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A New Cross-Linked Hyaluronic Acid Gel for Preventing Adhesion After Thyroid Surgery: An Animal Study

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ABSTRACT

Background: Revision of any neck surgeries is usually associated with increased rate of complications compared to the initial surgery due to adhesions. Especially, recurrent laryngeal nerve injury and hypoparathyroidism are most important postoperative complications of thyroid revision surgery. This study aimed to reveal anti-adhesive effects of cross-linked hyaluronic acid gel (NCHAG) in thyroid surgery.

Materials and methods: This study was performed in 16 adult male rats who underwent hemithyroidectomy in the right lobe and randomized into two experimental groups: Group I (control group) was given any substance and Group II (NCHA group) received NCHA spray into their perithyroidal area. The rats were sacrificed after three weeks of thyroidectomy for assessment.

Results: Gross adhesions score ($\bar{x} = 2.500$) was significantly higher in Group I than Group II ($\bar{x}$=1.750; $P = 0.031$). Group II showed significantly less fibrosis compared to the Group I ($P = 0.002$). The rate of inflammation was found to be significantly higher in group I ($P = 0.008$). Vascular proliferation was not different between two groups (p=0.083).

Conclusions: Our study showed that NCHA can reduce postoperative adhesion and might be effective in preventing fibrosis after the thyroidectomy. Although this study could not demonstrate that application of NCHA is able to reduce complication rate in revision neck surgery, it could be safely used after thyroidectomy and neck surgeries to prevent adhesions.

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Introduction

Fibroblasts start to migrate into the area of injury and proliferate following the surgical trauma. Collagen is synthesized by fibroblasts. Postoperative adhesions (PA) arise from the formation of these collagen fibers. Accumulation of collagen is regulated by certain collagenases and their inhibitors. The fibrin-rich exudate that builds up with the inflammation in the surgical site is degraded with the help of the proteolytic enzymes, and becomes eventually resorbed. Formation of a fibrin-rich exudate is involved in normal wound healing process. However, in cases where resorption of the fibrin-rich exudate is insufficient or the accumulation is prolonged, adhesions made of fibrin may develop. Collagen build-up and neovascularization with the preceding adhesions result in permanent fibrous adhesions.[1-3]

PA represents critical problems and significant challenges for all surgical operations, including neck surgeries. These adhesions affect patient's quality of life negatively by causing dysphagia and a sense of retraction in the neck. In addition, adhesions may prolong the duration of the surgery and the length of hospital stay during second revision surgeries, raise the rate of major complications such as recurrent laryngeal nerve injury and hypothyroidism, due to challenges in dissection.[4-7] Numerous clinical and experimental studies have been conducted for eliminating PAs and their complications. Although a number of drugs, commercial materials, and methods have been used, no widely-accepted and effective solution has been come up with.[8-10] Most of the studies for PA prevention involved abdominal operations.

Hyaluronic acid (HA) exists as an essential component in the extracellular matrix of all connective tissues and is a sulphate-free glycosaminoglycan that consists of repetitive disaccharide units (α-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine). HA has unique biological properties in wound healing. Different forms of commercial anti-adhesives made of HA have been used due to its excellent biocompatibility with positive results reported.[11-14] On the other hand, natural HA was reported to yield unsatisfactory post-operative outcomes. The reason might be its liquid form and rapid degradation (within 48 hours in vivo). However, cross-linking modification increases the viscosity of the super-high-molecular-weight material with a consequent delay of its degradation. As a result, it is achieved that novel cross-linked HA (NCHA) covers the surface during the critical time period for wound healing. Although NCHA is chemically cross-linked, the biological properties of HA are conserved.[15] NCHA was reported to significantly diminish the adhesions in the abdominopelvic cavity safely after laparoscopic gynecological surgeries.[16] In this study, our aim was to show whether NCHA could decrease post-thyroidectomy adhesions or not.

Materials and methods

Study design

This study was approved by Istanbul University Animal Experimentation Local Ethics Committee (Approval no: 2016/68).

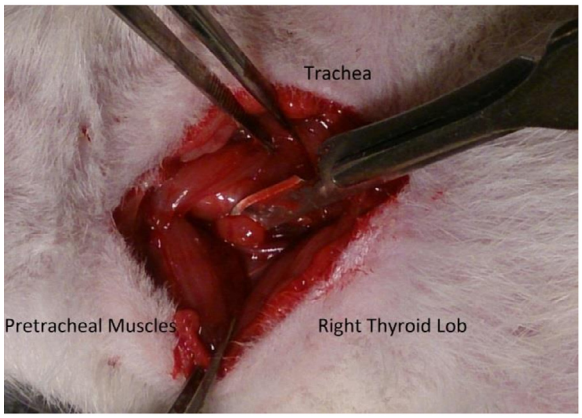

Fig. 1 – Intraoperative view of the thyroid dissection.

Table 1 – The adhesions grading of Evans.

Grade	Grading of adhesions
0	No adhesion
1	Spontaneously separating adhesions
2	Adhesions separating through traction
3	Adhesions separating through dissection

All animal experiments complied with the ARRIVE guidelines and the National Institutes of Health Guide for the care and use of laboratory animals. All animals were provided by Aziz Sancar Istanbul University Institute of Experimental Medicine, Department of Laboratory Animals Science, where the study was carried out.

In total, 16 albino Wistar rats (median weight: 275 g, median age: 16 weeks) were used in this study. Animals were randomized into 2 groups with 8 animals in each group. The rats were anesthetized with intramuscular administration of xylazine hydrochloride (10 mg/kg, Rompun, Bayer) and ketamine hydrochloride (90 mg/kg, Ketalar, Eczacibasi). All surgical operations were performed by a single surgeon (Aksakal N.) in semi-sterile conditions. A longitudinal incision of 2 cm in the anterior neck was followed by the traction of the muscles laterally and right hemithyroidectomy (Fig. 1). Group I (control group) animals did not receive any treatment but irrigated with normal saline; and in Group II (NCHA), respectively 1 ml to 2 ml NCHA (HyaRegen gel, BioRegen Biomedical, Changzhou, China) was sprayed onto the perithyroidal area. The muscles were repositioned to their anatomical positions and after that, the skin was closed with nonabsorbable 5 to 0 polypropylene monofilament suture. The animals were then followed up in single cages in the postoperative care room. They were provided with standard water and feed.

Scoring of the adhesions and histologic evaluation

Three weeks after surgery, all rats were sacrificed by sodium thiopental overdose. The operation field was assessed by two blinded surgeons (Goksoy B and Dogan S), (Fig. 2A-B). The adhesions were scored based on the Evans (Table 1). Then, the right perithyroidal area was totally excised for

Table 2 – Histopathologic fibrosis grading.

Grade	Grading of fibrosis
0	No fibrosis (no fibroblast and/or collagen fibers)
1	Low-level fibrosis (low number of fibroblast and/or collagen fibers)
2	Medium-level fibrosis (more fibroblast and/or collagen fibers)
3	Advanced fibrosis (high number of fibroblast and/or collagen fibers)

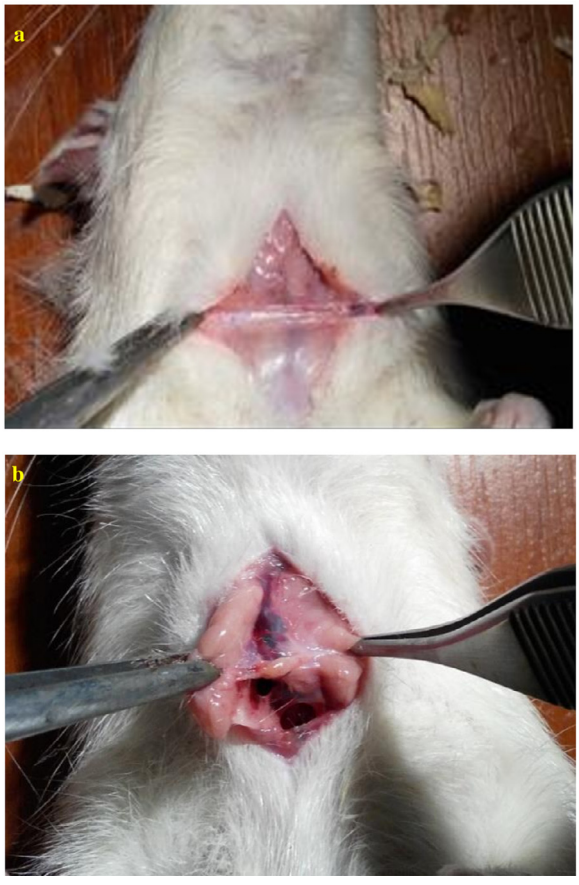

Fig. 2 – A-B. Three weeks after surgery. (A) Extensive and severe adhesion in the Group I; (B) partial adhesion in the Group II

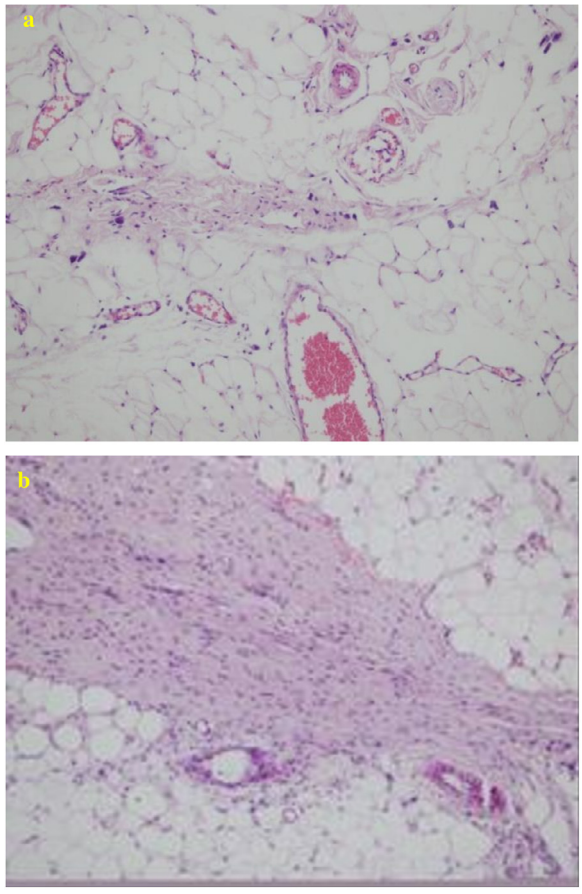

Fig. 3 – A-B. Microscopic findings of fibrosis (hematoxylin and eosin staining). (A) Moderately extensive adhesion in the Group II; (B) extensive adhesion in the Group I. (H&E; bar: 20 µm)

histopathological evaluation according to the fibrosis grading (Table 2). Histopathological evaluation was blindly performed by a pathologist (SYO). Excised tissues were fixed in formalin for 24 hours, embedded in paraffin and cross-sections of 5 micrometers were cut. Hematoxylin-eosin and Masson's trichrome were used for tissue staining.

Statistical analysis

The data of the study was analyzed by using Statistical Package for Social Sciences (SPSS) for Windows 22.0. Descriptive data were expressed as numbers and percentages or means and standard deviations, where appropriate. Parametric variables between the study groups were compared via *t*-test. Categorical variables were compared through chi-square test. Findings were evaluated at a significance level of 5% and a confidence interval of 95%. *P* values of less than 0.05 were considered significant.

Results

No mortality occurred in any group during the study. There were no complications in any animals. Gross adhesions score ($\bar{x} = 2.500$) was significantly higher in Group I than that in Group II ($\bar{x} = 1.750$; $P = 0.031$). Gross adhesion findings between two groups were shown in Table 3. Histopathologic fibrosis grading was significantly different between the two study groups. Group II showed significantly less fibrosis compared to that in the Group I ($X^2 = 12.667$; $P = 0.002$), (Fig. 3A-

Table 3 – ross adhesions scores of both groups.

Groups	Group I ($n = 8$)		Group II ($n = 8$)		t	P
	Mean	SD	Mean	SD		
Gross adhesions scores	2.500	0.535	1.750	0.707	2.393	0.031

SD = standard deviation.

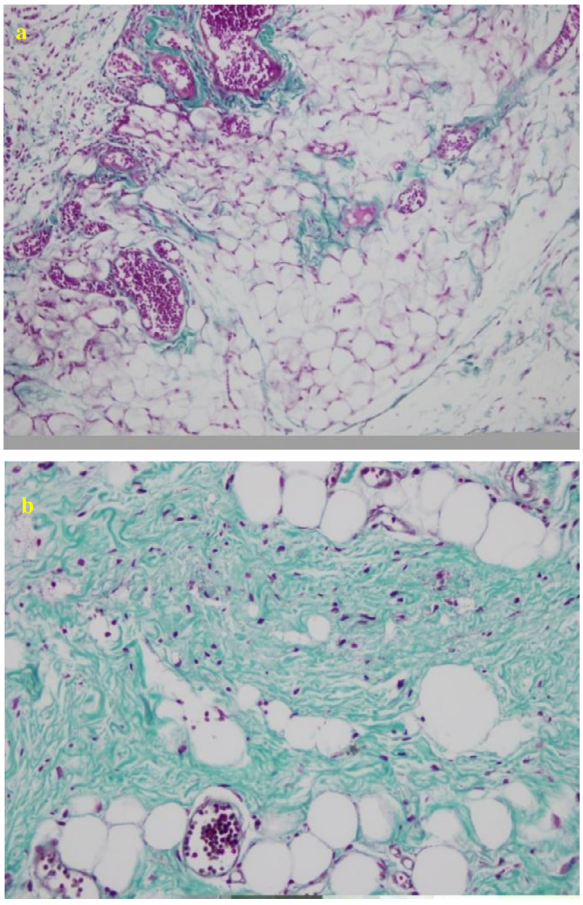

Fig. 4 – A-B. Microscopic findings of inflammation and fibrosis (Masson trichrome (MT) staining). (A) The Group I showed marked fibroblast infiltration and dense fibrosis; (B) minimal fibrosis was evident in the Group II; (MT; bar: 20 μm).

B). The rate of inflammation was found to be significantly higher in Group I compared to that in the Group II ($X^2 = 9.667$; $P = 0.008$). Group I showed marked fibroblast infiltration and dense fibrosis (Fig. 4A-B). The study groups did not differ in terms of vascular proliferation ($X^2 = 6.667$; $P = 0.083$). The comparison of histopathological findings between the two study groups was demonstrated in Table 4.

Discussion

Thyroidectomy is the most common intervention in endocrine surgery. Some of the most fearful and common complications of thyroidectomy are recurrent laryngeal nerve injury and hypoparathyroidism. Compared to primary surgeries, revision surgeries have a markedly increased risk of complications, even performed by experienced physicians. Rates of persistent and temporary recurrent laryngeal nerve injury was reported to be 5.6% and 9.4%, respectively. The other unwanted conditions, persistent hypoparathyroidism was reported in 6.6% and temporary hypoparathyroidism in 20.7%.[17-20] Surgical trauma and inflammation with regard to the surgical intervention lead to the development of PAs. The severity of PAs depends on several factors, including the harshness of the surgical trauma, applying some hemostatic foreign material in the wound, and presence of a hematoma or an infection. The main factor that increases the rate of complications is that normal anatomical boundaries are lost due to PAs and dissection becomes more difficult.

Numerous clinical and experimental studies have been conducted for reducing PAs and their complications. Although minimal invasive techniques, anti-inflammatory drugs, heparin, fibrinolytic agents, coverings, and fluids that build mechanical barriers have been used, a reliable and consistent success has not been achieved.[21-25] Most of the studies for PA prevention involved abdominal operations. Prevention of PAs related to thyroid surgeries have been the purpose of very few studies and most of them are animal studies. Makay O et al. reported that simvastatin prevented PAs effectively in one of their animal study in which they applied the agent to the surgical area.[26] Koyung SP et al. reported in their prospective clinical studies that an oxidized regenerated cellulose (Interceed) prevented the adhesion of skin to the trachea effectively and safely in patients who underwent thyroidectomy.[27]

In this study, we investigated the efficacy of NCHA in preventing PAs that develop after thyroidectomy, which has been used for the first time to the best of our knowledge. In fact, HA is a biological substance in liquid form that plays an important role in wound healing. Its viscous solution form was developed and used. However, its use has remained limited because of its low bioavailability caused by its rapid degradation.[28] HAs with different molecular weights were developed in order to enhance its bioavailability. Park WS et al. reported that the use of sodium hyaluronate and sodium carboxymethyl cellulose (HA-CMC) was safe but showed a questionable efficacy in their prospective randomized clinical study. Their reasoning for the questionable efficacy was that the drain which was located in the thyroidectomy site had sucked HA-CMC for 48 hours, thus, decreasing its efficacy.[29] In an animal study involving HA-CMC solution and 2 film barrier materials, HA-CMC solution reported to provide significantly less adhesions than that in the control group and numerically less adhesions compared to the film barrier group, albeit not significant. However, they advocated that using HA-CMC solution is technically more advantageous than film barriers. Film barriers are not only too

Table 4 – Histopathologic findings in the study groups.

		Group I		Group II		P
		n	%	*n*	%	
Histopathologic fibrosis grading	2	0	0.0	7	87.5	$X^2 = 12.667$ $P = 0.002$
	3	5	62.5	1	12.5	
	4	3	37.5	0	0.0	
Inflammation	Mild	3	37.5	1	12.5	$X^2 = 9.667$ $P = 0.008$
	Middle	0	0.0	6	75.0	
	Excessive	5	62.5	1	12.5	
Vascular proliferation	No	1	12.5	0	0.0	$X^2 = 6.667$ $P = 0.083$
	Mild	2	37.5	6	75.0	
	Middle	0	0	1	12.5	
	Excessive	5	62.5	1	12.5	

thin and fragile, but also have limited use since they could only be applied just under the incision line. On the other hand, solutions have several advantages both in open and laparoscopic surgeries since they are able to reach anywhere at the surgical site and do not require manipulation.[30]

In our study, we showed that NCHA significantly reduced gross adhesions compared to the control group (Table 3). Histopathologic assessment revealed that the control group had a greater inflammation, marked infiltration of fibroblasts, and a dense fibrosis (Table 4). An ideal anti-adhesive barrier should be biodegradable and absorbable. It should not cause any foreign body reaction, constitute an environment for infections, or prevent wound healing.[9] NCHA seem to possess the properties of an ideal anti-adhesive barrier. We did not observe any complications related to the use of NCHA. In the meantime, vascular proliferations of the two groups were not different which showed that NCHA did not have a negative impact on wound healing while it decreased the inflammation and adhesions. NCHA's gel form allows it to spread throughout the entire surgical site. Its cross-linked molecular structure and high viscosity prevent its rapid degradation, and thus provide appropriate time to prevent adhesions by remaining in the surgical area.

Conclusions

Further prospective clinical studies are necessary for demonstrating the efficacy of NCHAs in preventing PAs after thyroid surgery. Some of these studies will enable the evaluation of the undesired symptoms related to the adhesions while the clinical studies that involve perioperative evaluation (in case of reoperations) of PAs of patients who received NCHA would provide the adequate evidences supporting NCHA's anti-adhesive efficacy.

Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article. Nihat Aksakal, Beslen Goksoy, Semen Yesil Onder, Selim Dogan, Ali Fuat Kaan Gok, and Umut Barbaros declare that there is no conflict of interest.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the study conception and design.

Material preparation, data collection and analysis were performed by Nihat Aksakal, Beslen Goksoy, Semen Yesil Onder, Selim Dogan, Ali Fuat Kaan Gok, and Umut Barbaros.

The first draft of the manuscript was written by Nihat Aksakal and Beslen Goksoy and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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