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EFFECT OF INJECTABLE ALPHA CALCIUM SULFATE HEMIHYDRATE FOR REPAIR OF HILL-SACHS LESION IN A RABBIT MODEL

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Introduction

- Hill-Sachs lesion is located on the humeral head as a result of shoulder dislocation and impaction against the glenoid rim in subglenoid region.
- Repair of Hill-Sachs lesion requires open surgery from posterior approach, making the operation difficult.
- When Hill-Sachs lesion occupies 20-25% of area of the humeral head, engaging dislocation may often occur that both of the bone defect and the torn soft tissue should be repaired for shoulder stability recovery.
- Considering being accessible in arthroscopic surgery, the injectable material could be an immediate solution for repair of the bone defect.
- This study investigated the reparation in Hill-Sachs defect by injectable bilayer material of alpha calcium sulfate hemihydrate with 1% hydrochloric acid/15% alginate (αCSH+1%HCl/15% alginate).
- No injectable materials with proper physical and biological properties have been proposed to repair the Hill-Sachs lesion nor the established animal model.
- In this study, we set up an animal model of Hill-Sachs lesion on mature rabbits and hypothesized the novel αCSH+1%HCl/15% alginate injectable material can be applied directly on the Hill-Sachs lesion through arthroscopy in the future.

Materials and Methods

Material preparation

- Alpha calcium sulfate hemihydrate (αCSH) was produced by calcium sulfate dihydrate powder (CSD, J.T. Backer, Center Valley, PA). The alginate (Sigma-Aldrich, St. Louis, MO) was prepared from powders dissolved in water in a proportion of 15% wt. The testing samples were hardened by mixing the powders of αCSH with 1% HCl solution at a ratio of 1:1.

Material characterization

- Compressive strength
  The compressive strength of αCSH+1%HCl was tested by the materials testing system (MTS) with ASTM 451-99a.
- Structure and Morphology
  SEM (Hitachi S4100 Field Emission SEM, Hitachi, Japan) was used to observe the powder crystallization and cell attachment.
- Degradation test
  We soaked the specimens into 5 ml of pH 7.2 Hanks Balanced Salt Solution (HBSS, Gibco, Waltham, MA).

In vitro/ex vivo test

- Cell viability assay
  Human osteosarcoma cells (MG-63) were used for the in vitro biocompatibility test. MTS assay (Promega, Fitchburg, WI) was used to evaluate the cell viability of αCSH powders and αCSH+1%HCl cement.
- Cell adhesion assay
  The adhesion force between the material and bone in Hill Sachs defect was measured by push-out.

In vivo test

- Surgery procedure
  24 male adult New Zealand white were used. The area of the defect occupied 25% of the humeral head and a hole with 7.5 mm in diameter and 5 mm in depth was drilled. (Figure 1) Sterilized 15% alginate was then smeared at the bottom of the defect to secure the injected material. The mixture of αCSH+1%HCl was then injected into the defect site. Animals were sacrificed at 4 (N=6) and 12 (N=6) weeks and observed with CT and histological analyses.
- Micro-Computed Tomography (Micro-CT)
  After 4 and 12 weeks, the humerus from sacrificed rabbits was fixed in 10% formalin and scanned with micro-CT (SkyScan 1076, Bruker, Kontich, Belgium) to evaluate the degree of bone remodeling.

Results

- Morphology and mechanical properties of αCSH+1%HCl
  Crystals with needle-like structure had a size of 17.3±12.41 μm in width and 92.02±263 μm in length. (Figure 2A-2/3) The compressive strength of αCSH+1%HCl was 9.62±2.05 Mpa with 47% of porosity. (Figure 2B-2/3)
- Degradation of αCSH+1%HCl
  The pH value of the immersed solution with αCSH+1%HCl gradually dropped to 5.5-5.8. (N=6) (Figure 3A) The sponge-like structure of the αCSH+1%HCl did not conspicuously change in appearance during degradation. (Figure 3B)
- Cell viability assay
  The number of viable cells of αCSH+1%HCl and 15% alginate were close to the control at 1 and 3 days of culture. After 5 days of culture, the number of viable cells increased in αCSH+1%HCl. (N=6) (Figure 4A)
- Cells adhesion assay
  The αCSH+1%HCl and 15% alginate group showed no significant difference in cell morphology. (Figure 4C) Cells attached successfully and stretched out on αCSH+1%HCl scaffold after day 1. (Figure 4D-1) Cells spread on the surface and filled in the pores of the scaffold after day 3. (Figure 4D-2) Cells flattened into spindle shape with abundant filopodia and migrated into the center of the scaffold on day 5. (Figur4E 4D-3-4)

Histology

Samples were stained with hematoxylin and eosin (H&E) and Masson’s trichrome. Figure 1
Micro-computed tomography (Micro-CT)
2D transverse images were collected from the defect. (Figure 5A-1 to A-5) Bone volume per tissue volume (BV/TV) was 5.30% without injected materials after 4 weeks whereas BV/TV was significantly higher of 21.52% with αCSH+1% HCl/15% alginate injected after 4 weeks. (Figure 5C) 3D images showed better bone remodeling of the material group at 4 and 12 weeks compared to the control group. (Figure 5B-3/B-5) Figure 5

Hematoxylin and eosin stain
Osseointegration between material and bone defect begun after 4 weeks of material injection. (N=6) (Figure 6B) In contrast, the defect group (N=6) (Figure 6A) showed no bone formation and the defect still clearly existed. Figure 6

Masson’s trichrome stain
No collagen responses of bone formation in the defect group after 4 weeks. (Figure 7A) Collagen formation was observed at the edge of the defect, indicating the material had been resorbed and plenty of ostroblasts had migrated into the material after 4 weeks. (Figure 7B) The material was completely absorbed and the mature trabeculae had formed already in the material group compared to the control. (Figure 7C) Figure 7

Cadaver experiment
Arthroscopic images of (Figure 8A) Hill-Sachs defect created on the humeral head, (Figure 8B) the material injected in the defect and (Figure 8C) the defect covered by the material completely. (D=defect, C=αCSH+1% HCl, A=15% alginate) Figure 8

Discussion
Poly(methyl methacrylate) (PMMA) is a safe and effective bone filling material. However, exothermicity of PMMA might damage the surrounding tissue and the short working time is difficult to form the shape.

Studies have shown that αCSH is a bioactive material capable of osteoconducting and osteoinducting.

The working time for αSH+1% HCl is 10-14 minutes which is adequate for forming shape of material and clinically practical.

The combination of 15% alginate and αCSH+1% HCl is a bilayer structure promoting cell adhesion and enhancing the bonding strength between the material and defect.

Results of micro-CT analysis and histology demonstrated the defect had been repaired after 12 weeks.

Currently, there are still no animal models for Hill-Sachs lesion repair. In this study, 25% of the defect area is in compliance with clinical situation that causes the recurrent dislocation.

This is the first study to establish and investigate the animal model with the injectable material of αCSH+1% HCl/15% alginate applied to Hill-Sachs lesion repair.

Conclusions
We established the animal model of the injected αCSH+1% HCl/15% alginate to repair Hill-Sachs lesion in which the defect was repaired with favorable healing after 12 weeks. Moreover, αCSH+1% HCl/15% alginate has moderate hardening time which is promising for use under arthroscopy to treat Hill-Sachs lesion.