Intracordal Injection Technique: Materials and Injection Site

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Objective. Various materials have been used for intracordal injection to treat deficiencies in glottal closure. However, specific injection sites and materials are desirable for various conditions. Here, we reviewed injection sites and materials.
Methods. By considering the characteristics of injection materials used in Japan to date as well as the normal structure of the vocal cord, we have determined injection materials and sites suitable for different type of disease.
Results. Collagen obtained commercially for injection consists mainly of type I collagen, as does autologous fascia. When this material is injected into the superficial layer of the lamina propria mucosae of the vocal cord, the wave motion of the mucosa during phonation is disturbed. This material therefore should be injected deeper into the lamina propria. Indications for collagen injection include unilateral recurrent laryngeal nerve paralysis. Even when injected into the lamina propria, fat tissue does not disturb mucosal wave motion, and is useful for treating vocal cord atrophy or sulcus vocalis.
Conclusion. When intracordal injection is used in vocal rehabilitation, material and site should be selected, based on the cause of glottal dysfunction.

Key words: mucosal wave motion, deficiency of glottal closure, lamina propria mucosae, recurrent laryngeal nerve paralysis, vocal cord atrophy, sulcus vocalis

INTRODUCTION

Intracordal injection therapy has been used in vocal rehabilitation to treat deficiency of glottal closure from causes such as unilateral recurrent laryngeal nerve paralysis, vocal cord atrophy and sulcus vocalis. This procedure is intended to correct position and volume of the vocal cord and to attain glottal closure.

Since intracordal injection first was first described by Brünings (1) in 1911, injection techniques and injection materials have changed.

In Japan, silicone (2) and collagen (3) have been used for intracordal injection.

Silicone, which has been in use since the 1960s, is a polymer regarded as both suitable for injection and inactive within the human body. When this material is injected into the vocal cord, the tissue reaction is slight and injection effects are sustained and stable. Generally, connective tissue capsules are reported to form around silicone, and then persist stably (4). However, the injection site should be limited to within the muscle layer. When silicone is injected into the lamina propria mucosae, mucosal vibration is disturbed and voice quality is worsened. Moreover, since a report by the US Food and Drug Administration (FDA) in 1992 concerning the induction of human adjuvant diseases, room-temperature vulcanized silicone (RTVS) or silicone oil, which were used in the past, are now difficult to obtain.

Collagen for injection, extracted from bovine dermis, consists mainly of type I collagen. When collagen initially came to be recognized as an intracordal injection material, its characteristics were reported to differ from those of injection materials used in the past (5), possibly undergoing replacement by host tissue after injection. Furthermore, collagen has been reported to disturb vocal cord vibration less frequently than silicone, even when injected into the lamina propria (6).

In recent years, autologous tissues such as fat (7) or fascia (8) have also been used.

Technique using autologous fat for intracordal injection was first reported by Mikaelian (9) in 1991, and has been used clinically in Japan (8).

In augmentation using autologous fascia, clinical application preceded systematic study, and results have been reported (10). Histologic evaluation after injection was then carried out in experimental models, but no consensus has been reached regarding the change of volume from absorption after injection (11).

According to Arnold (12), the requirements for injection materials include minimal tissue response, absence of oncogenicity, ease of injection, nonabsorbability, and absence of migration. These conditions alone may not be sufficient if our aim is, to both correct vocal cord position as in patients with unilateral recurrent laryngeal nerve paralysis, and correct mucosal volume as in patients with vocal cord atrophy or sulcus vocalis.

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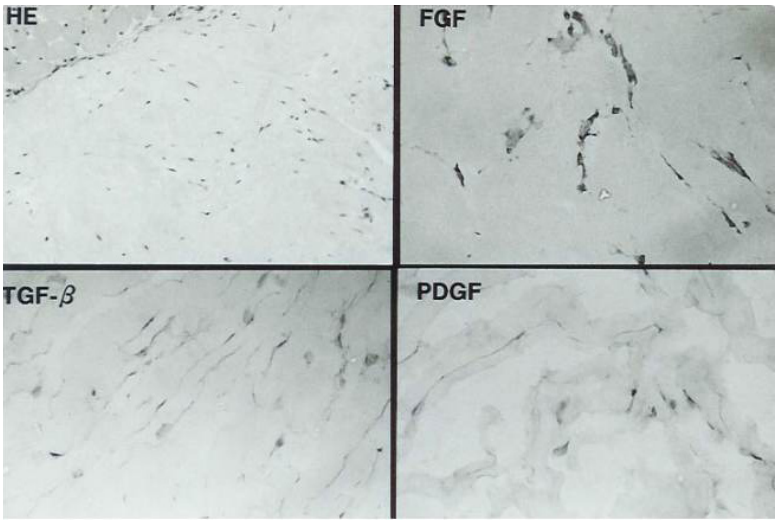

Fig. 1. Histologic findings in a dog after injection of 6.5% noncrosslinking-type collagen.
HE: HE-stained preparation (x66). Capsules consisting of collagen fibers formed around the gel, and infiltration of fibroblasts was present.
FGF: Immunostaining for FGF (x132). Cytoplasm of fibroblasts in the gel reacted with anti-FGF antibody.
TGF-β: Immunostaining for TGF-β (x 132). Cytoplasm of fibroblasts in the gel reacted with anti-TGF-β antibody.
PDGF: Immunostaining of PDGF (x132). Cytoplasm of fibroblasts in the gel reacted with anti-PDGF antibody. From reference 14.

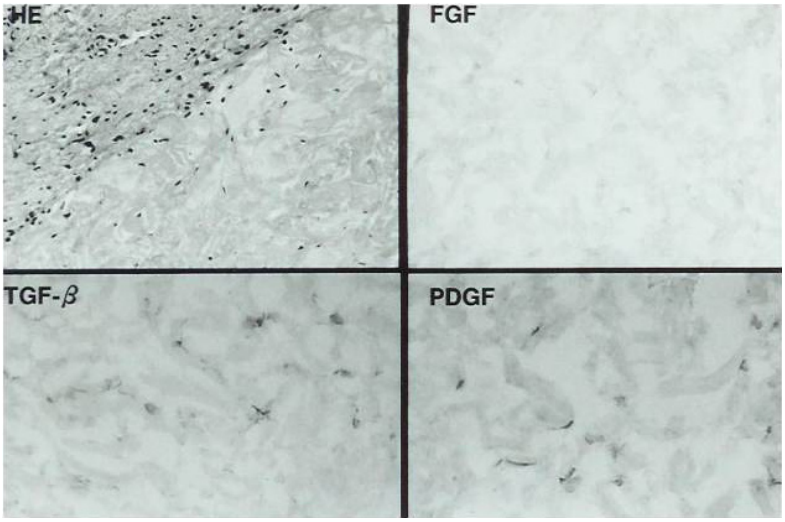

Fig. 2. Histologic findings in a dog after injection of 6.5% crosslinking-type collagen.
HE: HE-stained preparation (x 66). No capsule formation is evident surrounding the gel.
FGF: Immunostaining for FGF (x 132). Cytoplasm of fibroblasts in the gel showed no reaction with anti-FGF antibody.
TGF-β: Immunostaining for TGF-β (x 132).
Cytoplasm of fibroblasts in the gel showed a mild reaction with anti-TGF-β antibody.
PDGF: Immunostaining of PDGF (x 132).
Cytoplasm of fibroblasts in the gel showed no reaction with anti-
PDGF antibody. From reference 14.

We have studied the influence of materials on choice of injection site and on mucosal vibration during phonation, based on the immunological study of mucosae. Specifically, the desirable properties of injection materials should be defined while considering the anatomy of the vocal cord, which must vibrate at high speed.

We determined the injection materials and sites suitable for each underlying condition, such as unilateral recurrent laryngeal nerve paralysis, vocal cord atrophy and sulcus vocalis.

STRUCTURE OF LAMINA PROPRIA MUCOSAE

Immunohistochemical study of the lamina propria mucosae of the human vocal cord has shown that type III collagen is distributed widely in the superficial layer of the lamina propria, while type I collagen is present less superficially, deep in the laryngeal glands, and also near the anterior and posterior macular flava (13). This appears to correspond to the conditions of vocal cord vibration as evaluated by stroboscopic coronal imaging during phonation. Type III collagen, with high elasticity, is present in the superficial layer of the lamina propria mucosae where vocal cord vibration during phonation is greater, while type I collagen is prevalent at the fulcrum of vocal cord vibration and at sites with less vibration, representing function-related distribution.

COLLAGEN FOR INJECTION

The duration of beneficial effects after injection was studied indirectly in canine models by examining growth factors associated with wound healing (14). In dogs with recurrent laryngeal nerve paralysis, 6.5% noncrosslinking-type collagen or 6.5% crosslinking-type collagen (Koken) was injected into the paralyzed vocal cord, after which growth factors assessed immunohistochemically. These included fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-β. Figure 1 shows the gel in tissue at the 9th week after injection of noncrosslinking-type collagen. Similarly, a histologic preparation at 9 weeks after injection of crosslinking-type collagen is shown in Figure 2. In the noncrosslinking type collagen, immunoreactive growth factor was present in fibroblasts within the gel, while no such activity was seen with crosslinking-type collagen. As opposed to the noncrosslinking type, wound healing had ended by the 9th week after injection of crosslinking-type collagen, which appeared to remain

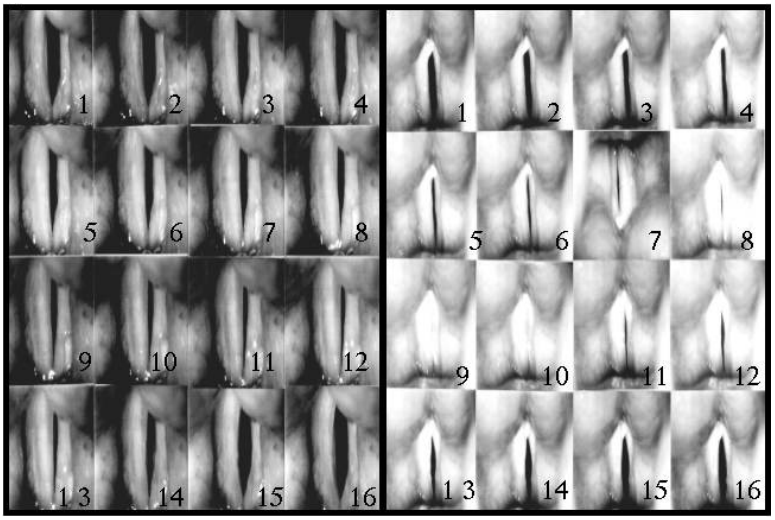

Fig. 3. Pre- and post-operative stroboscopic images during phonation (one cycle).
Left: Pre-operative image.
Amplitude of vocal cord vibration is small, and glottal closure is not seen.
Right: Post-operative image .
Glottal closure is observed at short-term follow-up. From reference 7.

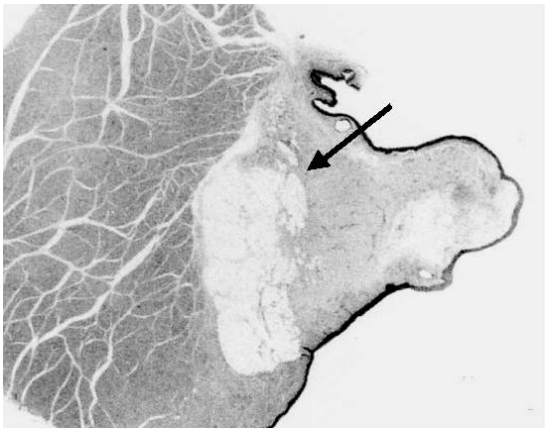

Fig. 4. Coronal section of vocal cord from a dog at 8 weeks after fat grafting.
Arrow, graft (HE staining x 2).
From the reference 7.

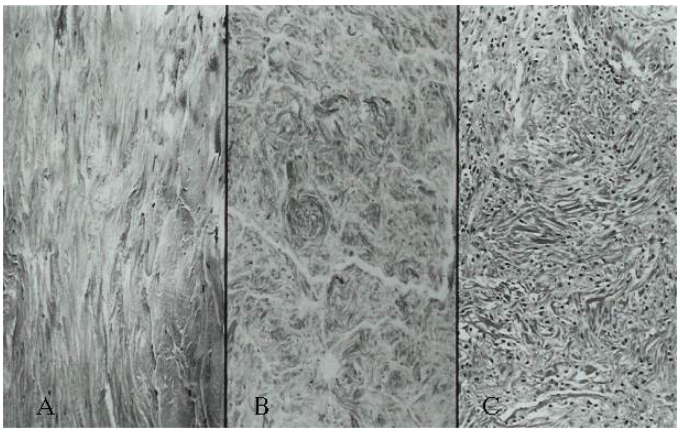

Fig. 5. Canine fascia lata.
A: Fresh fascia (HEstaining x50).
B: Freeze-dried fascia (HE staining x 50).
C: Fascia at 3 weeks after subcutaneous injection (HE staining x 50). From reference 16.

within the host tissues. Collagen without crosslinking was susceptible to host reaction after injection, and apparently difficult to retain in host tissues.

AUTOLOGOUS TISSUE

We have used autologous materials both in patients with unilateral recurrent laryngeal nerve paralysis and in patients with sulcus vocalis, and the results were satisfactory without disturbance of mucosal vibration after the procedure.

Figure 3 shows the results of autologous fat injection in a 67-year-old woman with sulcus vocalis. Stroboscopic images during phonation are shown before and at 8 weeks after the procedure are shown. Glottal closure was confirmed, and improvement was noted not only in voice quality but also in an aerodynamic examination.

However, the fat injection technique remains controversial because the degree of postoperative absorption differs according to the condition of fat injected (11). We therefore performed an experiment in dogs. The upper surface of the vocal cord was incised under a laryngomicroscope, and adipose tissues were grafted as a mass immediately above the muscle layer. In a coronal section of the vocal cord obtained at 8 weeks after grafting, the fat remained at the graft site, maintaining the same volume as at the time of grafting (Fig. 4) (15). This suggests that absorption after augmentation may be decreased by grafting as opposed to injection.

We then developed a method of preservation that uses freeze-drying, and absorption was evaluated after injection (15). Dogs were used in the experiment. After harvesting fascia lata specimens were processed by freeze-drying for 24 h at -65℃, and the minced in a food processor. Figure 5 shows specimens of fresh fascia, fascia after freeze-drying, and fascia at 3 weeks after subcutaneous injection in a mixture with 1% hyaluronic acid. The fascia before freeze-drying consisted mainly of collagen fibers, with few cellular components; fibroblasts were sparsely distributed. In the specimen of fascia after freeze-drying, no cellular components were recognized, but the collagen fiber representing the main component showed no apparent degeneration. After the fascia was injected subcutaneously, ingrowth of fibroblasts and new blood vessels, as well as infiltration by lymphocytes, was seen. However,

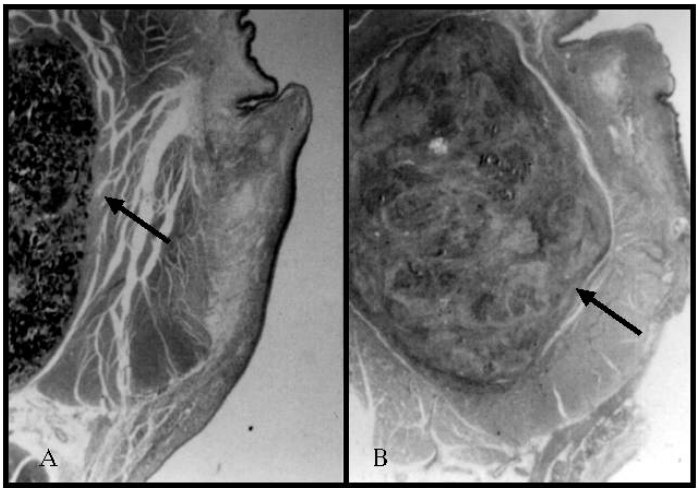

Fig. 6. Coronal section of canine vocal cord after injection into the muscle layer (HE staining x 2).
A: Three weeks after injection of freeze-dried fascia.
B: Four weeks after injection of fresh fascia.
From reference 16.

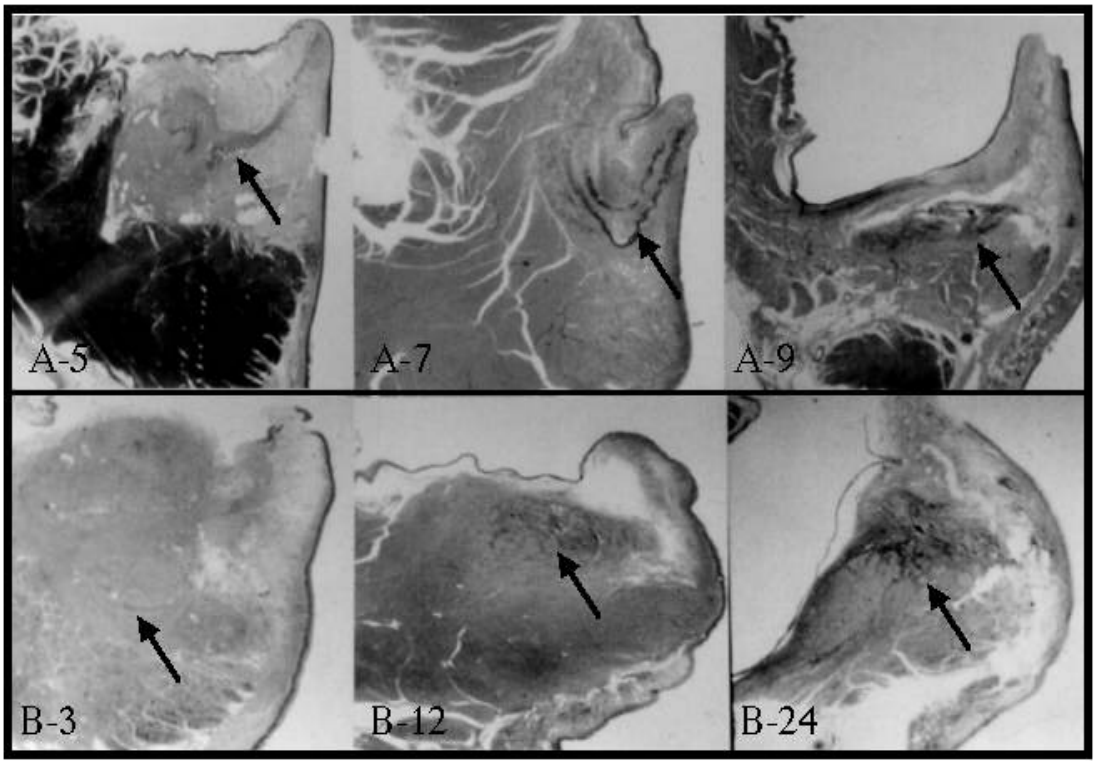

Fig. 7. Coronal section of canine vocal cord after injection into the lamina propria mucosae (HE staining; x 2) From reference 16.
A: After injection of freeze-dried fascia (A−5, A−7, A−9 : after 5, 7, and 9 weeks respectively).
B: After injection of fresh fascia (B−3, B−12, B−24: after 3, 12, and 24 weeks respectively)

neutrophils and necrosis were not seen. In an intracordal injection experiment in a dog with recurrent nerve paralysis, fascia remained present within the muscle layer as a mass, similar to findings after injection of fresh fascia (15).

Furthermore, 0.5 ml of freeze-dried fascia and 0.8 ml of fresh fascia were injected into the muscle layer and the lamina propria mucosae respectively. Assuming that the remaining fascial tissues took the form of a sphere, measurements were taken using a measuring ocular with a light microscope, and the volume was calculated. Figure 6 shows coronal sections of vocal cords at 3 and 4 weeks after injection into the muscle layer. Respective remaining volumes were 40% and 31% of injected volume. On the other hand, when freeze-dried fascia was injected into the lamina propria mucosae, the volume of the fascia decreased markedly over time (Fig. 7). Simple comparison is uninformative because the period of observation differed between lamina propria mucosae and the muscle layer, but the amount remaining in tissues tended to be higher when the injection was directed into the muscle layer, for both freeze-dried and fresh fascia (16).

CONCLUSION

The vocal cord has a layered structure. Waves of mucosal vibration reportedly originate mainly in the superficial layer of the lamina propria mucosae (17). Based on immunohistochemical results for collagen fibers in the lamina propria mucosae of the vocal cord,

we reported that the main component of collagen fibers in the superficial layer is type III collagen, which has high elasticity, while type I collagen is present at the fulcrum of vocal cord vibration and at deep sites showing less vibration (13). This suggests that, consideration of injection materials for intracordal injection should focus not only on histologic reaction after injection but also on vocal cord vibration.

We evaluated the histologic changes in injection materials after injection and examined injection sites based on the relationship between vocal cord constituents and vocal cord vibration.

Silicone, collagen, or fascia should be injected into a deep layer rather than into the intermediate layer of the lamina propria mucosae. These materials are suitable for patients with unilateral recurrent laryngeal nerve paralysis. Autologous fat does not disturb vocal cord vibration, even when injected into the superficial layer of the lamina propria.

We propose that autologous fat is superior to other materials for vocal rehabilitation in patients with unilateral recurrent laryngeal nerve paralysis and in patients with sulcus vocalis, as it gives satisfactory results without disturbing mucosal vibration.

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