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Antifibrotic effect of dexamethasone/alginate-coated silicone sheet in the abraded middle ear mucosa

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ABSTRACT

Silicone sheet is a material which is commonly used in middle ear surgery to prevent the formation of adhesions between the tympanic membrane and the medial bony wall of the middle ear cavity. However, silicone sheet can induce a tight and hard fibrous capsule in the region of the stapes, and this is particularly common in cases of eustachian tube dysfunction. As a result of the fibrous encapsulation around the silicone sheet, postoperative aeration of the stapes can be interrupted causing poor hearing gain. In this study, we performed an *in vitro* and *in vivo* evaluation of the antifibrotic effects of a dexamethasone and alginate (Dx/alginate) coating on silicone sheet. The Dx/alginate-coated silicone sheets were fabricated using a plasma-treatment and coating method. The Dx/alginate-coated silicone sheets effectively limited *in vitro* fibroblast attachment and proliferation due to the controlled release of Dx, which can be modified by manipulation of the alginate coating. For the *in-vivo* evaluation, guinea pigs (albino, male, weighing 250 g) were divided into two groups, with the control group (n = 5) implanted with silicone sheet and the test group (n = 5) receiving Dx/alginate-coated silicone sheet. Animals were sacrificed 3 weeks after implantation, and histological analysis was performed using hematoxylin and eosin (H&E) and immunohistochemical staining techniques. Dx/alginate-coated silicone sheets showed marked inhibition of fibrosis in both the *in vitro* and *in vivo* studies. Silicone sheet that incorporates a Dx/alginate coating can release Dx and inhibit fibrosis in the middle ear. This material could be utilized in middle ear surgery as a means of preserving proper aeration and hearing gain following ossiculoplasty.

1. Introduction

Middle ear pressure is regulated not only by the eustachian tube, but also by the middle ear mucosa. The eustachian tube plays a prominent role in middle ear pressure regulation through swallowing [1], but gas exchange within the middle ear mucosa assists in regulating middle ear pressure [2–5]. The purpose of tympanomastoidectomy is lesion removal and reconstruction of the anatomical structures with the intent of restoring middle ear aeration and maintaining stable, serviceable hearing. Postoperative middle ear aeration is an important factor for achieving optimal hearing outcomes after cholesteatoma resection. It is important to secure the pathway through the eustachian tube to the mastoid bone, and to preserve the middle ear mucosa as much as possible [1–5]. In prior studies, hearing outcomes following tympanoplasty or tympanomastoidectomy were significantly better in patients with well re-aerated middle ears than in those exhibiting poor re-aeration [6–8]. Postoperative aeration around the stapes is particularly important with regard to hearing outcomes [9].

Silicone sheet is a material which is commonly used in middle ear surgery to prevent adhesion formation between the tympanic membrane (TM) and the medial bony wall of the middle ear cavity. Tanabe et al. [10] reported that the use of large Silastic® sheets to cover the area extending from the bony eustachian tube and tympanic cavity to the epitympanum, aditus ad antrum, or antrum was found to be helpful in achieving recovery of mastoid aeration after complete resection of the mucosa and mastoid air cells.

Following implantation, biocompatible implant materials, such as silicone, are surrounded by a thin, dense layer of fibroblasts, collagen, and macrophages, and this forms a resistive interstitial pathway to electrical current flow [11]. Generally, silicone sheet used in middle ear surgery will be surrounded by a thin, dense layer of fibroblasts. However, this silicone sheet can induce severe side effects, including fibrous encapsulation which is character-

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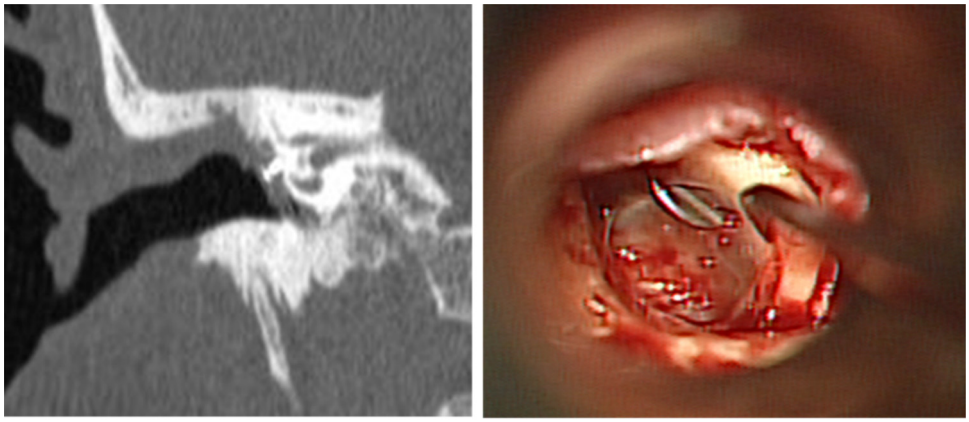

Fig. 1. (a) TBCT 6 months after canal-wall down mastoidctomy with total ossicular replacement prosthesis (TORP) with silastic sheet insertion, but marked fibrosis around the TORP with poor hearing gain. (b) Prominent fibrosis around the TORP in revision ossiculoplasty.

ized by an excessive foreign body reaction that forms a tight and hard fibrous capsule around the region of the stapes. This side effect is more common in eustachian tube dysfunction, and fibrous encapsulation of the silicone implant can interrupt postoperative aeration around the stapes and result in poor hearing gain (Fig. 1). Finding a solution to prevent, or at least minimize, fibrous capsule formation could also help in preserving aeration around the stapes following tympanomastoidectomy.

Dexamethasone (Dx) is known for its potent anti-inflammatory and immunosuppressant glucocorticoid effects, and the remarkable inhibition of fibroblast growth [12]. Transforming growth factor beta 1 (TGF-β1) is a key cytokine involved in the development and progression of fibrosis. It promotes the transcription of type I collagen and fibronectin in fibroblasts through an intricate signaling cascade [10]. Dx inhibits bleomycin-induced pulmonary fibrosis in mice [13], and local delivery of Dx may represent a potential solution to inflammatory or capsular fibrosis [14]. In this study, we evaluated the *in vitro* and *in vivo* antifibrotic effects of a Dx/alginate coating on silicone sheet.

2. Materials and methods

2.1. Materials

Silicone sheets (Hans Biomed, Seoul, Korea), Dx (5 mg/ml, Daewon Pharm Co., Seoul, Korea), and alginate (Sigma-Aldrich Corporation, Seoul, Korea) were used in this study.

2.2. Fabrication of dx/alginate-coated silicone sheets

To efficiently coat the silicone sheets with the Dx/alginate solution, the surface of the sheets were treated with oxygen plasma (CUTE-MP/R; Femto-Science Inc., Korea) for 4 h using established process conditions (power: 50 W, flow rate: 10 sccm, pressure: 5.2E–01 Torr) (Fig. 2). Following plasma treatment, the sheets were dipped into a 5 mg/ml Dx solution for 2 h, and then dried for 12 h at room temperature (Fig. 2). After coating the silicone sheets with Dx, an alginate coating was applied by submersion in a 7 wt% alginate solution. To crosslink the coated alginate, the coated sheets were immersed in a 2 wt% $CaCl_2$ solution for 1 min at room temperature. After the crosslinking process, the coated silicone sheets were washed three times with demineralized water for 5 min. To observe the drug loss during the 7 wt% alginate coating and crosslinking process, we indirectly measured the loss using the rhodamine-B solution. Through the simple measurement, we can find that the

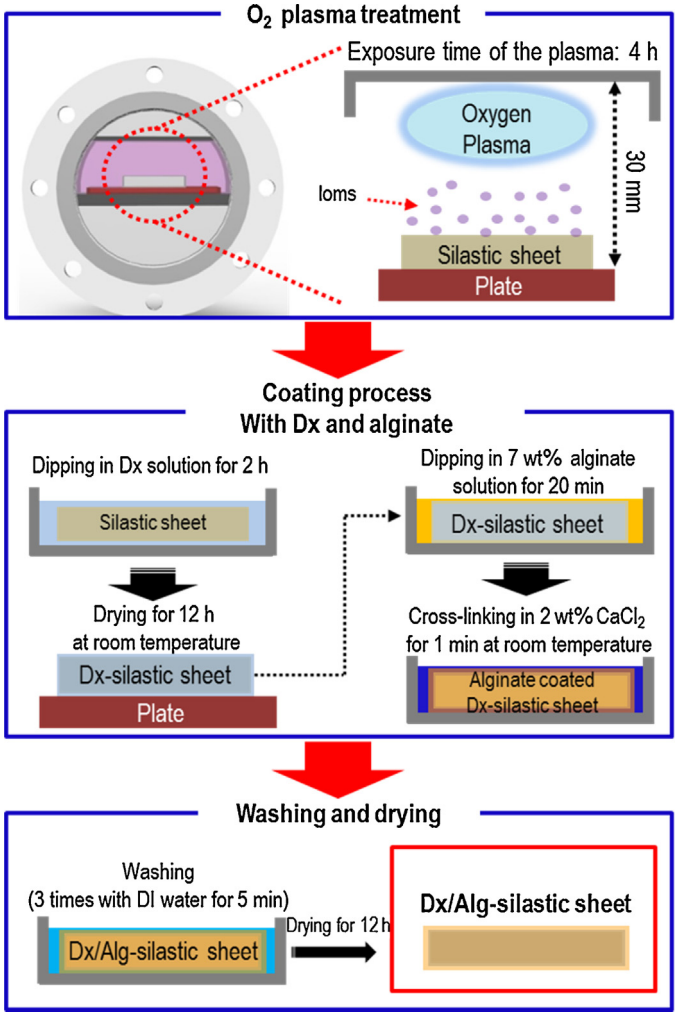

Fig. 2. Schematic of fabrication of the Dx/alg-silastic sheet.

percent rhodamine loss after the whole fabricating process was about 18.7 ± 0.4%.

2.3. In vitro *release test*

For the *in vitro* release testing, we utilized rhodamine-B (Sigma-Aldrich Corporation, St. Louis, MO, USA) since it has been widely