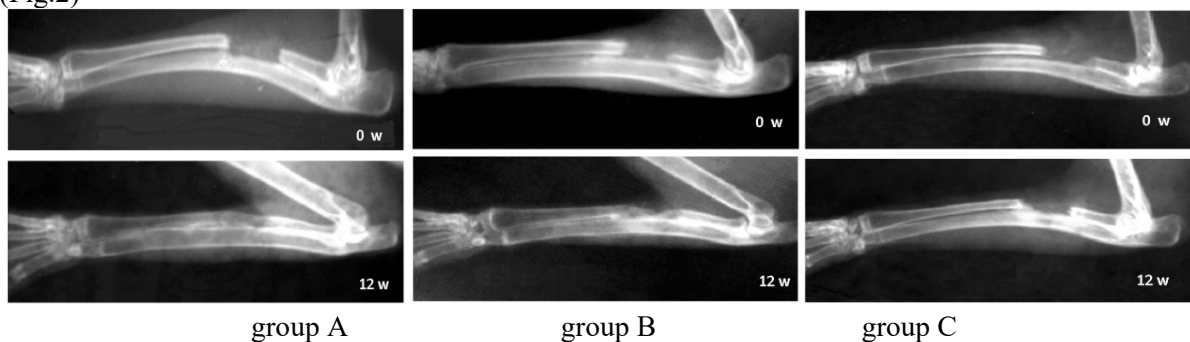


Fig.2 Radiographic examination of repair of bone defects

3.3. Bone density calculation

At 0, 4, 8, 12 weeks, the BMD of the defect were measured. The BMD of group A increased with time. From 4 to 12 weeks post-surgery, the average BMD value of group A was higher than group B and group C, and the differences were statistically significant ($P < 0.01$). At 12 weeks, The BMD value of group B was higher than group C ($P < 0.05$), too. The results showed that, comparing the repair effect, the calcium alginate hydrogel binding ASCs in group A was better than the scaffold in group B, and filling the defect with the hydrogel was superior to using no hydrogel. The calcium alginate hydrogel

had slight osteoconduction effects and could help partial bone formation in 12 weeks, even without ASCs. (Fig.3)

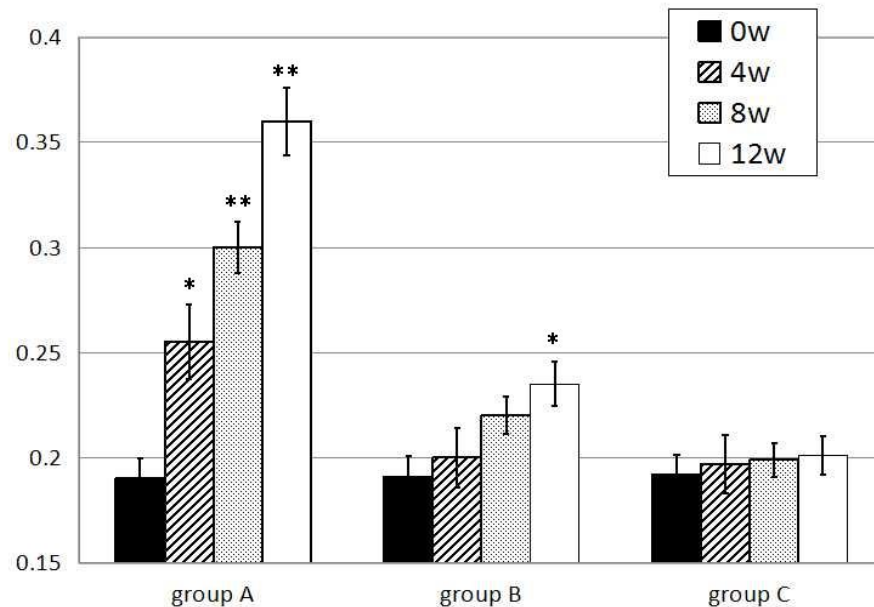


Fig.3 BMD score to assess the new bone formed of the groups(g/cm², n=6)
Compared to the control group C: *: $P < 0.05$, **: $P < 0.01$

4. Discussion

The differentiable stem cell and effective stents transplantation is a new option to treat bone defects. Without cell carriers, the implanted cells spread out easily, so it is hard to reach effective cell concentration at the implant site[6]. Suitable cell carriers materials can provide the cells with growth and reproduction space for bone tissue regeneration[7]. Now, biomaterials are the focus of research. Alginate is a kind of polysaccharide with bivalent anions extracted from seaweed. Its relative molecular mass is between 5000 and 15000, crosslinking with divalent cations calcium ions to form a three-dimensional network structure of calcium alginate hydrogel. Calcium alginate hydrogel has a good biocompatibility, hydrophilicity and shaping capability, is easy to make and maintains a different shape, and has been approved to be used in drug and food industry by the FDA[8]. By scanning electron microscope researchers found that calcium alginate hydrogel has a large number of the uniform mesh pore structure, 40 ~ 160 μm in diameter. It can act as adhesion stents for seed cells to grow.

ASCs are superstars in seed cells because they are undifferentiated precursor cells and their cell phenotype differentiation is not mature, allotransplantation rejection of is weak. MSCs from bone marrow were found that they not only provided osteogenic cells necessary for bone formation, but also secreted growth factors that promoted the repair of defects[9]. It is not very clear whether ASCs have the same function in repairing defects. So in this experiment, we used the conventional surgical procedure to acquire the fat from the donor rabbits, and the separated ASCs were fixed with calcium alginate hydrogel to form tissue engineering bone, and then the injectable composites were used to repair radial bone defect. The results showed that calcium alginate hydrogel fixed with ASCs had certain effect on bone regeneration. This finding will lay the foundation for ASCs as candidate of the seed cell of bone tissue engineering.

Calcium alginate hydrogel has strong plasticity, but it is too soft and has poor stereotyping ability. When it repairs bone defect, abnormal shape bone may be formed by external force. There are two cases of this in our experimental rabbits. In addition to that, long-term effects of allogeneic ASCs for

repair bone defect is uncertain, and the secretory properties of ASCs have not been studied, further exploration is needed.

5. Conclusions

In this experiment, the injectable composite of allogeneic ASCs and Calcium alginate hydrogel had osteogenic ability and were used to repair the segmental bone defect successfully. It is promising to serve as grafting material in bone tissue engineering.

Acknowledgments

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