New Technique for Preparing Cartilage for Intracordal Injection: The Freezing and Grinding Method

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Summary: Objectives/Hypothesis. We developed a technique for preparing harvested cartilage that creates finer, more uniform pieces by freezing with liquid nitrogen and grinding with a mortar and pestle. Herein, we report the application of this new technique for intracordal cartilage injection.
Study Design. Experimental study.
Methods. Human cartilage was obtained from surgical cases. In the standard method, harvested cartilage was prepared with scissors and a knife. In the experimental group, harvested cartilage was frozen with liquid nitrogen and ground with a mortar and pestle.
Results. It took an average of 60 minutes to manipulate cartilage using the standard technique, whereas it took an average of 10 minutes using the freezing and grinding method ($P < 0.001$). The average size of cartilage flakes generated by the standard and new techniques were 727 and 48.6 μm, respectively. The cartilage flakes produced using scissors and a knife were able to pass through a 19-gauge needle, whereas those created using the freezing and grinding method were able to pass through a 24-gauge needle.
Conclusions. Using the freezing and grinding method, cartilage was broken into fine, uniform pieces that could pass through a 24-gauge needle. This new technique will facilitate the production of commercial cartilage material for intracordal injection.
Key Words: Injection laryngoplasty–Intracordal cartilage injection.

INTRODUCTION

Surgical treatments of unilateral vocal fold paralysis include injection laryngoplasty, medialization laryngoplasty, arytenoid adduction, and nerve reinnervation. Injection laryngoplasty corrects the glottic gap by medializing the diseased vocal fold using various materials and is less of a burden to patients compared with other surgical procedures. The ideal material for injection laryngoplasty should be easy to inject, should not be absorbed or degenerated after transplantation, should not trigger an immune response, such as a foreign body reaction.

With the increasing interest in autologous implants, materials such as fat, collagen, fascia, and cartilage have been used for injection laryngoplasty.[1–3] Of these, cartilage has lower absorption rates after implantation in the human body.[4] Lee et al performed intracordal injection with autologous cartilage in a canine paralyzed vocal fold model and proved that injected autologous cartilage remained in the vocalis muscle for 3 years.[5] Subsequently, they reported that intracordal cartilage injections using allogeneic cartilage in a canine paralyzed vocal fold model could be used to correct a glottic gap.[6] As described above, although autologous and allogenic cartilages are effective for volumetric augmentation, injection laryngoplasty with cartilage has several limitations. Using a knife and scissors, it takes a long time to prepare small, uniform pieces of harvested cartilage. In addition, it is difficult to make cartilage flakes that are small enough to pass through a needle smaller than 19 gauge. Consequently, intracordal cartilage injection could not be performed under local anesthesia, as injection laryngoplasty requires a small needle.[7,8] Accordingly, a simple technique was required to prepare cartilage pieces small enough to pass through a 19-gauge needle. To meet this need, we developed a technique for preparing harvested cartilage for intracordal cartilage injection using freezing with liquid nitrogen and grinding with a mortar and pestle to create finer, more uniform pieces than the standard method.

MATERIALS AND METHODS

Preparation of harvested cartilage

After the Pusan National University School of Medicine Review Board approved this study, human nasal septal cartilage was obtained from surgical cases. Two methods were used to convert the harvested nasal septal cartilage into injectable cartilage flakes. In the control group, the harvested cartilage was manipulated in the standard fashion using scissors and a knife.[6,7] In the experimental group, harvested cartilage was prepared using our freezing and grinding technique. The harvested cartilages were placed in a mortar, frozen using liquid nitrogen, and then ground manually with a pestle (Figure 1). Then, the obtained cartilage flakes were passed through a 150-μm strainer (Figure 2). We measured the size of the flakes and evaluated their ability to pass through different sized needles.

Outcome measurement

For both methods, the time required to prepare the cartilage flakes was measured and the size of cartilage flakes was measured using 200-fold magnification. After hematoxylin and

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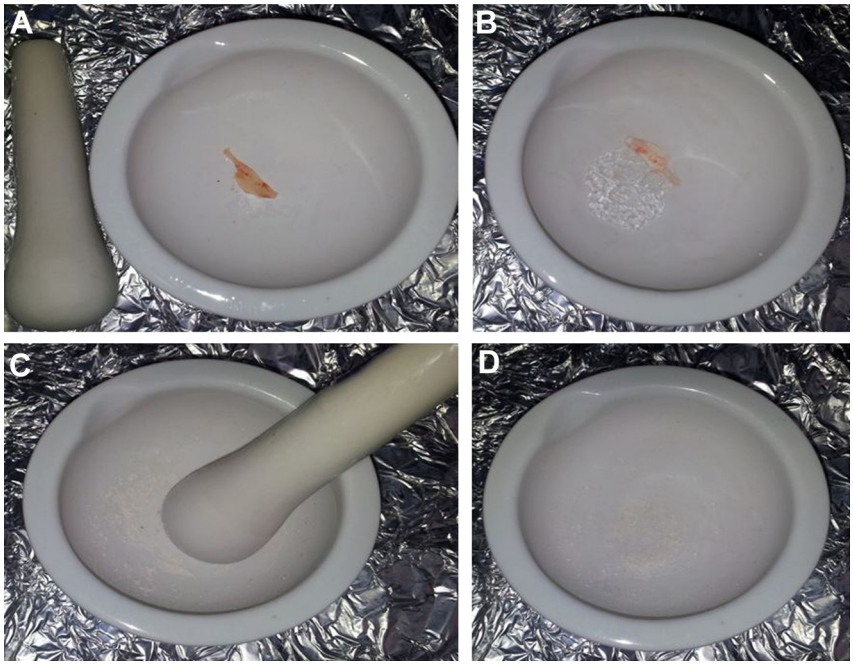

FIGURE 1. The freezing and grinding method. (**A** and **B**) Harvested cartilage was placed in the mortar and frozen in liquid nitrogen. (**C** and **D**) After grinding the frozen cartilage using a pestle, fine, uniform pieces of cartilage were obtained.

eosin staining, the cartilage flakes were examined cytologically. Finally, the flow properties of the cartilage flakes were assessed by attempting to pass through the needles of different sizes.

RESULTS

Twenty nasal septal cartilages were obtained from surgical cases for this study. It took an average of 60 minutes to manipulate the cartilage using the standard preparation technique with scissors and a knife, whereas it took an average of 10 minutes using freezing and grinding ($P < 0.05$). Because nasal septal cartilage is hyaline cartilage, it is difficult to make it into fine, uniform cartilage flakes. During the preparation process using a scissors and a knife, cartilage pieces were lost because their rigidity and stiffness made them difficult to handle. The size of the cartilage flakes produced using both techniques was measured under ×200 magnification (Figure 3). The average size of cartilage flakes created using the standard technique was 727 versus 48.6 μm with the freezing and grinding method. The difference was significant ($P < 0.05$). The freezing and grinding method created finer pieces than the standard technique (Table 1).

Flow properties (needle passing test)

Quantifying the flow properties, cartilage flakes created with the standard technique could pass through a 19-gauge needle, whereas flakes produced using our freezing and grinding method easily passed through a 24-gauge needle (Table 2). As the largest piece produced by the freezing and grinding technique was 220 μm, a blockage formed when attempting to inject these through a 25-gauge needle. Although irregularly shaped, narrow, but long (220 μm) pieces of cartilage could pass through the 150-μm strainer, they failed to pass through a 25-gauge needle due to their length.

Histologic finding

The cartilage flakes were observed under ×200 magnification after hematoxylin and eosin staining. The flakes produced using the freezing and grinding method were finer and more uniform

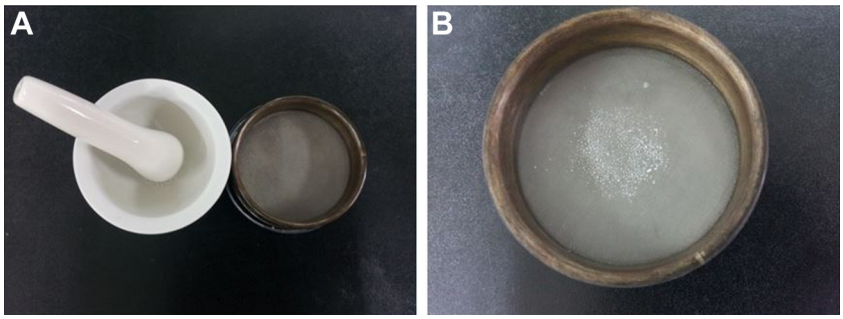

FIGURE 2. After freezing and grinding (A), the obtained cartilage flakes were passed through a 150-μm strainer to harvest the finest flakes (B).