

## Effect of Composite Gel Cryoprotectant on Cryopreservation of Cartilage Tissues during Biological Reconstruction with Frozen Osteochondral Autograft

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### **Background:**

Biological reconstruction with frozen osteochondral autograft is a reliable method for high-grade bone malignancy around the joint. However, the long-term complication of cartilage deterioration limited the clinical application. Sustainable integrity of cryopreserved cartilage determines the prognosis of reconstruction surgery. However, the most common DMSO-based cryoprotectants in liquid phase do not sustain the preserved tissues from cryogenic damage during the surgery. We developed as innovated composite gel cryoprotectant (CGC) in gel phase with high adhesive and hydrophilic properties.

### **Questions/Purposes:**

This study aimed to evaluate the cryoprotective effectiveness of CGC on liquid nitrogen-treated cartilage tissues.

### **Patients and Method:**

Fresh specimens of articular cartilage cancellous bone of condyle were harvested from distal femur of the rates. Three groups, control group (without cryoprotectant), DMSO(10% DMSO)-embedded group, and CGC-embedded group, of cylindrical tissues in a dimension of 5×5×2mm were investigated. The studied cartilage tissues were treated in liquid nitrogen for 20 minutes followed by 15-minute thaw at room temperature and subsequent irrigation with normal saline for 15 minutes. Standard histological microscopy (HE staining), TUNEL assay, western blotting analysis and colorimetric were applied to examine the cryoablation-mediated apoptosis of cartilage cells.

### **Results:**

In comparison with the controlled and DMSO group, the results of HE staining revealed higher counts in viable cells among the CGC group. Roughing of the cartilage surface were found in control and DMSO group. Tear between the cartilage layers could be seen after cryoablation in all groups, the distance were  $2.1\pm 0.5$  mm in control group,  $1.8\pm 0.8$  mm in DMSO group and  $0.45\pm 0.2$  mm in CGC group. ( $p<0.01$ ) Quantitation of the fragmented DNA in TUNEL assay suggested that liquid nitrogen induced cell death. Significantly lower apoptotic rates in the composite gel group (100% in control group, 87% in DMSO group and 23% in composite gel group,  $p<0.01$ ) were detected by TUNEL assays. Results of western blotting indicated that apoptosis-mediating molecules, including caspase-9 and the cleaved caspase-3, were decreased after the cryogenic treatment. By colorimetric grey scales, the level of cartilage luminance decrease after cryoablation was  $4.8\pm 0.2$  in control group,  $5.1\pm 0.3$  in DMSO group and  $0.7\pm 0.3$  in CGC group ( $p<0.01$ ).

**Conclusions:**

We demonstrated the evidence on supporting that composite gels could protect cartilage grafts from cryogenic apoptosis mediated by liquid nitrogen-cryoablation. Compared with liquid phase DMSO, composite gel cryoprotectant could stick to the cartilage surface firmly and provide certain degree of insulation. The application of composite gel during frozen osteochondral autograft in rates model could protect innocent cartilage tissue. Future clinical trial to decrease the complication of cartilage deterioration should be performed based on current result.

Figure 1. Photography of Composite Gel Cryoprotectant (CGC). CGC could stick to the cartilage surface firmly and provide certain degree of insulation during cryosurgery.



Figure 2 . HE staining revealed higher counts in viable cells among the CGC group. (\*) and roughing of the cartilage surface were found in control and DMSO group. (black arrow)

