ECRONICON OPEN ACCESS

DENTAL SCIENCE
Research Article

Cytological Evaluation of Hyaluronic Acid on Wound Healing Following Extraction

Gocmen Gokhan[1]*, Gonul O[1], Oktay NS[2], Pisiriciler R[2] and Goker K[1]

[1]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Marmara University, Turkey*

[2]*Department of Biochemistry, Faculty of Dentistry, Marmara University, Turkey*

***Corresponding Author:** Gocmen Gokhan, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Marmara University, Turkey.

Received: February 12, 2015; **Published:** February 28, 2015

Abstract

Purpose: The purpose of this study is to measure the efficacy of high molecular weight HA on epithelial wound healing considering cytological parameters after extractions. We compared leucocytes, collagen and necrosis cytologically between control and HA group.

Patients and Methods: 40 patients were included in this study. 0, 2 ml 0, 8% HA immediate after M3 removal was applied in to the extraction within the HA group (n:20). Nothing was applied to the control group (n:20). The primary outcome variables were; leukocyte, necrosis and collagen content. Che-square Test was used to assess the differences between the HA and control groups for each study variable.

Results: There was no statistically significant difference between groups regarding leukocyte and collagen parameters immediate after extraction. Necrosis in HA group showed less "none" and "slight" then control group ($p < 0, 05$).

Conclusion: Our results do not confirm the hypothesis that HA has anti-inflammatory effect and improve the repair capacity following tooth extraction. Further studies examining different parameters with different examination techniques are necessary to present more useful outcomes.

Keywords: *Hyaluronic acid; Wound healing; Extraction*

Introduction

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide of the extracellular matrix. As a major molecule in the extracellular matrix, it affects inflammation regulation, angiogenesis, granulation formation, and re-epithelialization. The unique viscoelastic nature of exogenous HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications including; supplementation of joint fluid, assisting wound regeneration and dermal filling [1,2].

Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. HA, can take place at any stage of these phases or indirectly associated with accompanying proceedings. These potential interactions raise the question if there is a correlation between HA's existence and clinical outcomes of inflammatory reaction [3,4].

The purpose of this study is to measure the efficacy of high molecular weight HA on epithelial wound healing considering cytological parameters after extractions. We have compared leukocytes, collagen and necrosis cytologically between controls and HA group.

Materials and Methods

We implemented a double blind, randomized, and controlled clinical trial. The tissue samples were obtained from 40 patients (Female patients n = 20), who underwent surgical removal of third molar (M3) in Departments of Oral and Maxillofacial Surgery at Marmara University between January 2011 and January 2012. Erupted or half impacted but without bony retention and vertically positioned M3 were included to the study. All patients were healthy and classified as the ASA I-II groups. The mean age was 26, 6 +/- 6, 3. The following patients were excluded from the study : those with signs of pericoronitis/pain before surgery, those in whom extraction time lasted for more than 30 min, those who had undergone antibiotic or any other medication therapies during the preceding 2 weeks, those who had active carious lesions and/or periodontal diseases.

Extractions, 0.8% Hyaluronic acid gel (GENGIGEL PROF®, Milano, Italy) application, obtaining samples and follow-up were performed by the same operator. Marmara University, Department of Histology and Embryology performed the cytological assessment of the samples. The clinical research ethics committee of Marmara University approved the study protocol (Protocol number: 2011-1)

Tissue samples, about 2 mm^3 in volume, were obtained following extraction. All samples were taken from the buccal wound edge of extraction socket. Wound closures were made with 3.0 silk sutures. Subjects were randomly assigned to receive HA application. HA group (n:20) was applied 0, 2 ml 0, 8% HA immediately after M3 removal to the edge of extraction socket. Control group (n:20) was applied nothing. Tissue samples were taken from the same region after one week. Each of the swap samples were stained with May-Grunwald Giemsa dye. They were observed by an Olympus BH-2 light. The primary outcome parameters were leukocyte, necrosis and collagen content.

The grading of parameters was established by randomly selecting and counting fields of leukocyte infiltration, necrosis and collagen deposition. Scoring was made in the following manner: "0", when none of the fields show parameters; "Slight", when at least 5 fields contain parameter that occupy < 50% of the field; "Mild", when at least 5 fields show parameter that occupy > 50% of the field; and "Intense", when all 10 fields evaluated show parameters that occupy > 90% of the field.

The results were analyzed using the Statistical Package for the Social Sciences (SPSS version 12.0; SPSS, Chicago, IL). Che-square Test was used to assess the differences between the HA and control groups for each study variable. The level of statistical significance was set at p less than 0.05.

Results

There was no statistically significant difference between groups regarding all parameters immediate after extraction (Figure 1). Leukocytes and collagen content were not statistically significant between groups after one week (Table 1, Table 2). Necrosis was statistically significant between groups after one week (Table 3). Necrosis in HA group showed less "none" and "slight" then control group (Figure 2 and Figure 3).

Collagen		**Control Group**	**HA Group**	*p
		n (%)	**n (%)**	
Immediate After Extraction	**None**	1 (%5)	0 (%0)	397
	Slight	14 (%70)	12 (%60)	
	Mild	5 (%25)	8 (%40)	
	Intensive	0 (%0)	0 (%0)	
After 1 Week	**None**	10 (%50)	10 (%50)	822
	Slight	8 (%40)	9 (%45)	
	Mild	2 (%10)	1 (%5)	
	Intensive	0 (%0)	0 (%0)	

Table 1: *Collagen parameters.*
**Che-square Test.*

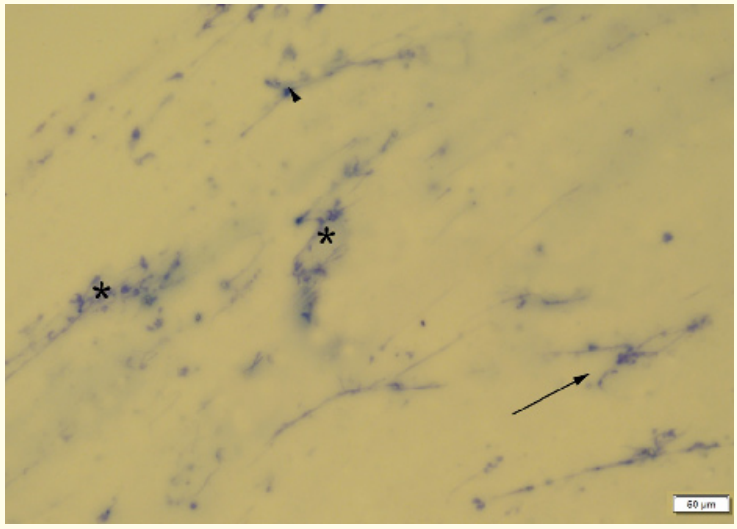

Figure 1: *Immediate after extraction gingival swap May Grunwald- Giemsa stain X200; collagen (arrow), fibrocyte (*), leukocyte (arrowhead).*

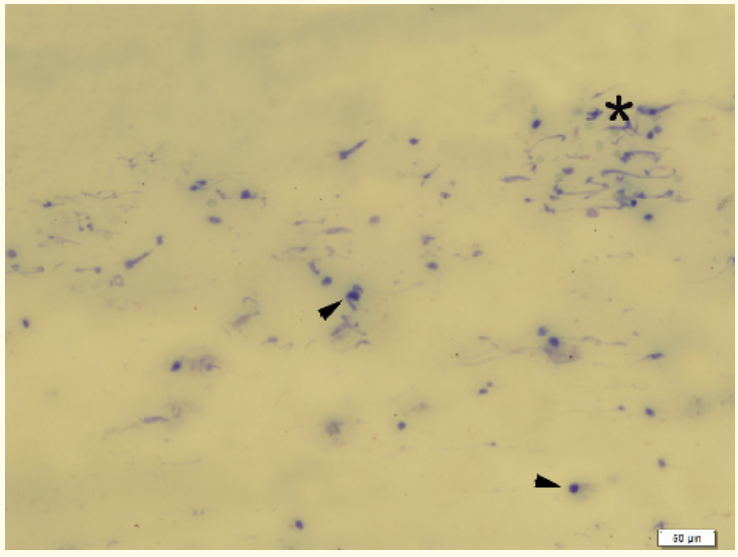

Figure 2: *HA group 7. Day gingival swap May Grunwald-Giemsa stain, X200 Collagen (*), leukocyte (arrowhead).*

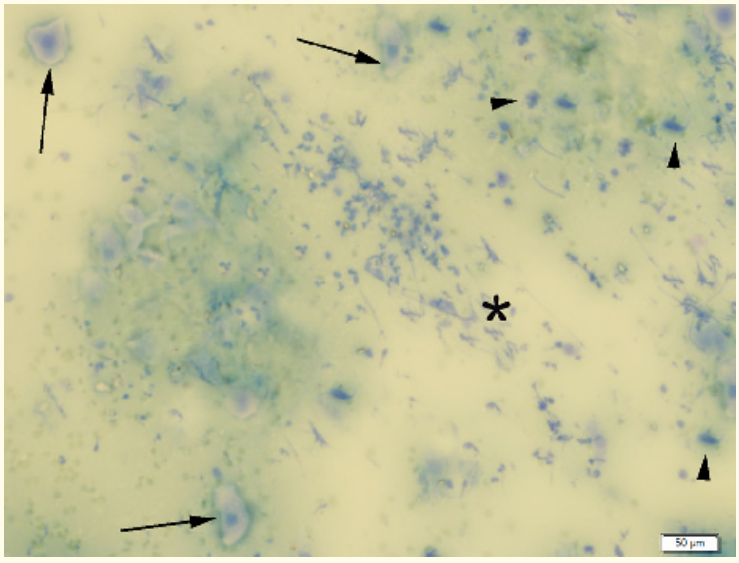

Figure 3: *Control group 7. Day gingival swap May Grunwald-Giemsa stain, angiogenesis (arrow), collagen (*), leukocyte (arrowhead).*

Leucocyte		**Control Group**	**HA Group**	***p**
		n (%)	**n (%)**	
Immediate After xtraction	**None**	9 (%45)	9 (%45)	≅ 1
	Slight	11 (%55)	11 (%55)	
	Mild	0 (%0)	0 (%0)	
	Intensive	0 (%0)	0 (%0)	
After 1 Week	**None**	0 (%0)	1 (%5)	330
	Slight	12 (%60)	9 (%45)	
	Mild	8 (%40)	8 (%40)	
	Intensive	0 (%0)	2 (%10)	

Table 2: *Leukocyte parameters.*
** Che-square Test.*

Discussion

The purpose of this study was to measure the efficacy of high molecular weight HA, on the wound healing after M3 extraction. We aimed to measure effects of HA on wound healing cytologically in terms of leukocytes, collagen and necrosis parameters.

HA has been shown to promote the migration and maturation of keratinocytes in mucosal re epithelialization [5]. It is reported that topically applied hyaluronan accelerates cutaneous wound healing. Additionally, hyaluronan acts as a promoter of early inflammation, production of pro-inflammatory cytokines and formation of granulation tissue [4,6,7]. Leukocyte infiltration is one of the primary inflammatory responses of tissue during wound healing. The action mechanism of HA's inhibition of cell migration, was not clearly understood. Various mechanisms have been proposed to explain the effect of HA on the inflammatory process. HA has been shown to be the main ligand of CD44 receptor and subsequent ligand-receptor interaction is involved in number of cell-to-cell interactions. Some studies reported that HA, especially high-molecular-weight HA, acts like anti-CD44 antibody, inhibiting cell phagocytosis and chemotaxis by

influencing the intracellular cytoskeleton through its receptor [8]. In our study, there was no significant difference between cytological outcomes of HA and control group considering leukocyte infiltration.

HA can modulate cellular activity in fetal wound, which is persistently enriched with HA. It is claimed that HA can modulate fibroblast function prevent fibrosis and scar formation in the early phases of wound healing [9,10]. Abdalla *et al*. [11] reported increase in new vessel formation and decrease in collagen deposition in Hyaluronic acid based hydrogel-injected groups using haematoxylin-eosin and vascular endothelial growth factor (VEGF) staining. Friedrich *et al*. [12] indicated that anti-TNF- α conjugated to HA can be an effective treatment for reducing secondary necrosis and improving healing outcomes with collagen composition. In our study, there was less necrosis in the HA group but no significant difference between HA and control group considering collagen content.

Necrosis		**Control Group**	**HA Group**	***p**
		n (%)	**n (%)**	
Immediate After Extraction	**None**	19 (%95)	19 (%95)	≅ 1
	Slight	1 (%5)	1 (%5)	
	Mild	0 (%0)	0 (%0)	
	Intensive	0 (%0)	0 (%0)	
After 1 Week	**None**	6 (%31,6)	19 (%95)	<, 0001
	Slight	13 (%68,4)	1 (%5)	
	Mild	0 (%0)	0 (%0)	
	Intensive	0 (%0)	0 (%0)	

Table 3: *Necrosis parameteres.*
**Che-square Test.*

Conclusion

In conclusion, our results do not confirm the hypothesis that HA has anti-inflammatory effect and improve the repair capacity following tooth extraction. Further studies examining different parameters with different techniques are necessary to present more useful outcomes.

Bibliography

1. Teh BM., *et al*. "A review on the use of hyaluronic acid in tympanic membrane wound healing". *Expert Opinion on Biological herapy* 12.1 (2012): 23-36.
2. Price RD., *et al*. "The role of hyaluronic acid in wound healing: assessment of clinical evidence". *American Journal of Clinical Dermatology* 6.6 (2005): 393-402.
3. Prosdocimi M and Bevilacqua C. "Exogenous hyaluronic acid and wound healing: an updated vision". *Panminerva Medica* 54:2 (2012): 129-135.
4. Chen WY and Abatangelo G. "Functions of hyaluronan in wound repair". *Wound Repair and Regeneration* 7.2 (1999): 79-89.
5. Colella G., *et al*. "Molecular insights into the effects of sodium hyaluronate preparations in keratinocytes". *Clinical and Experimental Dermatology* 37.5 (2012): 516-520.
6. Wisniewski HG., *et al*. "TSG-6, a glycoprotein associated with arthritis, and its ligand hyaluronan exert opposite effects in a murine model of inflammation". *Pflugers Archiv* 431.6 Suppl 2 (1996): R225-226.
7. Kobayashi H and Terao T. "Hyaluronic acid-specific regulation of cytokines by human uterine fibroblasts". *American Journal of Physiology* 273.4 pt 1 (1997): C1151-C1159.
8. Tamoto K., *et al*. "Effects of high-molecular-weight hyaluronates on the functions of guinea pig polymorphonuclear leukocytes". *Seminars in Arthritis and Rheumatism* 22.6 Suppl 1 (1993): 4-8.

9. Mast BA., *et al*. "Hyaluronic acid modulates proliferation, collagen and protein synthesis of cultured fetal fibroblasts". *Matrix* 13.6 (1993): 441-446.
10. Croce MA., *et al*. "Hyaluronan affects protein and collagen synthesis by in vitro human skin fibroblasts". *Tissue Cell* 33.4 (2001): 326-331.
11. Abdalla S., *et al*. "Hyaluronic acid-based hydrogel induces neovascularization and improves cardiac function in a rat model of myocardial infarction". *Interactive Cardiovascular and Thoracic Surgery* 17.5 (2013): 767-772.
12. Friedrich EE., *et al*. "Local Delivery of Antitumor Necrosis Factor-alpha Through Conjugation to Hyaluronic Acid: Dosing Strategies and Early Healing Effects in a Rat Burn Model". *Journal of Burn Care and Research* 36.2 (2014): e92-101.

Volume 1 Issue 2 February 2015