

## Original Article

# A bioabsorbable membrane (Seprafilm®) may prevent postoperative mediastinal adhesions following mediastinoscopy: an experimental study in rats

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**Abstract:** Introduction: The aim of this experimental study was to investigate the anti-adhesion property of a bio-absorbable membrane following mediastinoscopy in a rat model. Methods: The study was conducted in 20 male Sprague-Dawley rats. Mediastinoscopy was performed all of them. Rats were divided into two groups; control group (n=10); mediastinoscopy alone, study group (n=10); mediastinoscopy and sodiumhyaluronate-carboxymethylcellulose film (Seprafilm®; Genzyme Corporation, Cambridge, Mass. USA). It was used to the mediastinal surface at the end of the surgical procedure in study group. Re-mediastinoscopy was performed after 7 days. Adhesion and vascularity grade description scores were analyzed. The parameters evaluated were presence of polymorphonuclear leucocyte, macrophage, lymphocyte, fibroblasts, edema, neovascularisation, collagenisation, and foreign body reaction. Results: All the rats survived uneventfully until being sacrificed without any postoperative complications. The mean adhesion score was found to be significantly higher in control group ( $n=2.5\pm 0.5$ ) compared with study group ( $n=1.0\pm 0.1$ ) ( $P=0.007$ ). Vascularity grade description score was significantly higher in control group ( $n=2.3\pm 0.6$ ) than in study group ( $n=1.4\pm 0.6$ ) ( $P=0.009$ ). There were no statistical differences between the groups with regard to edema, lymphocyte and macrophage infiltration, fibroblast proliferation and foreign body reactions ( $P>0.05$ ). Conclusion: The used of Seprafilm® during the primary procedure can reduce to the mediastinal adhesions. However, further studies are required to assess the precise impact of the anti-adhesive agents on adhesion.

**Keywords:** Mediastinoscopy, mediastinal adhesion, sodiumhyaluronate-carboxymethylcellulose, re-mediastinoscopy

## Introduction

With the improvement of neoadjuvant treatment protocols in lung cancer surgery, a need for reassessment of tumor stage after chemoradiotherapy is emerging [1, 2]. Wound healing involves overlapping steps of inflammation, cell migration and proliferation, neovascularisation, extracellular matrix production and remodelling giving rise to the adhesion formation [3]. When mediastinal pleura is damaged, the basal membrane of the mesothelial layer becomes exposed to surrounding tissues and the sequence of adhesion mechanism noted above is formed. These mechanisms end with the development of adhesions which constitute significant cause of postoperative complications.

In all reoperative procedures performed for locoregional recurrences, metastasis or surgi-

cal treatment of postoperative complications the thoracic surgeon encounters with the formation of adhesions. Re-mediastinoscopy can be a difficult procedure due to fibrosis in the mediastinum. The presence of mediastinal adhesions significantly increases the morbidity and mortality of reoperative mediastinal staging procedures.

Some clinical studies have demonstrated that sodium hyaluronate-carboxy methylcellulose (NaH/CMC) (Seprafilm®; Genzyme Corporation, Cambridge, Mass. USA) significantly decreases the incidence and severity of adhesion formation [4-6], although there was no clinical study of specific to the mediastinal surgery. In our study we aimed to reduce the formation of adhesions by limiting tissue apposition during the critical stages of mesothelial repair. Therefore we evaluated the effect of Seprafilm®

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**Table 1.** Adhesion score and vascularity grade description score

Grade	Thickness-Tenacity	Vascularity
0	No adhesion	-
1	Filmy, easily separable	No bleeding
2	Moderate, thick, moderate tension required	Petechial bleeding
3	Severe, cohesive, sharp dissection required	Bleeding that require suture or tamponade

**Table 2.** Histopathologic evaluation

	Control Group		Study Group		p value
	Mean	SD	Mean	SD	
Adhesion score	2.50	.53	1.00	1.15	.007**
PMNL	.00	.00	.10	.32	.739
Macrophages	1.60	.70	1.20	.42	.123
Lymphocytes	1.30	.67	.90	.57	.218
Fibroblasts	1.00	.67	.90	.74	.796
Edema	.70	.67	1.00	.47	.315
Neovascularisation	.10	.32	.40	.70	.436
Collagenisation	.30	.48	.20	.42	.739
Foreign Body Reaction	.70	.82	.30	.48	.353
TOTAL	5.70	2.26	5.00	2.26	.315
Vascularity grade description score	2.30	.67	1.40	.69	.009**

SD: Standard Deviation, PMNL: Polimorphonuclear leukocytes. Histopathological analysis was performed to study the effect of the anti-adhesive agent. Scores were calculated based on collagen fibrosis, fibroblasts, granulation tissue, muscle alterations/inflammation, histiocytes, mononuclear giant cells, inflammation and vascular proliferation. \*\*This difference is found to be statistically significant.

### Surgical procedure

The induction of anesthesia was performed with injection of ketamine (100 mg/kg i.m. of Ketalar; Eczacıbaşı, Istanbul, Turkey) and chlorpromazine (0.75 mg/kg i.m. of Largactil; E.R.P. Istanbul, Turkey). The surgical procedures were performed under sterile conditions.

After the hair removal, the trachea was cleaned with 70% alcohol and 1% betadine solution. The muscles of the neck were separated and the pretracheal fascia with underlying surface was exposed after a vertical neck incision. The anterior and lateral aspects of the trachea were dissected to the level of the carina. In the control group, the pretracheal surface was abraded for five times and the overlying tissues were closed without application of a material. In the study group, we installed a 1x0.3 cm Seprafilm® over the abraded pretracheal surface before closure of the layers. This bioabsorbable membrane is based on a chemically modified form of hyaluronic acid which is a natural glycoaminoglycan and of carboxymethylcellulose that's a derivative of cellulose [8-10].

After seven days, pretracheal fascia was re-exposed and the degree of mediastinal adhesion formation was noted as technical difficulty in dissection. Adhesion formation is initiated by surgical trauma and any procedure for inhibition should begin during the surgical procedure and persist until postoperative third to fourth days [7]. Major steps of fibrosis formation is seemed to be completed during the first week. Thus we have chosen a 7 day period for mediastinal histologic examination.

on mediastinal surface postoperatively in a standardized rat model.

### Material and methods

The study was conducted after approval by the Marmara University School of Medicine Experimental and Investigative Animal Laboratory (Laboratory Ethic Committee Certification No: 7.3.2002). Twenty sexually mature Sprague-Dawley rats were used weighed 300 gr. Animals were kept under standard laboratory conditions at room temperature with a relative humidity of 50-70% and a day cycle of 14 hours light and 10 hours dark with free access to food and water ad libitum. Animals were treated in accordance with the standards of National Institutes of Health as described in the Guide for Care and use of Laboratory Animals.

The rats were randomly divided in two groups with 10 rats in each group; Group 1 (Control group): Mediastinoscopy alone (n=10); Group 2 (Study group): Mediastinoscopy and installation of 1x0.3 cm Seprafilm® (n=10).

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The examinations were reported blindly. The incision area was examined after the dissection. The ease of tissue separation, the need for sharp dissection of fibrous bands and the degree of bleeding were recorded. The adhesion score was reported for each sampling on a scale of 0 to 3 in terms of thickness-tenacity and vascularization as described in the literature [8-10] (**Table 1**).

The subjects were humanely killed with an intravenous injection of an overdose of sodium pentobarbital. Histological sampling for microscopic examination was performed. Random biopsies were taken from pretracheal surface. Histologic classification and scoring was reported according to adhesion formation model in rats which was described by Milligan and Raftery [3, 11]. Histopathologic examination was performed by a board-certified pathologist blindly. The parameters evaluated were presence of polymorphonuclear leucocyte, macrophage, lymphocyte, fibroblasts, edema, neovascularisation, collagenisation, and foreign body reaction.

### Statistical analysis

The mean and the standard deviation of all values were measured. Mann-Withney-U test was used for statistical analysis. A value of  $P < 0.05$  was accepted as statistically significant.

### Results

All animals tolerated surgical procedure. All the rats survived uneventfully until being sacrificed without any postoperative complications. The mean adhesion score was found to be significantly higher in control group ( $n = 2.5 \pm 0.5$ ) compared with study group ( $n = 1.0 \pm 0.1$ ) and this difference was found to be statistically significant ( $P = 0.007$ ). Vascularity grade description score, which represent fibrosis, was significantly higher in control group ( $n = 2.3 \pm 0.6$ ) than in the study group ( $n = 1.4 \pm 0.6$ ) ( $P = 0.009$ ).

The comparison of two groups in for histopathological evaluation are shown in **Table 2**.

No polymorphonuclear leukocytes (PMNL) infiltration was detected in control group, whereas PMNL infiltration was  $0.1 \pm 0.3$  cell per 100 high field in the study group ( $P = 0.739$ ). The degree of macrophage, lymphocyte infiltration and the

mean fibroblast count was found to be statistically indifferent between the control and study group ( $P > 0.05$ ). However the incidence of edema was diminished in control group, although the difference was not found to be statistically significant ( $P = 0.315$ ) (**Table 2**).

Neovascularisation was observed slightly more in the study group ( $n = 0.4 \pm 0.7$ ) than the control group ( $n = 0.1 \pm 0.3$ ), although there was no statistically significant difference ( $P = 0.436$ ).

Furthermore, collagenisation and foreign body reaction were similar between the two groups ( $P = 0.739$  and  $P = 0.353$ , respectively).

### Discussion

Precise staging of the mediastinum after induction therapy for non-small cell lung cancer with mediastinal lymph node metastasis (N2 disease) is important as the nodal stage will determine the patient survival. In a recent study, sensitivity of remediastinoscopy was reported to be 71%, with 84% accuracy [12]. However, the adhesions form a significant impediment for re-mediastinoscopy after neoadjuvant therapy for N2 disease [1, 2]. In general, any surgical wound can form postoperative adhesions that might cause postoperative complications or make reoperative procedures challenging [13, 14]. The difficulty in reexploration, prolongation of the operation time and dangerous bleeding are the major problems encountered in remediastinoscopy [1, 15, 16]. Van Schil et al. [1, 16] reported that a complete mediastinal exploration was not usually possible due to fibrous adhesions and scar tissue after mediastinoscopy. There is no material in clinical use to prevent mediastinal adhesions following mediastinoscopy in spite of the fact that remediastinoscopy are not uncommon.

The clinical methods of preventing postsurgical adhesions have limited success [8, 17]. Limited number of investigations have reported on the ability of topically applied gel compounds to reduce the formation of mediastinal adhesions after mediastinoscopy [18]. Although the exact mechanism by which these compounds prevent adhesions is unknown, it appears that they may act as an extracellular matrix substitute. In order to reduce postoperative adhesion formation many surgeons have developed a variety of surgical techniques and have used

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several agents. However, several studies have detected 55-86% adhesion rate in the postoperative period [7, 8, 19].

Our study was designed to evaluate the role of Seprafilm® on the adhesion, tissue response to mediastinal surgery in terms of fibroblastic proliferation neovascularization, macrophage and lymphocyte infiltration in experimental setting. These parameters defined the surgical reexploration of the previously de-epithelized pretracheal mediastinal tunnel [20, 21].

Seprafilm® is proposed to provide an optimum remediastinoscopy by inhibiting adhesion formation without distorting the wound healing process after the first surgical intervention. The material sticks to the moist surface, rapidly changes to the agar form. It's absorbed within a week and totally excreted from the body in 28 days. It is widely used in abdomino-pelvic surgery and is the only biosynthetic material that has been studied in well designed randomized controlled clinical studies. It has been shown to reduce postsurgical adhesion formation by inhibiting tissue desiccation and abrasive mesothelial damage [9, 22, 23]. The surgically traumatized surfaces are kept covered during mesothelial regeneration thus preventing adherence of adjacent structures and reducing adhesion formation [24].

In the present study, the mean adhesion and vascularity grade description scores were decreased by NaH/CMC application. Although, there was no significant reduction in other histopathological parameters for example foreign body reaction and edema, the mean adhesion score indicates that surgical reexploration was easy after application of the membrane. Adhesion score has been defined as the end point aim of the application. The encouraging results of this study suggest that topical Seprafilm hold promise in the prevention of postoperative mediastinal adhesions.

The exact mechanism of adhesion and the importance of other histopathologic parameters yet to be clarified. In addition we were unable to assess tissue factors, cytokines and chemokines which were reported to initiate and mediate the inflammatory events in the surgical trauma.

In conclusion, patients who potentially require remediastinoscopy in the future may benefit

from bioabsorbable membrane placement at the initial operation. We observed that, technical difficulties in dissection, bleeding after the dissection of fibrous bands were diminished in the NaH/CMC treated group. NaH/CMC gel are efficacious in the prevention of mediastinal adhesions in this model, and appear safe in doses one times the amount need to prevent adhesions. Further studies investigating the mechanism by which Seprafilm® prevent adhesions, its optimal dose and method of application, and documentation of their safe use in humans are warranted.

### Disclosure of conflict of interest

None.

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