

## Araştırma Makalesi / Research Article



## Yeni Bor Ester Türevlerinin Yara İyileşmesi Üzerindeki Etkisi

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## The Effect of Novel Boron Ester Derivatives on Wound Healing

## ÖZET

Bor fiziksel ve kimyasal özellikleri ile yeni biyolojik özelliklere sahip bor içeren moleküller oluşturmayı olanaklı kılar ve moleküler tasarım ile tıbbi uygulamalar yeni alanlarının araştırılması sonucunda insanlığa ve bilim insanlarına fırsatlar sunar.

Bu çalışmada; bir pilot çalışma ile etkinliği göreceli test edilmiş bor içerikli 2 adet sentez molekülün yara iyileşmesine etkisini daha geniş örneklem ile test etmek için faz 0, *in vivo*, *in vitro* çalışmalarını gerçekleştirerek yeni ürün geliştirmek amaçlandı.

Yara iyileşme açısından molekül 1'in yara iyileşme yüzdesi diğer tüm gruplardan daha yüksekti ( $p = 0.001$ ) ve kronik bir cilt altı enflamatuvar reaksiyona yol açmadı ( $p < 0.05$ ). Kolajen üretimi açısından molekül 1'in 7. günde ( $p < 0.05$ ) ve molekül 2'nin 14. günde ( $p < 0.05$ ) kolajen üretimi daha yüksekti.

Bor içerikli sentez içerikli molekülleri, yara iyileşmesini makroskopik (yara iyileşme yüzdesi) ve mikroskopik (mikrobiyolojik, histopatolojik ve immünohistokimyasal) olarak desteklemektedir. Çalışma sonucunda 2 adet patent başvurusu yapılmıştır.

**Anahtar kelimeler:** Yara iyileşmesi, Yara bakımı, Bor

## ABSTRACT

Boron's unique physical and chemical characteristics make it possible to create novel boron-containing molecules resulting in a wide range of distinct biological properties. This paves the way for exciting prospects in various scientific disciplines and medical applications through advanced molecular design and research.


In this study, two novel boron-containing molecules were synthesized and their effect on wound healing property were tested in a pilot study as phase 0, *in vivo* and *in vitro* in order to test them on a larger sample.


The wound healing percentage of molecule 1 was higher than that of all other groups in terms of wound healing percentage ( $p = 0.001$ ), and it did not lead to a chronic subcutaneous inflammatory reaction ( $p < 0.05$ ). Collagen production of molecule 1 and molecule 2 on day 7 ( $p < 0.05$ ) and that of molecule 2 on day 14 ( $p > 0.05$ ) were higher.


In this study, obtained boron containing compounds show promising effect on wound healing macroscopically (wound healing percentage) and microscopically (microbiological, histopathological, and immunohistochemical). As a result of the study, 2 patent applications were made.

**Keywords:** Wound healing, Wound care, Boron

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## INTRODUCTION

The number of compounds containing boron is increasing every day, because of their capacity for natural production and innovative synthesis procedures (Liu et al., 2020). Evidence regarding the biological effects of natural and synthetic boron-containing molecules dates back to ancient times. In recent years, the mechanisms of action of these compounds have been determined, and they can be used in the treatment and alleviation of certain diseases (Estevez-Fregoso et al., 2021).

Wounds, whose the specific prevalence of which is not yet known, are becoming one of the greatest and most growing problems of the world. In particular, the rapid increase in the elderly population and the increase in the prevalence of diabetes mellitus and other chronic diseases have caused the incidence of chronic wounds to rise. Research on wound treatment has become widespread worldwide. In this context, there is a need to develop effective wound care materials that do not carry the risk of infection, reduce bacterial colonization, provide rapid angiogenesis to carry oxygen and nutritional substances to cells in the wound area, support epithelization, induce granulation, and prevent hypertrophic scar formation (Metcalf & Ferguson, 2007a; Zhao et al., 2015; Kuzay & İlçe, 2023).

Boron compounds, which can be involved in biochemical processes, induce numerous pharmacological responses in organisms. The antibacterial (Temel et al., 2022), hypolipidemic (Apdik et al., 2015; I H Hall et al., 1989; Sood et al., 1992), anti-inflammatory (Iris H Hall et al., 1996; Sood et al., 1992), antineoplastic (Altinoz et al., 2019), and antiosteoporotic effects (Al-Rawi et al., 2013; Liu et al., 2018; Racu et al., 2021) of boron compounds have been demonstrated. The protective effects of boron on reproductive health, long life, brain functions, prostate cancer (Wang et al., 2019), breast cancer (Seneviratne et al., 2022), cervical cancer, lung cancer, and cardiovascular diseases have been revealed in a few studies (Kuru & Yarat, 2017). The use of boron-containing compounds will increase in the coming years and will be considered an important element in

the development of certain drugs (Hosmane, 2012). Boron plays a role in cell membrane functions and various regulatory enzymatic systems. Free oxygen radicals can interact with cells through cell membranes and organelles, causing serious damage to cells and tissues (Aysan et al., 2017; Ince et al., 2010). Boron containing compounds also used as an antioxidant agent that prevents oxidative damage (Aysan et al., 2017; Borrelly et al., 1991). Studies have shown the effects of boron-containing compounds on wound healing, both *in vivo* and *in vitro*. It has been reported that boron changes the extracellular matrix and increases tumor necrosis factor-alpha (TNF $\alpha$ ) expression (Nzietchueng et al., 2002a). The positive effects of topical form of boron containing ingredients on wound healing have been demonstrated in various studies. In the first study conducted in 1941, boric acid and potassium permanganate were found to be effective against infected car accidents and war wounds (Hosmane, 2012). In another study conducted by Borrelly et al. (1991), it was reported that boron expedited wound healing in wounds with deep tissue loss and shortened the duration of intensive care unit stay (Blench et al., 1990).

In a study in which a hydrogel wound dressing with boric acid content was used, it was reported that hydrogels were quite flexible and strong antimicrobial properties, and it was recommended the use of boron as a wound dressing care material (Roy et al., 2010). In another study, in a case where systemic antibiotic treatment proved ineffective as wounds that develop in the lower extremities due to diabetes caused weak blood circulation, wound dressings containing boron content were found to be effective in wound healing (Kurtoğlu & Karataş, 2009a). In a study conducted by Demirci et al. (2015), certain active biological polymers were combined with sodium pentaborate and pentahydrate to form a gel. This gel has been reported to promote wound healing and display antimicrobial activity (Demirci et al., 2015). In a study conducted by Akgun et al. (2015), boron-based gel was used in dermatitis induced by radiation in rats and was shown to alleviate dermatitis (Akgun et al., 2015). Boron also improved wound healing and exerted an antimicrobial

effect on a diabetic wound models in rats (Demirci et al., 2016). An *in vitro* study conducted by Chupakhin et al. (2017) reported that hydrogels with silicone-boron content increased wound healing, regeneration, and antimicrobial activity (Chupakhin et al., 2017). In a randomized controlled trial by Kapukaya and KulaHCI (2020), it was determined that polyurethane sponge containing boric acid had a superior effect in the treatment of wounds with tissue defects at all stages of the wound compared to silver sponge (Kapukaya & KulaHCI, 2020).

The effect of boron on wound healing is still not well understood; however, it may play a role in the expression and synthesis of extracellular matrix molecules and the creation of proteoglycan, collagen, and cytokines. Boron increases Vascular Endothelial Growth Factor (VEGF), which promotes angiogenesis, which is important for wound healing (Dousset et al., 2002; Hosmane NS, 2012). In addition, boron increases the expression and synthesis of TNF $\alpha$ , which stimulates the release of fibroblasts, collagen, collagenase, and prostaglandin E2 (Nzietchueng et al., 2002b). It has been stated that despite its known effects on wound healing, more studies are needed to show the effect of boron on wound healing for boron to be used in routine wound treatment (Chupakhin et al., 2017; Hosmane NS, 2012).

In this study, based on the effects of boron on human health, we aimed to test the effect of two boron-containing containing compounds on wound healing through *in vitro* and *in vivo* studies to include boron in the health industry.

## MATERIALS AND METHODS

### Study Type

Two boron-containing molecules, whose effect on wound healing had been relatively well tested in a pilot study, were studied in phase 0, *in vivo* experimental study and *in vitro* in order to test them on a larger sample.

### Synthesis of the Molecules

Certain physical and spectroscopic properties of boron-containing Molecule 1 (2-(5- ethylpyridine -2-il) ethyl di((E)-octadec-9-en-1-il) borate)-(C<sub>45</sub>H<sub>82</sub>BNO<sub>3</sub>) and Molecule 2 (2-(5- ethylpyridine -2-il) ethyl dinonyl borate)-(C<sub>45</sub>H<sub>86</sub>BNO<sub>3</sub>) compounds, which we reported for the first time in te literature with this study, were determined.

### In Vitro Studies

The synthesis molecules were applied in vitro to L929 cells, which consists of rat fibroblast cell line, at different doses, and their IC50 values were determined. Subsequently, genotoxicity studies were performed using comet, micronucleus assay, Wound Healing Assay and Polymerase Chain Reaction (PCR).

### In Vivo Studies

A total of 60 male wistar albino rats were used in this study. The animals were housed in our temperature- and light-controlled vivarium with access to food and water as desired.

### Wound model

In the wound model, a full-thickness excisional wound (skin defect) was created using a 6 mm biopsy punch in rats under anesthesia (ketamine (100 mg/kg) + xylazine (100 mg/kg)). The wounds were opened at 5 mm distances on the backs of the rats, with four wounds on each rat (Masson-Meyers et al., 2020; Saeed & Martins-Green, 2007). The excisional wounds created symmetrically in full thickness were washed with 0.9% SF, and the rats were observed under anesthesia. The rats were then placed in solitary cages. No complications related to anesthesia developed.

Wound care was applied as a sterile open dressing, twice daily. The wounds were measured on days 0, 7, 14, and 30, and photographs of the wound area and closing percentage, color, depth, scar size, and

complication development in the healing process were taken from a distance of 15 cm with the researcher's smart phone with a 16MP camera and saved. The saved wound photos were analyzed in ImageJ software, which is a Java-based video processing program developed in National Health Institutes and Optical and Calculational Instrumentation Laboratory.

Biopsy samples were collected for histopathological and immunohistochemical analysis. Rats from which biopsies were obtained were not included in the study. Swabs were collected from the wound surface on certain days for microbiological analysis.

### Experimental Groups

Animals were divided into 6 groups. Group 1, molecule 1 + hydrogel containing cream was used and consisted of 12 rats. Group 2, molecule 2 + hydrogel containing cream was used and consisted of 12 rats. Group 3, molecule 2 + DMSO containing cream was used and consisted of 12 rats. Group 4, molecule 1 + hydrogel containing cream was used and consisted of 12 rats. Group 5 also used hydrogel and consisted of 8 rats. Group 6 also used DMSO and consisted of 8 rats.

In the study, both molecules were used in *in vivo* experiments with control groups. The molecules were examined in a total of six groups, including four control groups (hydrogel solvents of boron molecules and molecule 1 + hydrogel and molecule 2 + hydrogel and solvents of hydrogel and dimethyl sulfoxide (DMSO) and two experimental groups (solvents of boron molecules and their pure form molecule 1+DMSO and molecule 2+DMSO). Hydrogel (Hydrosorgel) used in standard wound care and DMSO, which is used as a solvent in *in vitro* studies and whose cytotoxic effect (micro) is not seen, were used as solvents to obtain the pure forms of our molecules. With their use in pure forms, we aimed to differentiate the sole effects of boron-containing molecules.

### Histopathology

Samples were fixed in 10% buffered formalin, processed and embedded in paraffin, and then cut. The sections were stained with hematoxylin & eosin (H&E) and Gomori trichrome stain. Histological evaluation was done by a pathologist in a blind randomly numbered fashion. Re-epithelization, collagen deposition in healing tissue and connective tissue remodeling were analyzed. The amount of collagen was rated on a subjective scale of 0 to 4, with 0, representing no collagen; 1, minimal collagen; 2, little collagen; 3, moderate collagen; 4, abundant collagen.

### Immunohistochemistry

Paraffin sections of 3- $\mu$ m were obtained, deparaffinized and rehydrated for immunohistochemical staining. Immunostaining was performed using the Leica Bond-Max automation and Leica Refine detection kit (Leica Biosystems, Newcastle, UK). Sections were processed with Anti-EGFR (ab32077 at 1/200 dilution, Abcam), anti-TNF $\alpha$  (ab199013 at 1/150 dilution, Abcam), anti-IL6 (ab9324 at 1/250, Abcam), anti-FGFR1 (ab10646 at 1/200 dilution), Anti-VEGFA antibody (ab39250 at 1/100 dilution, Abcam), anti IL-1 beta (ab205924 at dilution 1/500 dilution, Abcam).

Rat skin tissue for anti-EGFR, human lymph node for anti-TNF  $\alpha$ , human spleen tissue for anti-IL6, human umbilical cord for anti-FGFR1, human angiosarcoma for Anti-VEGFA, and mouse kidney tissue for IL-1 were used as positive controls. Negative controls, in which the primary antibodies were replaced by phosphate buffer saline, were carried out for each primary antibody. Staining intensity for all markers was divided as follows: negative (0), weak (1), moderate (2), and strong (3). For each immunohistochemical marker, the staining intensity in epidermis, vascular endothelial cells and fibroblasts were scored separately. The total score was divided by three to obtain a single staining intensity score for each lesion.

## Ethical Considerations

Ethical approval for the *in vivo* experiments was obtained from the Abant İzzet Baysal University Experimental Animal Ethics Board (2019/07). This study was carried out by the Republic of Turkey Ministry of Energy and Natural Resources, TENMAK Boron Research Institute with the support of project number 2018-31-07-15-004.

## Statistical Analysis

The wound sizes and wound closing percentages were recorded in a computer environment, and the wound sizes and mean values were calculated. The data were coded in a statistical program, and for normally distributed data, parametric tests of ANOVA and t-test were used, while for the data without normal distribution, non-parametric tests of Kruskal-Wallis and Mann-Whitney U test were used. To determine whether the data were normally distributed, skewness and kurtosis values (values between -2 and +2 were accepted as normal) were used (Lohana et al., 2019). Statistical significance was set at  $p < 0.05$ . To determine the difference in significance between the groups in the ANOVA test results, post hoc tests of Freidman, Tukey, and Games-Howell test were used.

Kruskal-Wallis test for multiple comparisons and Mann-Whitney U test for binary comparisons were used for histopathological. Chi-square test was used to compare epithelialization data. Epithelialization was divided into two as complete or incomplete. On the 7th day, epithelial hyperplasia was examined (graded as none, mild, moderate and severe). Acute inflammation (none, mild, moderate and apparently intense).

## RESULTS

### Synthesis of the Molecules

The molecules were synthesized in one step using basic esterification reaction conditions. In the reaction environment, 1 equivalent of boric acid, 1 equivalent of pyridine alcohol derivative, and 2 equivalents of oil

alcohol in toluene were refluxed by using a Dean–Stark apparatus. The obtained compounds were characterized using IR and NMR spectroscopy and they are stable at room temperature for months.

### *In Vitro* Studies

For *in vitro* studies, I929 cells, which form a rat fibroblast cell line, were reproduced and passaged in a culture environment as experimental and control groups. Their cytotoxic effects were evaluated using the trypan blue and XTT methods, and genotoxic effects were assessed using the micronucleus test. Wound Healing Assay test was applied in order to evaluate wound healing properties of the molecules. In this regard, different methods have been attempted in lengthy studies. In light of these methods, 500  $\mu\text{M}$  concentration was determined as IC50 value, and no genotoxic effect was observed at this concentration. No cytotoxic data were obtained in the Flow Cytometry test of cells treated with a 500  $\mu\text{M}$  concentration. The *in vitro* results for these molecules were similar.

### *In Vivo* Studies

#### Wound Sizes and Healing Percentages

When wound sizes were compared in the wounds opened using the standard method on day 0, no difference was observed between the groups; in other words, the wounds were homogenous in terms of size ( $p \geq 0.05$ ) (Figure 1). No depth developed in the wounds on day 7 when red granulation tissue and pink epithelization areas were observed, white/yellow flow appearance, which indicates infection, or black debridement areas were not observed. Wounds were closed on day 14, on day 30, complications, such as hypertrophic scars and keloids, were not observed.

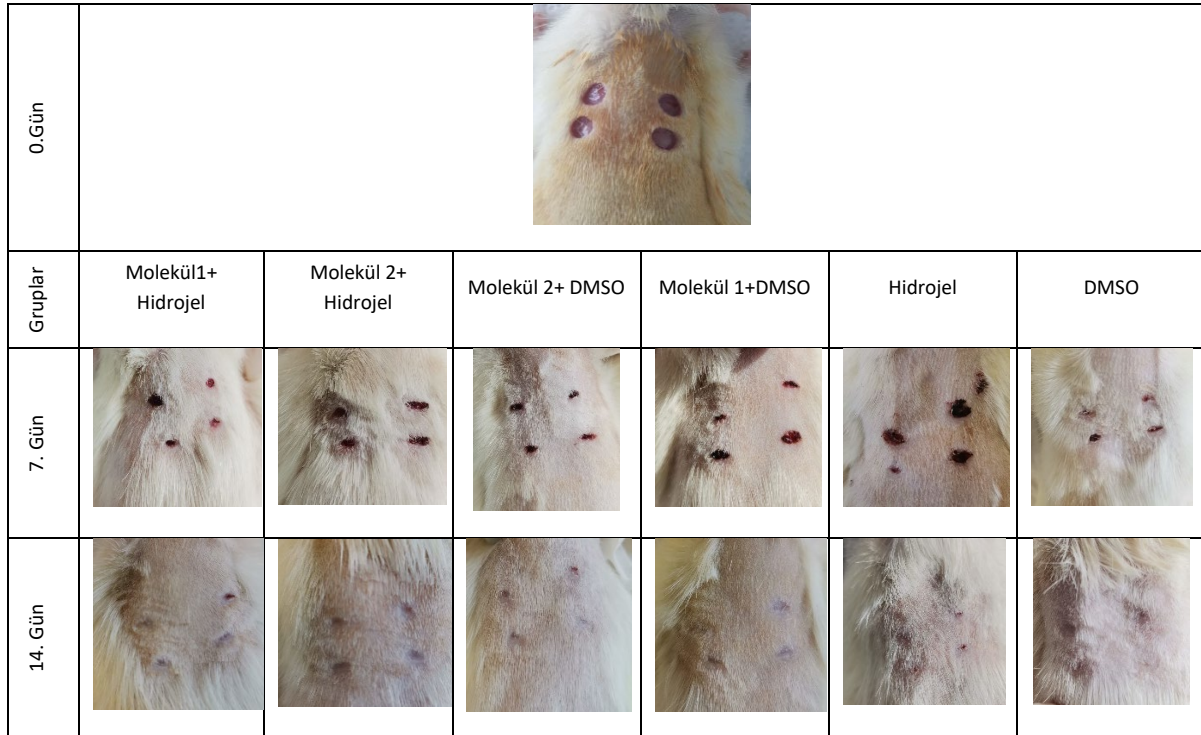
The results of the experiments showed that the healing percentage of molecule 1 on day 7 was higher than that of the other groups ( $p = 0.001$ ), the healing percentage of the pure form of molecule 1 with DMSO was higher than that of the other groups ( $p = 0.01$ ) and did not



cause any chronic subcutaneous inflammatory reactions ( $p < 0.05$ ), and full epithelization of pure forms

of both molecules without ulceration was found to be higher ( $p > 0.05$ ) (Table 1).

**Figure 1.** Photos of the wounds in the experimental and control groups on days 0, 7, 14, and 30.



**Table 1.** Comparison of Wound Healing Percentages in Rats

Measurement Time	Groups	N	$\bar{X}$	Ss	F	p
Day 7	Molecule 1 Hydrogel	12	80,41	5,6	13,43	0,000 <sup>a</sup>
	Molecule 2 Hydrogel	12	72,35	8,5		
	Molecule 2 DMSO	12	72,53	8,8		
	Molecule1 DMSO	12	68,56	10,3		
	Hydrogel	8	46,81	15,6		
	DMSO	8	60,57	9		
Day 14	Molecule 1 Hydrogel	12	84,22	5,6	5,625	0,000 <sup>a</sup>
	Molecule 2 Hydrogel	12	86,09	3,9		
	Molecule 2 DMSO	12	85,47	4,5		
	Molecule1 DMSO	12	88,69	3,5		
	Hydrogel	8	79,05	3,8		
	DMSO	8	76,85	11,6		

<sup>a</sup>Games-Howell post hoc test

### Histopathology

In terms of epithelial hyperplasia (epithelialization), the DMSO group was found to be different on day 7, although there was no statistically significant difference between the groups on day 14 ( $p>0.05$ ). Full epithelialization without any ulceration was higher in the

1+DMSO and molecule 2+DMSO groups than in the other groups (Table 2).

Collagen production was found to be higher in the molecule 1 and 2 groups on day 7 ( $p<0.05$ ) and in the molecule 2 group on day 14 ( $p>0.05$ ) (Table 3).

**Table 2.** Results of Chi-square test performed in order to determine the change in hyperplasia value by groups

Groups	Day 7			Statistical analysis	Day 14			Statistical analysis
	Full	Ulcerated	N		Full	Ulcerated	N	
Molecule1+Hydrogel	12	0	12		11	1	12	
Molecule2+Hydrogel	11	1	12	$\chi^2:24,000$	10	2	12	$\chi^2:0,410$
Molecule2+DMSO	11	1	12	df:5	12	0	12	df:5
Molecule1+DMSO	10	2	12	p:0,000	12	0	12	p:0,134
Hydrogel	2	6	8		4	0	4	
DMSO	4	0	4		4	0	4	

**Table 3.** Results of Kruskal-Wallis test performed in order to determine the change in collagen value by groups

Groups	Day 7			Day 14		
	N	X $\pm$ sd	p	N	X $\pm$ sd	p
Molecule1+Hydrogel	12	2,00 $\pm$ 0,85		12	2,50 $\pm$ 0,52	
Molecule2+Hydrogel	12	1,83 $\pm$ 0,38		12	2,58 $\pm$ 0,51	
Molecule2+DMSO	12	2,00 $\pm$ 0,42	0,057	12	2,75 $\pm$ 0,45	0,183
Molecule1+DMSO	12	2,16 $\pm$ 0,38		12	2,58 $\pm$ 0,51	
Hydrogel	8	1,37 $\pm$ 0,51		4	2,75 $\pm$ 0,50	
DMSO	4	1,75 $\pm$ 0,50		4	2,00 $\pm$ 0,00	

### Immunohistochemistry

In this study, a significant difference was observed in the wound cytokine results for molecules 1 and 2 (epidermal growth factor receptor [EGFR], tumor necrosis factor alpha [TNF $\alpha$ ], Interleukin-6- IL6, Interleukin-1- IL1, Fibroblast Growth factor receptor [FGFR], and vascular endothelial growth factor

receptor [VEGF]). The results were statistically significant. (Table 4). EGFR ( $p:0,391$ ), TNF- $\alpha$  ( $p:0,026$ ), IL-1( $p:0.054$ ), IL-6 ( $p>0.,104$ ), and VEGF ( $p>0,130$ ) levels were higher in the molecule 1 group on day 7.

In the pairwise comparison of molecules, TNF $\alpha$ , IL-1, IL-6, VEGF, and FGFR values were not statistically significant on day 14 ( $p>0.05$ ) (Table 4).

**Table 4.** Mann-Whitney U test results for wound cytokines on day 7 and 14

		Groups	N	$\bar{X} \pm sd$	U	z	p
Day 7	EGFR	Molecule 1+Hydrogel	16	1,99±0,43	106,00	-,857	0,391
		Molecule 2+Hydrogel	16	1,85±0,34			
	TNF $\alpha$	Molecule 1+Hydrogel	16	1,85±0,38	71,00	-2,219	0,026
		Molecule 2+Hydrogel	16	1,51±0,45			
	IL6	Molecule 1+Hydrogel	16	1,95±0,31	88,00	-1,624	0,104
		Molecule 2+Hydrogel	16	1,76±0,33			
	FGFR	Molecule 1+Hydrogel	16	2,87±0,27	120,00	-4,479	0,632
		Molecule 2+Hydrogel	16	2,91±0,22			
	VEGF	Molecule 1+Hydrogel	16	2,01±0,31	90,50	-1,502	0,133
		Molecule 2+Hydrogel	16	1,84±0,32			
	IL1	Molecule 1+Hydrogel	16	1,12±0,56	78,50	-1,929	0,054
		Molecule 2+Hydrogel	16	0,74±0,41			
		Groups	N	$\bar{X} \pm sd$	U	z	p
Day 14	EGFR	Molecule 1+Hydrogel	16	2,20±0,26	100,50	-1,085	0,278
		Molecule 2+Hydrogel	16	2,05±0,38			
	TNF $\alpha$	Molecule 1+Hydrogel	15	1,57±0,36	109,00	-,454	0,650
		Molecule 2+Hydrogel	16	1,64±0,39			
	IL6	Molecule 1+Hydrogel	15	2,37±0,56	101,00	-,783	0,434
		Molecule 2+Hydrogel	16	2,45±0,60			
	FGFR	Molecule 1+Hydrogel	15	3,00±0,00	120,00	,000	1,000
		Molecule 2+Hydrogel	16	3,00±0,00			
	VEGF	Molecule 1+Hydrogel	16	1,89±0,39	82,00	-1,804	0,71
		Molecule 2+Hydrogel	16	2,14±0,36			
	IL1	Molecule 1+Hydrogel	15	0,99±0,25	109,00	-,468	0,640
		Molecule 2+Hydrogel	16	1,01±0,37			

### Microbiology

In terms of microbiological findings, only the proliferated bacterial groups are shown in the table. No methicillin-resistant Staphylococcus aureus (MSRA) was detected in the microbiological analysis. All Staphylococcus aureus (s. aureus) bacteria were

determined to be methicillin-sensitive S. aureus (MSSA). No proliferation was observed in mold and yeast fungi.

As shown in Table 5, Coagulase-Negative Staphylococcus (CNS) bacterial colonization significantly changed with time (p = 0.022). In the



intergroup comparisons, there was no statistically significant difference between the groups in terms of the mean colony numbers on all three measurement

days. The colony number was very low. The absence of wound infections and closure of all wounds on day 14 supports this finding.

**Table 5.** Bacteria proliferation results on day, 0, 7, and 14

Bacteria	Day 0	Day 7	Day 14	p <sup>a</sup>
<b>S. aureus (MSSA)</b>	0(0-332)	100(50-250)	0(0-100)	0.678
<b>CNS</b>	62(33-360)	466(129-654)	277(127-1000)	0.022
<b>Streptococcus</b>	0(0-0)	43(4-263)	12(0-368)	0.056

<sup>a</sup>Freidman test Median (min-max).

## DISCUSSION

When studies conducted with different boron-containing compounds were examined, Dogan et al. (2014) demonstrated that in in vitro and in vivo experiments, sodium pentaborate significantly improved wound healing by increasing the migration capacity of cells and fibroblast activity (Doğan et al., 2014). Benderdour et al. (2000) found that among different boron compounds, a solution containing 3% boric acid affects the extracellular matrix and improves wound healing (Benderdour et al., 2000). In another study, it was reported that the use of boron-containing gel created significant healing on the skin of patients who received radiation therapy for breast cancer and developed dermatitis after the stimulation of cell reproduction and migration (Aysan et al., 2017). Regarding wound healing percentage in the present study, while hydrogel molecule 1 had a higher percentage of healing on day 7 compared to the other groups ( $p = 0.001$ ), the pure form of DMSO molecule 1 had a higher wound healing percentage on day 14 compared to the other groups ( $p = 0.001$ ), it did not lead to chronic subcutaneous inflammatory reaction ( $p < 0.05$ ), and full epithelization of the pure forms of the molecules without ulceration was found to be high ( $p > 0.05$ ). As it was expected that the wound healing effects of boron would occur in the proliferative phase, according to the literature, we concluded that the boron-containing molecules we used were effective in wound healing.

Wound healing involves a series of complex cellular damage and healing processes. This process includes consecutive molecular and biochemical events and includes the phases of hemostasis, inflammation, proliferation, and wound closure (restructuring) (Abbas et al., 2005). The proliferation phase is the phase in which fibroblast movement, collagen synthesis, and re-epithelization start (Dhivya et al., 2015; Velnar et al., 2009). Collagen is an important signaling molecule that plays a key role in all phases of wound healing (Penelope et al., 2018). In the present study, collagen formation by new boron-containing molecules was examined in terms of thickness and intensity. Wound samples were collected on days 7, 14, and 30 for histochemical and immunohistochemical staining. Collagen formation in the molecule 1+hydrogel, molecule 2+hydrogel, and hydrogel groups on days 7 and 30 was significantly higher than that in the control groups. On day 14 of healing, only the molecule 2+hydrogel group contained a significantly higher ratio of collagen than the control groups.

Collagen is the main extracellular protein in the granulation tissue of a healing wound, and an increased collagen concentration provides a tissue matrix with strength and integrity (Alqahtani et al., 2020). Fibroblasts are stimulated for collagen production, and the synthesized collagen is the most important element of connective tissue (Gupta et al., 2019; Shrivastav et al., 2018). In an in vitro study conducted with live cells, it was found that although it

did not have a direct effect, boron increased fibroblast activity (protease activity with trypsin-like activity, protease, collagenase, and cathepsin D) and TNF $\alpha$  stimulation (Penelope et al., 2018). Ramanathan et al. reported that collagen-coated nanofibrous scaffolds expedited the wound healing process (Ramanathan et al., 2017). In addition, in studies conducted, the effects of agents aiming to increase collagen synthesis in wound healing have been demonstrated, and various collagen-containing topical and oral products have been recommended for chronic wounds because of their great contributions to the wound healing process (Albaugh et al., 2017; Chattopadhyay & Raines, 2014).

Clinical formulations such as boron oxygen-sensing nanoparticles (BNPs) can provide a promising solution to directly map oxygen levels in wounds. The clinical use of films has the potential to track the healing process of chronic wounds by guiding care decisions (N & P., 2019).

EGFR plays a critical role in epidermal regeneration during wound healing, and stimulates granulation tissue formation. As a primary stimulant and regulator, EGFR is effective in fibroblast migration and wound closure (Abbas et al., 2005; Aysan et al., 2017; Repertinger et al., 2004). This is expected to increase in the case of open wounds. Significantly higher levels of EGFR in the molecule 1, molecule 2, and hydrogel groups compared to the other groups ( $p < 0.05$ ) support wound healing, and this result is consistent with the data we found in terms of collagen. Although our FGFR results were not significantly different between the groups, they were highly consistent with the collagen results in all groups, molecule 1, molecule 2, and the hydrogel groups. In addition to fibroblast stimulation, FGFR is an effective factor for new vein formation (Abbas et al., 2005; Aysan et al., 2017).

Although there is no clear correlation between specific cytokine synthesis and collagen synthesis in the literature, it was observed in the present study that increased cytokine synthesis correlated with -increase in collagen amount. IL-1, IL-6, and TNF- $\alpha$  cytokines are important in acute inflammation. In the acute phase, TNF- $\alpha$  increased, and in our study, TNF- $\alpha$  significantly

increased in the acute phase in the molecule 1 and molecule 2 groups. TNF- $\alpha$  stimulates IL-1, and IL-1 stimulates IL-6. IL-6 stimulates plasma proteins including fibrinogen (Kurtoğlu & Karataş, 2009; Kuru & Yarat, 2017; Metcalfe & Ferguson, 2007). IL-1, IL-6, and TNF $\alpha$  were found to be significantly higher in the molecule 1+hydrogel, molecule 2+hydrogel, and hydrogel groups than in the other groups. Collagen synthesis actively starts with the proliferation process and increases to the maximum level in 4 to 21 days (Penelope et al., 2018). In addition to their contributions to the acute phase, IL-1, IL-6, and TNF- $\alpha$  cytokines also stimulate collagen synthesis. Significantly high collagen and cytokine levels were found in molecule 1+hydrogel, molecule 2+hydrogel, and hydrogel groups corroborate each other.

In wound infections, we mostly encounter opportunistic infections by bacteria present in the skin flora (Edwards-Jones, 2016). Coagulase-negative staphylococci (CNSs) are the most common bacteria found in the skin flora (Avcioglu et al., 2019). In the present study, it was determined that CNSs significantly changed over time. In the microbiological comparison of bacteria between the groups, no statistically significant difference was found in terms of mean colony numbers on the three measurement days for each group. The colony counts were found to be low. There was also no proliferation of mold and yeast fungi. The absence of wound infection and wound closure on day 14 support this finding.

## CONCLUSION AND RECOMMENDATIONS

It was determined that molecule 1 could be used in the treatment of open wounds and wound support products, as it is advantageous in terms of wound closing percentage and IL-1, IL-6, TNF $\alpha$ , EGFR, FGFR, and VEGF cytokines.

It was also found that molecule 2 could be used in many cosmetic products such as hand-body cream/care creams and rash creams after performing dermatological tests, as it is advantageous in terms of collagen production in addition to its effects similar to

those of molecule 1. As a result of the study, 2 patent applications were made.

In this study, the effect of innovative boron compounds on wound healing was demonstrated. For wound healing, which follows a complex process, new alternative products can be used in different wounds in different individuals instead of traditional uses.

### Conflict of Interest

The authors declare no conflicts of interest.

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