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ORIGINAL ARTICLE

Antimicrobial Effects of Nanocrystalline Silver-Loaded Wound Dressings in Full-Skin Thickness Rat Burn Wounds

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Abstract

Introduction: Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care to minimize mortality and morbidity. Despite major advances in the management of burn patients, infectious complications remain a major contributor to morbidity and mortality. In this experimental study, the effect of two different nanocrystalline silver dressings on Candida albicans-contaminated full-thickness burn wounds in rats was analyzed.

Methods: A full-thickness skin burn was formed in a total of 24 female 200-230 gr Sprague-Dawley rats. After the burn wound was seeded with 10^8 CFU/ml standard strain of Candida albicans ATCC90028, the animals were separated into three groups, and the antifungal activity of nanocrystalline silver-containing nanofiber dressing and Acticoat dressing was compared. All rats were sacrificed on the seventh day. Biopsies were obtained from the center of the burn eschar and the paravertebral muscles beneath the burn eschar. Blood was drawn from the left ventricle, and lung biopsies were examined by performing thoracotomies.

Results: There was no significant difference in Candida and bacteria growth on the burn eschar, muscle, lung tissue, and blood cultures among the groups ($p>0.5$).

Discussion and Conclusion: Nanocrystalline silver dressings facilitate wound healing and have wide antimicrobial effects. Although these effects are well known, the antifungal and antibacterial activity of the nanocrystalline silver dressings used in our study could not be revealed.

Keywords: Acticoat dressing; Burn; Nanocrystalline silver.

One of the common and destructive types of trauma is burns. To minimize mortality and morbidity, patients with severe thermal injuries require urgent professional care. Despite tremendous advancements in the management of burn patients, infectious complications after burn injuries remain a common cause of morbidity and mortality. When a bacterial infection occurs, using broad-spectrum topical and systemic antimicrobials and

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isolating the patient have been successful approaches for treatment. However, the incidence of severe fungal infections has risen among burn patients. Candida infections increase mortality in systemic infections through burn wound colonization, which delays wound healing and causes persistent infections.

Moyer et al.[1] were the first to report the antimicrobial effect of silver. In its metallic form, silver does not exhibit any antimicrobial activity; however, upon ionization, it becomes active in solutions, thereby affecting the cell wall, altering proteins, and inhibiting transcription by DNA binding. Therefore, silver demonstrates potential antibacterial and antifungal properties.

The biological estimation of silver is remarkably enhanced through nanotechnology and the ability to release silver from a nanocrystalline construction[2]. Nanocrystalline silver creates a wide surface area for reaction with water. In moisturized conditions, nanocrystalline silver continues to release silver ions into the environment[3], thereby exhibiting potential antimicrobial effects. Nanosilver exerts antifungal activity by disrupting the cell membrane structure, thereby inhibiting the fungal budding process[4]. Nanofibers have high porosity and specific surface areas and mimic the extracellular matrix. When an adequate drug is combined with nanofibers, it can be delivered into the healing tissue in an evenly diffused and controlled manner[5]. Additionally, nanofibers promote wound healing by enabling adequate oxygen, allowing water vapor diffusion, and protecting the wound from infection and loss of hydration. Due to these properties, a new wound dressing concept involves a nanofiber construction and includes nanosilver particles as the active material.

The nanofiber wound dressings that were developed and applied in this experiment consist of three layers (Fig. 1): upper and lower polycaprolactone protective sheets and a middle polyethylene oxide sheet including the active substance. The nanofibers were produced through electrospinning. The active substance comprised 1% nanosilver. Polycaprolactone and polyethylene oxide were used as base substances due to their biocompatibility and biodegradability. Nanofiber layers contain 0.24±0.04 mg of silver per milligram (approx. 99.99% silver) and include 20–50 nm nanosilver particles (Figs. 2-6).

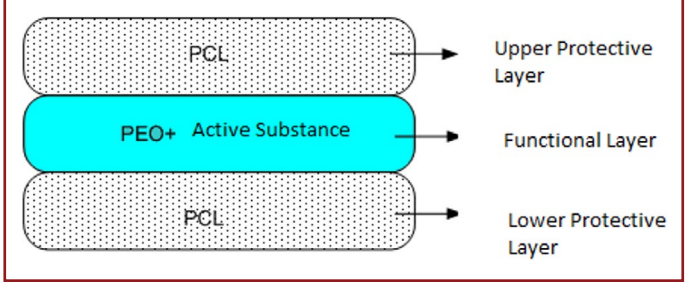

Figure 1. Structure of the Nanofiber Wound Dressing Developed in This Study. PCL: Polycaprolactone; PEO: Polyethylene Oxide.

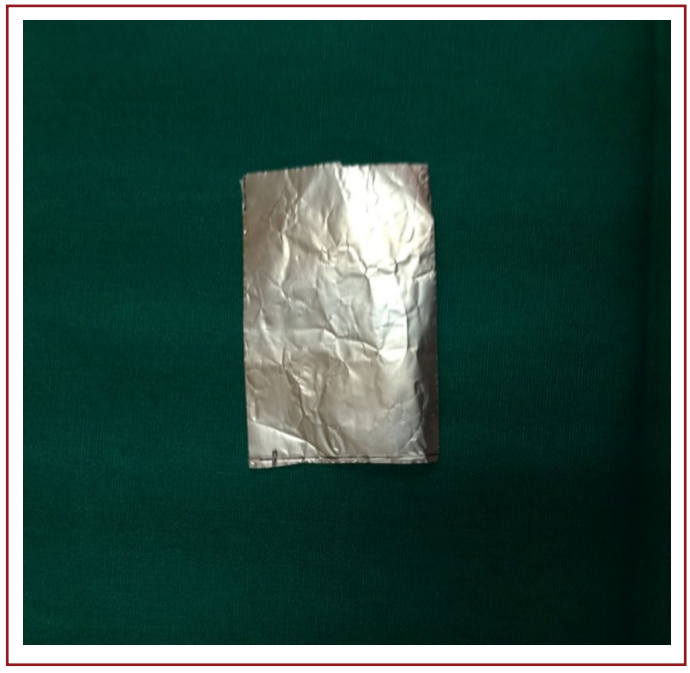

Figure 2. The Nanofiber Wound Dressing Developed in This Study (Front View).

Figure 3. The Nanofiber Wound Dressing Developed in This Study (Back View).

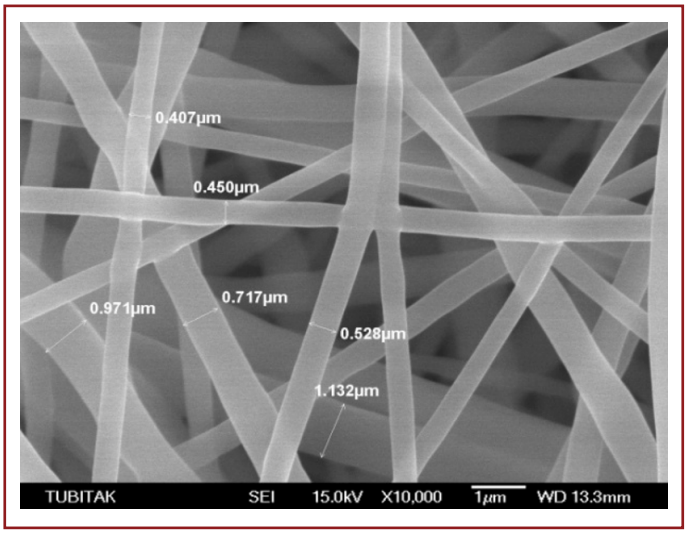

Figure 4. Polycaprolactone–Polyethylene Oxide–Polycaprolactone Nanofiber Section Electron Microscope Images (Dry).

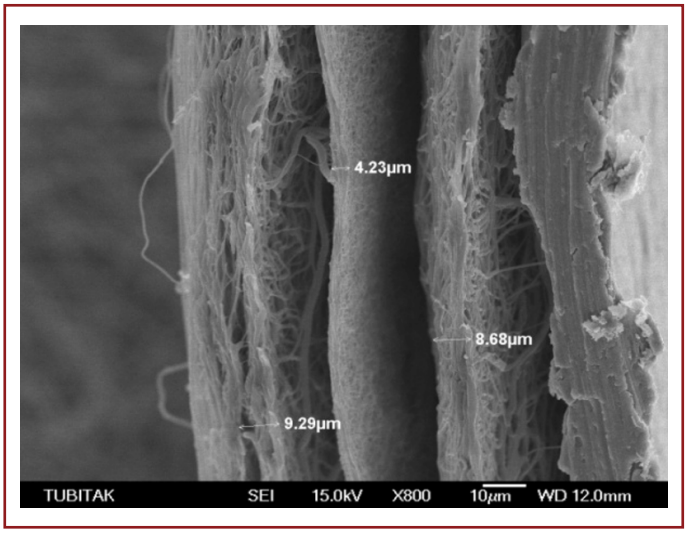

Figure 5. The Nanofiber Wound Dressing Surface Electron Microscope Images (Dry).

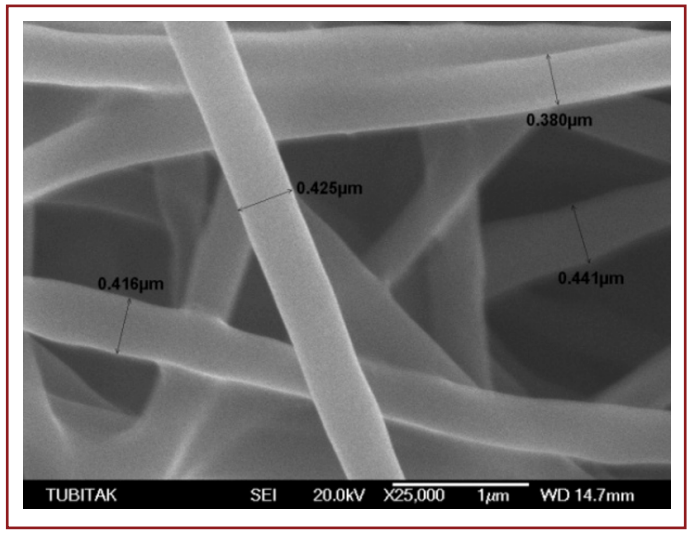

Figure 6. Electron Microscope Images of the Polycaprolactone–Polyethylene Oxide–Polycaprolactone Nanofiber (Wet).

The Acticoat dressing is a three-layered protective garment developed by Dr. Burrell. This dressing consists of a hydrophilic polyester core between two silver-coated, high-density polyethylene sheets. These sheets are bound together with ultrasound welds. The high-density polyethylene sheets of Acticoat contain 0.24±0.04 mg of silver per milligram (approx. 99.99% silver). They also take the form of columns containing nanocrystals and are highly porous[6]. This physical structure, along with oxygen atoms and molecules trapped inside the crystal lattice, contributes to the increased solubility of the films, continuing to release silver up to 66 mg/L, a concentration 50–100 times greater than that expected from normal silver metal. Polyethylene layers containing the hydrophilic polyester core and silver crystals on both sides of the core provide the moisture required for silver release[6]. Additionally, this silver dressing initiates a continuous Ag+ release when it is wetted with sterile water. When released, ions combine with bacteria and proteins in the exudate, and silver is released again to restore balance. Thus, as noted in recent publications, this silver release completely and rapidly destroys all pathogens on the wound surface. Silver acts on bacteria by attaching to the cell membrane and inactivating intracellular proteins and enzymes[7]. Acticoat does not require frequent dressing changes, as its antimicrobial activity lasts longer.

This study sought to compare the antimicrobial efficiencies of a novel nanofiber wound dressing containing nanosilver and the Acticoat dressing as active substances for the treatment of Candida albicans-contaminated full-thickness burns in rats.

Materials and Methods

The research procedure was approved by the Marmara University Animal Studies Ethical Committee. Twenty-four female Sprague–Dawley rats weighing about 200–230 g were used.

Intraperitoneal injection of ketamine (75–100 mg/kg) was used to anesthetize the rats. After weighing, their backs were shaved. Burn wounds, covering >15% of the rats' body surface area, were created through immersion in water at 100°C for 8 seconds. Full-thickness tissue injuries were confirmed with histological evidence. Lactated Ringer's serum (2 mL/100 g) was given subcutaneously for resuscitation, and paracetamol (200 mg/kg per day)

was added to their water for analgesia. Ten minutes after submersion in hot water for burn induction, the wound on each rat was seeded with 0.5 mL broth containing 10^8 colony-forming units (CFUs) of *C. albicans* (ATCC 90028). Throughout the study, the rats were housed individually at ambient room temperature and provided with sufficient water and laboratory chow.

The rats were randomly separated into three groups after 24 hours. Each group consisted of eight rats as follows: Group 1—control group (no topical agent was used), Group 2—nanosilver-containing nanofiber dressing group, and Group 3—Acticoat dressing group. The dressings were replaced every other day for groups 2 and 3. Initially, the dressings were moistened with sterile physiological serum, and then immobilized with sterile gauze and skin staples.

On the seventh day, all rats were sacrificed. A high dose of ketamine was injected intraperitoneally. Their weights were recorded. A weight loss of >7.5% of their total body weight was indicative of systemic disease. Tissue samples were taken from the middle of the burn eschar and the paravertebral muscles underneath the burn eschar. Thoracotomies were performed, blood was aspirated from the left ventricle of the heart, and lung biopsies were obtained. Tissue weighing 0.25 g, 0.15 g, and 0.10 g was collected from the lung, eschar, and paravertebral muscles, respectively. All samples were obtained under aseptic conditions and prepared for culture in the clinical microbiology laboratory. The tissue samples were weighed in sterile petri dishes on a microbalance and placed in 2-mL brain-heart infusion broth. The specimens were diluted in saline, and 0.1-mL portions were seeded on Sabouraud dextrose agar and 5% sheep blood agar plates using a calibrated loop. Additionally, a smear was prepared for each specimen for Gram staining. After 24–48 hours of incubation at 35°C, colonies were enumerated. Gram staining was performed to identify all detected colonies. The Miles and Misra formula was used to determine the quantitative numbers for all colony types.

Pediatric blood culture bottles were seeded for blood cultures and monitored with a BacT/Alert 3D (BioMerieux, France) automated system. All bottles that tested positive by the device were subcultured on 5% sheep blood agar and Sabouraud dextrose agar and incubated for 24–48 hours at 35°C in aerobic conditions. The presence of *C. albicans* was confirmed with a germ tube test (Tables 1 and 2). The results among groups were compared statistically.

Table 1. Candida albicans growth numbers in the study groups (CFU/g of tissue)

	Control Group (n=8)				Acticoat Group (n=8)				Nanofiber Wound Dressing Group (n=8)			
	Eschar	Muscle	Lung	Blood	Eschar	Muscle	Lung	Blood	Eschar	Muscle	Lung	Blood
1	21×0^4	1×10^3	2×10^2	-	1×10^4	5×10^3	1×10^3	-	1×10^8	3×10^3	1×10^3	-
2	1×10^8	2×10^3	-	-	1×10^8	1×10^4	5×10^3	-	2×10^4	3×10^3	-	-
3	1×10^8	1×10^4	-	-	1×106	1×10^4	-	-	1×10^8	-	-	-
4	1×10^8	1×10^4	1×10^3	2×10^2	1×10^8	2×10^3	-	-	1×10^8	2×10^3	1×10^3	-
5	1×10^8	4×10^2	-	-	1×10^8	2×10^4	1×10^4	-	1×10^8	6×10^3	2×10^2	-
6	1×10^4	1×10^3	-	-	1×10^4	4×10^3	6×10^2	-	1×10^8	5×10^3	-	-
7	1×10^8	1×10^3	-	-	1×10^8	-	-	-	1×10^4	4×10^2	-	-
8	1×10^8	4×10^2	-	-	5×10^3	4×10^2	-	-	1×10^8	5×10^3	-	-

Table 2. Bacteria growth numbers in the study groups (CFU/g of tissue)

	Control Group (n=8)				Acticoat Group (n=8)				Nanofiber Wound Dressing Group (n=8)			
	Eschar	Muscle	Lung	Blood	Eschar	Muscle	Lung	Blood	Eschar	Muscle	Lung	Blood
1	1×10^8	-	-	1×10^8	1×10^8	2×10^4	4×10^2	-	1×10^8	1×10^4	-	1×10^8
2	1×10^8	2×10^4	-	-	1×10^8	1×10^7	9×10^3	1×10^8	1×10^8	1×10^6	1×10^5	1×10^8
3	1×10^8	1×10^7	2×10^3	1×10^8	1×10^8	1×10^3	-	1×10^8	1×10^4	8×10^3	4×10^2	1×10^8
4	1×10^8	2×10^5	2×10^3	1×10^8	1×10^8	7×10^3	3×10^3	1×10^8	1×10^8	1×10^4	-	-
5	1×10^8	2×10^3	8×10^2	1×10^8	1×10^8	1×10^8	2×10^2	1×10^8	1×10^8	2×10^4	4×10^2	1×10^8
6	3×10^3	6×10^2	-	1×10^8	1×10^6	1×10^4	-	1×10^8	1×10^8	2×10^3	6×10^2	1×10^8
7	1×10^8	-	-	1×10^8	1×10^8	2×10^4	6×10^3	1×10^8	1×10^8	2×10^3	-	1×10^8
8	2×10^2	-	-	-	1×10^8	1×10^3	-	-	1×10^8	6×10^3	-	1×10^8

Table 3. Number of rats with Candida albicans colonization in each group

	Control (n=8) Median (Min-Max)	Acticoat (n=8) Median (Min-Max)	Nanofiber Wound Dressing Group (n=8) Median (Min-Max)	p
Eschar	1×10^8 (1×10^4–1×10^8)	$5{,}05 \times 10^7$ (0–1×10^8)	1×10^8 (0–1×10^8)	0.420
Muscle	4×10^2 (0–1×10^4)	$4{,}5 \times 10^3$ (0–2×10^4)	3×10^3 (0–1×10^4)	0.254
Lung	0 (0–1×10^3)	1×10^8 (0- 1×10^8)	0 (0–1×10^3)	0.409
Blood	0 (0–2×10^2)	-	-	0.368

Table 4. Number of rats with bacterial colonization in each group

	Control (n=8) Median (Min-Max)	Acticoat (n=8) Median (Min-Max)	Nanofiber Wound Dressing Group (n=8) Median (Min-Max)	p
Eschar	1×10^8 (200–1×10^8)	1×10^8 (1×106–1×10^8)	1×10^8 (1×104–1×10^8)	0.640
Muscle	$1{,}3 \times 10^3$ (0–1×10^7)	$1{,}5 \times 10^4$ (1×103–1×10^8)	9×10^3 (2×104–1×10^6)	0.359
Lung	0 (0–2×10^3)	$1{,}7 \times 10^3$ (0–9×10^3)	2×10^2 (0–1×10^5)	0.273
Blood	1×10^8 (0–1×10^8)	1×10^8 (0–1×10^8)	1×10^8 (0–1×10^8)	0.785

Statistical evaluation was conducted using SPSS for Windows statistical software. In addition to descriptive statistical methods (median, minimum, maximum, frequency, and rate), the Kruskal-Wallis variance test was performed to compare variances with non-normal distribution between groups. The Mann-Whitney U test was applied to compare the groups with variations. The significance level was set at $p<0.05$ (Tables 3 and 4).

Results

All rats were sacrificed on the seventh day. Tissue samples were taken from the middle of the burn eschar and the paravertebral muscles underneath the burn eschar. Blood was aspirated from the left ventricle of the heart, and lung biopsies were examined by performing thoracotomies.

No animal deaths occurred during the research prior to sacrifice. *C. albicans* growth was observed in the eschar samples and paravertebral muscle biopsies of each rat in the control group. The number of fungal colonies ranged from 4×10^2 to 1×10^8 CFU/g of tissue. *C. albicans* growth was also encountered in two lung biopsies and one blood culture in the control group, with numbers ranging from 2×10^2 to 1×10^3 CFU/g of tissue.

C. albicans growth was observed in eight eschar tissues and seven paravertebral muscle biopsies in the groups treated with nanosilver-containing nanofiber dressing and Acticoat dressing. No fungal proliferation was found in the blood cultures of either group. However, *C. albicans* growth was recorded in four lung biopsies in the Acticoat dressing group and three lung biopsies in the nanosilver-containing nanofiber dressing group, with proliferation amounts ranging from 2×10^2 to 1×10^8 CFU/g of tissue. Furthermore, varying degrees of bacterial colonization were observed in almost all of the eschar, muscle, lung, and blood cultures in all groups.

Statistical evaluation found that no significant differences were observed among the three groups in terms of bacterial and fungal colonization in eschar, muscle, lung, and blood cultures ($p>0.05$).

Discussion

Degradation of the skin barrier in patients with burns, co-occurring with depressed local and systemic immune responses, leads to infectious complications. Following the deterioration of skin integrity in these patients, microorganisms originating from their own skin flora,

gastrointestinal and respiratory tract flora, or the hospital environment quickly colonize burn wounds, resulting in microbial invasion. Burn trauma leads to tissue destruction, and avascular burn eschars create a favorable environment for infections that can progress to septicemia[8,9]. Infection may worsen with immunosuppression, which is often observed in burn patients[10].

Infection rates vary depending on the size of the burn wound, wound care applied, and host factors such as nutritional status, age, immunosuppression, and comorbidities. Thus, the management of burn wound infectious problems greatly impacts the morbidity and mortality of these patients. Fungal pathogens, particularly Candida strains, cause opportunistic infections with increased antifungal drug resistance in patients with burns treated with broad-spectrum topical and systemic agents.

Topical and systemic antimicrobial substances are used in the management of patients with burns, especially to prevent infectious complications. Antiseptic, antimicrobial, anti-inflammatory agents, and broad-spectrum antibiotics, as well as silver, have been used for this purpose since ancient times. Topical ointments containing silver and silver dressings have been included in these antimicrobial agents.

Compared with other forms of silver, silver nanoparticles exhibit more effective antimicrobial activity against microorganisms due to their high surface area. Nanosilver particles are efficacious at concentrations 30 times lower than normal silver ions. Nanoparticle forms of silver adhere to and permeate bacterial cell membranes by interacting with proteins that include sulfur in the membrane. When silver nanoparticles penetrate bacteria, the bacterial cell content clusters in the middle to protect the DNA from the silver ions. Nanoparticles primarily attack the respiratory chain and inhibit cell division, eventually leading to cell death[11].

A similar study was conducted in 2012 with the novel wound dressing design used in the present study. In the 2012 study, the efficacies of topical silver sulfadiazine and two newly engineered nanofiber dressings consisting of nanosilver and silver sulfadiazine as active substances were analyzed. Although this study reported that the antifungal activity of nanosilver particles was known to be much higher, the silver sulfadiazine-loaded nanofiber dressing was found to be more effective in preventing fungal infections than other agents[12]. This was believed to be due to the lower nanosilver content (0.11 mg/cm^2) in the designed nanofiber dressing than that in currently widely used nanosilver dressings. Thus, the nanosilver content was increased to comparable levels as other nanosilver dressings (0.84–1.34 mg/cm^2) and evaluated for its efficacy in our study. However, our study found no statistically significant differences among the nanofiber wound dressing, Acticoat dressing, and control groups in terms of *C. albicans* and bacterial growth ($p>0.05$). This result is interesting because the antimicrobial efficacy of nanosilver particles is well known. In addition, the efficacy of the Acticoat dressing has been demonstrated in various key studies in the literature.

The antimicrobial effects of silver have been firmly established in the literature, and silver is widely used as a clinical agent. Nanocrystalline silver is a more rapid and efficacious antimicrobial material than normal silver ions. The efficacy of nanosilver has been demonstrated many times in vitro and in animal and even human experiments. Wound dressings with nanofibers also improve wound healing, as they release strong drug properties. Despite all these, the superiority of both the Acticoat dressing and nanosilver-added nanofiber dressings to the control group could not be established ($p>0.05$). The reason for this may be the difficulty of conducting animal studies or the inadequacy of animal numbers and parameters used in the experiment. If the number of animals in the groups were increased, and the effects of these dressings on wound healing examined, a statistically remarkable result might be achieved.

Conclusion

In conclusion, in this study, we compared the antifungal and antibacterial effects of two nanocrystalline silver wound dressings, one of which was newly designed and the other frequently used in clinical applications. We aimed to demonstrate the reliability of the newly developed nanofiber dressing with nanosilver. However, our experiment found no statistically remarkable differences among study groups in terms of *C. albicans* and bacterial growth. Although the antimicrobial and positive wound healing effects of silver have been observed in burn wounds, the antifungal and antibacterial effects of the nanosilver dressings could not be demonstrated in this study. Thus, more extensive studies are needed to demonstrate the effectiveness of the new nanosilver nanofiber wound dressing.

Ethics Committee Approval: The research procedure was approved by the Marmara University Animal Studies Ethical Committee.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: A.İ.İ., N.S.Ç.; Design: A.İ.İ., Z.B., N.S.Ç.; Supervision: N.S.Ç., S.A.; Fundings: A.İ.İ., Z.B.; Materials: A.İ.İ., Z.B., S.C.A.; Data Collection or Processing: A.İ.İ., S.C.A., Z.B.; Analysis or Interpretation: A.İ.İ., N.S.Ç., S.A.; Literature Search: A.İ.İ., Z.B.; Writing: A.İ.İ., N.S.Ç., Z.B.; Critical review: A.İ.İ., N.S.Ç., S.A.

Conflict of Interest: None declared.

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