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Use of hyaluronic acid matrix in dorsal augmentation rhinoplasty

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ABSTRACT

Augmentation rhinoplasty sometimes is required for patients with saddle nose deformity caused by failed rhinoplasty or facial trauma; finding appropriate grafting material remains a significant problem for this procedure. We investigated hyaluronic acid matrix as an allograft for dorsal augmentation rhinoplasty in a rabbit model. We performed an osteotomy on the nasal bones of eight rabbits. Four animals were sham operated as the control group and four were administered a mixture of saline-gelled hyaluronic acid matrix and sliced cartilage. Ultrasonography and three-dimensional reconstruction tomography were performed at the end of the experimental period. After sacrifice of the animals, nasal tissues were examined for histopathology, and both collagen scores and number of capillaries were compared between the two groups. Increased collagen and capillaries were apparent in the hyaluronic acid matrix group compared to controls. The median collagen score was significantly greater for the hyaluronic acid matrix group than for the control group. Although the number of capillaries for the hyaluronic acid matrix group was greater than for the control group, the difference was not statistically significant. Three weeks is sufficient for adhesion of ends of fractures in clinical practice; however, we found no ossification at this time in either group. A hyaluronic acid matrix may be a useful alternative supplement for dorsal augmentation rhinoplasty. Development of collagen was commensurate with membranous ossification; however, assessment of complete ossification requires a longer experimental period.

KEYWORDS

Augmentation; extracellular matrix; hyaluronic acid; nasal dorsum; rabbit; rhinoplasty

According to the American Society of Plastic Surgeons (ASPS 2020), rhinoplasty was the most performed cosmetic procedure in 2020. Augmentation rhinoplasty may be indicated in patients with saddle nose deformity caused by either suboptimal rhinoplasty surgery or facial trauma (Lee, Hong et al. 2019); unfortunately, an appropriate grafting material for this procedure remains problematic. Allograft materials or autografts can be used to elevate the nasal dorsum (Alshehri et al. 2020). Although auricular and costal cartilage grafts are common autograft sources for dorsal augmentation rhinoplasty, harvesting either of these grafts is invasive and painful, and a deformity could result in the donor area. By contrast, allografts are easier to procure, but may present rejection problems; therefore, more efficacious materials are required (Yıldırım et al. 2013; Kim and Park 2018).

Hyaluronic acid is a component of mammalian extracellular matrix that is composed of repeating disaccharide chains (Zhang et al. 2019). This polysaccharide facilitates migration and concentration of mesenchymal cells during development. Hyaluronic acid also plays multiple roles in skeletal biology and participates in bone remodeling by controlling osteoclast, osteoblast and osteocyte behavior (Bastow et al. 2008).

The nasal bone is formed by intramembranous ossification during the prenatal period (Sandikcioglu et al. 1994). Intramembranous ossification is direct ossification; osteoprogenitor cells from the mesenchyme accumulate without a cartilage template. As a natural biological material found in different parts of the human body, hyaluronic acid commonly is used as a filling material for procedures for the head and neck region, such as nonsurgical shaping of the nose (Stupak et al. 2007; Humphrey et al. 2009).

We have found no report concerning the use of hyaluronic acid to elevate the nasal dorsum. Consequently, we investigated the use of hyaluronic acid matrix (Figure 1) as an allograft for dorsal augmentation rhinoplasty using a rabbit model and assessed outcomes using histopathologic and radiologic criteria.

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Figure 1. Fragment, 2 × 1 cm, of hyaluronic acid matrix prior to gelatinizing.

Material and methods

Animals and study design

Our study was approved by the ethical committee of Giresun University for animal experiments (no. 2017/20). We used eight 2,000–2,500 g 8–12-year-old New Zealand rabbits. The experiment was carried out in the laboratory for animal experiments of Giresun University. Rabbits were housed at 21 °C, 40–60% humidity with a 12 h light:12 h dark cycle. Animals were provided rabbit pellets and water *ad libitum*. Rabbits were divided randomly into two groups of four: an untreated control group and a matrix group for which a mixture of small cartilage pieces and saline-gelled hyaluronic acid matrix was applied to the osteotomy area as described below.

Surgery and experimental procedure

General anesthesia was induced by intravenous injection of 7.5–15 mg/kg xylazine hydrochloride (Rompun®, Bayer, Germany) and 40–60 mg/kg ketamine hydrochloride (Ketalar®, Pfizer, NY, NY). Body temperature of the rabbits was maintained at 37.5–39 °C during general anesthesia using an electric heater. The nasal dorsum of the rabbits was exposed by a lateral incision (Figure 2). A partial osteotomy was performed on the nasal dorsum using a conventional median osteotome. A 2 × 1 cm hyaluronic acid matrix (Hyaloss™; Fab-Fidia-Advanced Bioplymers-Pd, Abano Terme, Italy) was gelled by adding 1 ml saline. A cartilage graft was harvested from the auricle, then sliced into small pieces and added to the saline-gelled

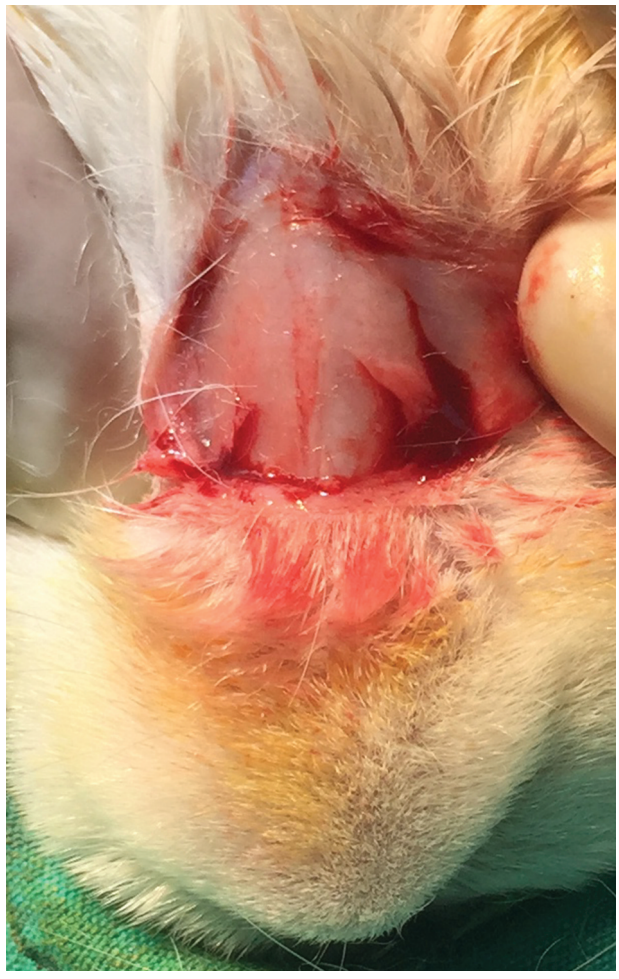

Figure 2. Exposure of the nasal bone of a rabbit.

hyaluronic acid matrix to add mass to the gel. The mixture of cartilage pieces and saline-gelled hyaluronic acid matrix was placed onto the osteotomy area of the four rabbits of the matrix group. Stabilization of the grafts was achieved by a skin sutures only; no additional suture was used. Osteotomy sites of the remaining four rabbits were not treated to constitute a control group. We administered 0.5–2 mg/kg methylprednisolone sodium succinate (Prednol L®; Mustafa Nevzat, Türkiye) intramuscularly on day 1 postoperatively and 10 mg/kg cefazolin sodium (Cefozin®; Bilim, Türkiye) was administered twice daily on days 1–5 post-operatively to prevent post-operative edema and infection. We administered 0.1–0.5 mg/kg butorphanol (Butomidor; Richter Pharma AG, Wels, Austria) intravenously twice/day for post-operative pain. Prior to sacrifice at the end of week 3, rabbits were examined by ultrasonography (USG) and three-dimensional (3D) computerized tomography (CT) under anesthesia. The USG device was an Aplio 500 (Canon, Otawara, Japan) with a linear array transducer and a 5–14 MHz frequency range. The CT scanner was a 16 slice

spiral Somatom Emotion scanner (Siemens, Berlin, Germany) with 1 mm pitch, 0.8 sec rotation time, 1.2 mm slice thickness and 130 kVp.

At the end of week 3, all animals were sacrificed by overdose of anesthesia. The nasal dorsum of the rabbits was exposed by a lateral incision and a 2 × 1 cm sample that included the fracture area was excised using bone scissors and placed in neutral buffered formalin.

Histopathology

Excised tissue samples were fixed with 10% neutral buffered formalin for 4 days, then placed in a Shandon TBD-2 decalcifier (Thermo Scientific, Runcorn, UK); decalcification progress was checked daily. The solution was checked for cloudiness; if cloudy, the tissue was still releasing calcium into the decalcification solution. Decalcification was completed when the decalcification solution remained transparent; tissue softness was assessed using a pin.

After complete decalcification, samples were kept in fixative solution for 2 more days, then dehydrated through a 70, 80, 90 and 100% alcohol series, cleared with xylene and embedded in paraffin wax. Serial sections were cut at 5 μm using a microtome and affixed to adhesive slides. Two sections from each rabbit were deparaffinized, rehydrated and stained with Masson's trichrome (5022-100; Gul Biology Laboratory, Istanbul, Türkiye) to assess general connective tissue features and type I collagen distribution. Stained sections were dehydrated through an alcohol series, cleared with xylene and mounted with Entellan. Collagen density in the fracture area was examined using a Zeiss Imager A-2 light microscope (Axio, Jena, Thüringen, Germany) at 200 × magnification. Collagen density was scored using the system reported by Tandelilin et al. (2006) as follows: 1, low density; 2, moderate density; 3, high density. Preserved capillary profiles throughout the recovery zone of the osteotomy were counted at 200 × magnification. Capillaries were counted in the entire fracture zone with intact bone edges as the border. Capillary profiles with rounded or defined borders were counted; capillaries with no definite borders were not included in the count. To avoid double counting, the entire fracture zone was scanned under the microscope and points were marked as reference; the capillaries within these areas were counted.

Statistical analysis

Data are medians. The SPSS v. 23.0 software for Windows (SPSS Inc., Chicago, IL) was used for the statistical analysis. Because the data were ordinal and the number of the samples was < 30, we used the nonparametric Mann-Whitney U test for comparisons. Values for $p \leq 0.05$ were considered statistically significant.

Results

Based on histopathological analysis of sections stained with Masson's trichrome, it was apparent that complete ossification had not occurred after 3 weeks in either the matrix or control group. Both groups, however, exhibited collagen formation and development of capillaries, which are important indicators of membranous ossification. Collagen fiber formation was more distinct, the density of newly formed collagen was greater and the fibers were organized more regularly throughout the fracture line in the matrix group compared to controls.

Figure 3 shows a 3D CT image of the osteotomy area. The USG image of a rabbit from the matrix group clearly shows that tissue integrity was reconstructed in the osteotomy zone (Figure 4). The median collagen scores were 3 for the matrix group and 2 for the control group. The median collagen score was significantly greater in the matrix group compared to the control group ($p = 0.028$) (Figure 6). We found that the median number of capillaries was 9 in the matrix group and 4 in the control group; however, the difference was not statistically significant (Figures 5, 6). Table 1 shows median collagen scores and number of capillaries for both groups.

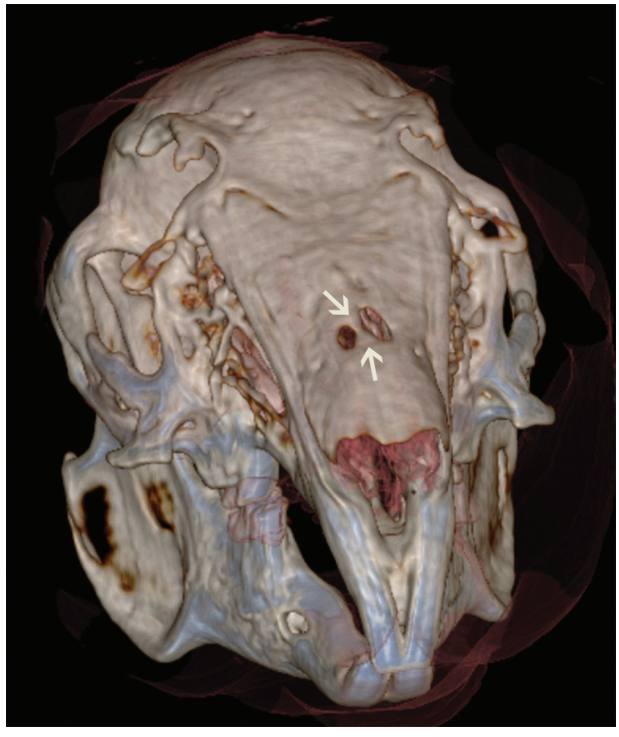

Figure 3. 3D CT image of osteotomy area. Arrows, tissue integrity in the osteotomy area.

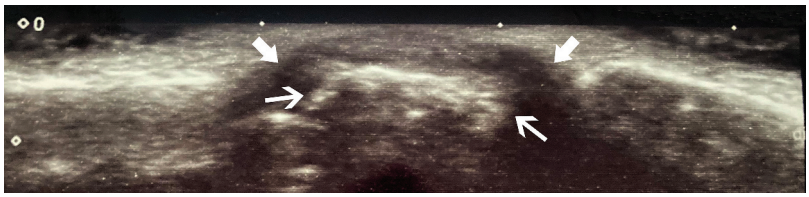

Figure 4. Ultrasonography of the osteotomy area. Thick arrows, boundaries of osteotomy area; thin arrows, the graft with gelatinized hyaluronic acid matrix.

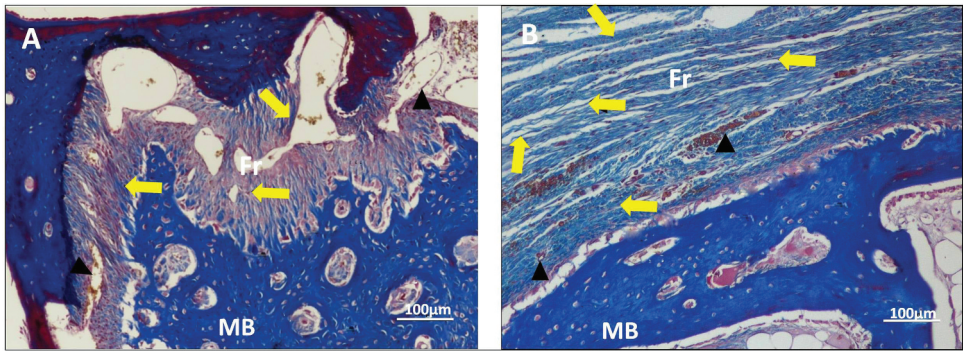

Figure 5. Representative photomicrographs of sections of nasal bones of the experimental groups. A) Control group. Newly formed collagen fibers; orientation is irregular. B) Matrix group. Collagen fibers were organized and elongated parallel to fracture area. Yellow arrow, collagen fibers; arrowhead, capillary; Fr, fracture area; MB, mature bone. Masson's trichrome stain. Scale bars = 100 µm.

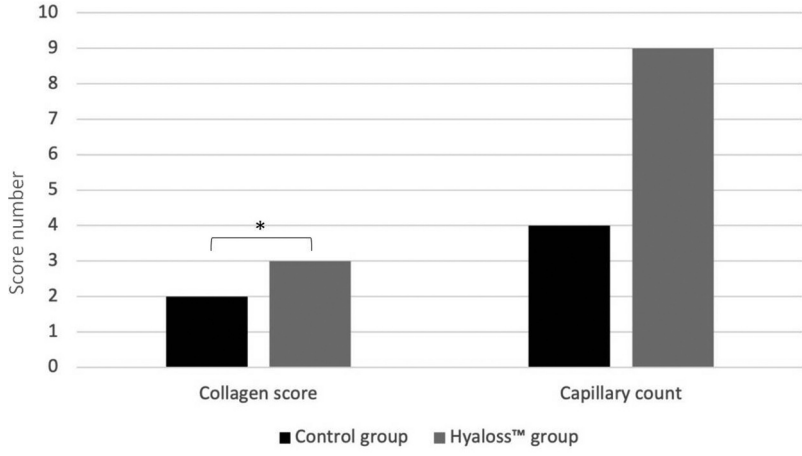

Figure 6. Semiquantitative median scores for collagen density and number of capillaries for control and matrix groups. *$p < 0.05$.

Table 1. Median collagen scores and numbers of capillaries.

Experimental group	Collagen score	Capillary number
Matrix group	3	9
Control group	2	4
*p	0.028	0.442

*Matrix group compared to control group.

Discussion

We investigated the effects of a mixture of a saline-gelled hyaluronic acid matrix and cartilage slices on collagen density and capillary development in the osteotomy zone of rhinoplasty in rabbits. Compared to the control group, animals administered hyaluronic acid matrix exhibited more significant collagen development. Because type I collagen is the major component of bone tissue, collagen scoring was based only on type I collagen formation. The matrix group exhibited a greater number of capillaries compared to the control, although the difference was not statistically significant. These findings suggest the onset of membranous ossification. Consistent with this, our 3D CT sections of the osteotomy zone of the samples and USG images verified tissue integrity in the osteotomy zone. Our findings suggest that hyaluronic acid matrix

with cartilage graft material may be useful for dorsal augmentation rhinoplasty.

Rhinoplasty is a common facial esthetic operation; some patients may require intervention for correction and/or augmentation. No graft material is ideal in every way; therefore, the optimal material for augmentation rhinoplasty is problematic. Autografts are biocompatible for augmentation; hyaluronic acid based products may be useful for maintaining the volume and shape of cartilage grafts.

Hyaluronic acid is a natural polysaccharide found in the extracellular matrix of the human body. Main components of hyaluronic acid include β-glucuronic acid and *N*-acetyl-D-glucosamine (Jang et al. 2008). Hyaluronic acid stimulates release of endogenous growth factors, epidermal growth factors, insulin growth factor, tumor necrosis factor and vascular endothelial growth factor (Saliba et al. 2014). Hyaluronic acid contributes to viscoelasticity and regulation of hydration of soft tissues (Güneri et al. 2003); It can be used safely and easily in clinical applications (Jang et al. 2008).

Hyaluronic acid also can be used to support wound healing (Simman et al. 2018). It stimulates fibroblast migration and angiogenesis, and facilitates cell proliferation (Chen and Abatangelo 1999). Hyaluronic acid also increases development of granulation tissue in the wound area and has been used to treat skin, tendon, muscle and bone defects (Dessy et al. 2013; Simman et al. 2018). Hyaluronic acid also can be used for diploic bone tissue and periosteum (Dessy et al. 2013). It has been used as a filling material for correction of scars and augmentation of soft tissues (de Lacerda and Zancanaro 2007; Stupak et al. 2007). Despite its great utility, however, we have found few reports concerning use of hyaluronic acid as an alternative or supplemental filling material for augmentation (Redaelli 2008; Tanaka et al. 2011; Kim and Ahn 2012; Xue et al. 2012; Won et al. 2018). Soft tissue filling materials, such as hyaluronic acid derivatives and human collagen, have been reported to be useful for correcting various deformities of the nose (Redaelli 2008; Rokhsar and Ciocon 2008; Mehta and Fridirici 2019). Soft tissue filler injection also has been reported to be safe for dorsal nasal augmentation (Lee et al. 2019). Hyaluronic acid is a safe and easily applicable filling material for nonsurgical augmentation rhinoplasty (Bravo et al. 2018; Santorelli and Marlino 2020). Irregularities after surgery can be corrected by subdermal injection of hyaluronic acid (Liapakis et al. 2013). Owing to branches of facial artery and the anastomoses of dorsal nasal vessels, use of filling materials in this way risks vascular embolization.

We developed a novel method for intra-operative use of hyaluronic acid by combining sliced cartilage in a saline-hyaluronic acid gel matrix using Hyaloss™. Hyaloss® matrix is a biodegradable, fast gelling adjunct composed of loose fibers made of hyaluronan-based biodegradable polymers (HYAFF®), an esterified derivative of hyaluronic acid. The polymer used is a hyaluronic acid derivative that releases the hyaluronic acid molecule, which enriches the implantation site with hyaluronic acid. The advantages of using Hyaloss® matrix as the graft include rapid gelling properties when in contact with sterile saline for mixing with cartilage or bone fragments, formation of an easy-to-apply paste, straightforward placement by the surgeon in the appropriate dimensions to the fracture area, and easy formation of a smooth surface. Hyaluronic acid is a naturally occurring extracellular matrix molecule; therefore, the risk of allergy and rejection is low. Consequently, hyaluronic acid possesses both autograft and allograft potential (Ballini et al. 2009; Baldini et al. 2010).

The ossification zone was incomplete after 3 weeks in our rabbit experimental model. In clinical practice, this interval is considered sufficient for adhesion and plaster is removed at this time. It is clear that the experimental period for our rabbit model must be extended to > 3 weeks to monitor ossification.

The short study period is a limitation of our study. Another limitation of our study was the small sample size. We intend to extend our investigation using larger groups and longer periods to confirm our current findings. Nevertheless, significant collagen development and capillary organization, though not statistically significant, in the matrix group suggests that future investigations involving extended times are justified. Our 3D CT images exhibited a smooth contour of the hyaluronic acid matrix treated osteotomy region.

Hyaluronic acid matrix appears to be a useful supplemental material for dorsal augmentation rhinoplasty, because the biocompatibility of hyaluronic acid matrix reduces the risk of rejection.

Disclosure statement

The authors declare no conflict of interest.

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