Effects of functional poly(ethylene terephthalate) nanofibers modified with sericin-capped silver nanoparticles on histopathological changes in parenchymal organs and oxidative stress in a rat burn wound model

Yaşar ŞAHİN[1,a,✉], Zehra GÜN GÖK[2,b], Mehmet Eray ALÇIĞIR[3,c], Miyase ÇINAR[4,d]

[1]Kırıkkale University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Kırıkkale, Türkiye; [2]Kırıkkale University, Engineering and Architecture Faculty, Bioengineering Department, Kırıkkale, Türkiye, [3]Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, Kırıkkale, Türkiye, [4]Kırıkkale University, Faculty of Veterinary Medicine, Department of Biochemistry, Kırıkkale, Türkiye

[a]ORCID: 0000-0001-5936-4210; [b]ORCID: 0000-0002-6024-9129; [c]ORCID: 0000-0002-5165-5854; [d]ORCID: 0000-0003-3806-9938

ARTICLE INFO

ABSTRACT

In this study, it was aimed to investigate the effect of a poly(ethylene terephthalate)-g-poly(hydroxyethyl methacrylate) (PET-g-HEMA) nanofiber wound dressing modified with sericin-coated silver nanoparticles (S-AgNPs) on internal organs, oxidative stress, and biochemical parameters. To establish a burn model, the backs of anesthetized rats were shaved and then third-degree burns were created with a round-bottomed stainless steel rod 2 cm in diameter kept in 100 °C water for 20 seconds. The wounds of the negative control group (G1) were covered with standard bandages; the wounds of the positive control group (G2) were covered with silvercel, used as burn wound material; and the wounds of the experimental group (G3) were covered with PET-based dressing material. Histopathological changes in organs (liver, kidneys, heart, pancreas, lungs), total oxidant status (TOS), total antioxidant status (TAS), nitric oxide (NO), and biochemical parameters (serum aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma glutamyl transpeptidase [GGT], creatine kinase, lactate dehydrogenase [LDH], total protein, albumin, globulin, urea) were examined. Compared with the G1 group, plasma AST, ALT, and GGT levels were found to be significantly decreased in G2 and G3 (P<0.001). Plasma TAS was found to be significantly increased in G2 and G3 compared to G1 (P<0.05). Compared to the G1 group, degenerative and necrotic changes in the liver, kidneys, and pancreas were found to be significantly reduced in G2 and G3 (P<0.05). In conclusion, this work demonstrates that the synthesized PET-based wound dressing material has the capacity to be used commercially.

Introduction

The skin is the largest organ of the body and has an important role in sensing the environment and maintaining body homeostasis and temperature control, as well as protecting against pathogens such as viruses, bacteria, toxins, and environmental contamination (42, 50). Wounds occur with the deterioration of the integrity of the skin as a result of deliberate or accidental causes or diseases (42). In general, wounds can develop due to many factors and conditions, including surgery, pressure, cuts, and diseases (diabetes or vascular) (33). Burn injuries are traumas whose time and place cannot be predicted,

affecting many organs in addition to the skin (16, 25). They may be caused by friction, freezing, radiation, electrical currents (high voltage electrical), or chemicals. However, most burn injuries are caused by heat produced by extremely hot solid and/or liquid substances (25). Although it is difficult to classify burn wounds, they are generally divided into 4 groups according to the degree of damage to the layers of the skin (32). These are first-degree burns of the skin, second-degree superficial partial-thickness burns, third-degree full-thickness burns, and fourth-degree burns that affect deep tissues such as muscle or bone (25).

After an injury for any reason, the skin must immediately regain its integrity in order to continue its functions (13). The wound healing process occurs in three phases, which are interrelated and ordered consecutive steps. The first of these is inflammation. This phase is followed by the proliferative phase, and the final phase entails tissue maturation and remodeling (13, 17). After burn damage to the skin, which is the body's external barrier, the immune system deteriorates and susceptibility to infections increases, causing a delay in wound healing processes (47). In addition, after severe burns, free radicals (reactive oxygen species) are released, which exert local or systemic effects as a result of physiopathological responses. Thus, the release of increased levels of free radicals can cause immunosuppression, infection, sepsis, tissue damage, and multi-organ failure (29).

It is often difficult to choose the appropriate dressing after a burn wound; this decision depends on various factors such as the depth of the burn, the condition of the wound bed, the desired moisture retention and drainage of the wound, and the frequency of dressing changes (47). There are many specialty dressings available, some of which are designed for special occasions and for ease of use (22). Wound dressing products such as gauze, plasters, and bandages are used, but there are also modern dressing products. Among these modern products, there are many newly developed products such as bioactive dressings and medicated dressings (15). In recent years, wound dressing products have been created using nanotechnology (52). Silver compounds have been used in medical fields for centuries, but silver's importance as a suitable treatment option has recently increased in applications against infections in cases of burns, open wounds, and chronic ulcers (6, 41). However, it has recently been reported that silver compounds, which have serious cytotoxic activity against various host cells, may also delay the wound healing process (6, 27, 36). In addition to silver compounds such as silver sulfadiazine, widely used in burn treatment today, there are various newly developed compounds such as silver-coated nanoparticles (27, 36, 41). In general, silver has both beneficial properties and harmful cytotoxic effects that raise concern. It may pose a potential biohazard for health when used in various products. In addition, extensive production and application of silver will increase its release into aquatic environments such as rivers and lakes, thereby posing an environmental hazard (14). In our previous in vivo study, we obtained a functional and antimicrobial biomaterial surface to be used for wound dressing. Nanofiber membranes obtained from a poly(ethylene terephthalate)-g-poly(hydroxyethyl methacrylate) (PET-g-HEMA) copolymer with 55% HEMA grafting were coated with green synthesized sericin-coated silver nanoparticles (S-AgNPs) and the biomaterial was then successfully administered to experimental groups (19).

Subsequently, in the present study, S-AgNP-coated PET-g-HEMA nanofibers were used to cover burn wounds in a rat burn model. It was aimed to investigate the effects of the wound dressing material on internal organs and on oxidative stress and biochemical parameters.

Materials and Methods

Materials: In this study, to cover the burn wounds in the experimental group, PET-g-HEMA nanofibers coated with 10 mM S-AgNPs were used. The modified PET-g-HEMA nanofibers were synthesized and characterized as explained in our previous study (19). To cover the burn wounds in the positive control group, SILVERCEL® was used. Silvercel is a commercial alginate-based dressing material containing silver and it was obtained from Systagenix (UK). OctaCare® dressings (gauze) were used to cover the burn wounds of the negative control group.

Experimental Design and Rat Burn Model: Twenty-four male Sprague-Dawley rats (average weight: 275±25 g) were used. For sedation, xylazine (10 mg/kg, Xylazinbio 2%®, Bioveta, Czech Republic) and ketamine (90 mg/kg, Vetaketam®, Vetagro, Poland) were injected intraperitoneally just before the creation of burn wounds in the animals. Third-degree burn wounds were created in anesthetized rats using a round-bottomed stainless steel rod 2 cm in diameter, which was held in 100 °C water for 20 seconds (31). Animals were divided into 3 groups: a negative control group (G1; standard bandages), a positive control group (G2; silvercel, a commercial dressing material containing silver), and an experimental PET-based dressing material group (G3). The animals were randomly assigned to these groups after the burn wounds were created, with each group consisting of 8 rats. The period for the treatment of burn wounds in each group was 21 days and dressing materials were changed every 3 days. At the end of the study, blood was collected from anesthetized rats, and then the animals were immediately euthanized. Organ-tissue samples were taken from euthanized rats for histopathological examination.

Sample Collection and Analysis: The blood samples were centrifuged at 3000 rpm and 4 °C for 10 min to separate the plasma. The obtained plasma samples were stored in eppendorf tubes in a deep freezer at -80 °C until analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), total protein, albumin, total oxidant status (TOS), and total antioxidant status (TAS) in the plasma were determined with an autoanalyzer (Mindray BS400, China) using commercial test kits (Rel Assay Diagnostics, Türkiye). Nitric oxide (NO) levels were determined with a spectrometer (Shimadzu UV-1700, Japan) using a commercial test kit (Elabscience, USA).

Histopathological Examination and Scoring of Lesions: In order to detect cellular damage and any histopathological changes, the collected tissues were fixed in a buffered neutral formalin solution (pH 7.2-7.4). After being trimmed and placed in disposable cassettes, a routine procedure was followed and tissues were embedded in paraffin wax. Sections of 5 μm in thickness were cut from each paraffin block. Routine hematoxylin and eosin (H&E) staining was applied for histopathological examination (34). The results were evaluated with a digital light microscope (Olympus BX51, Japan) in the bright field. Images at different magnifications were obtained from the required fields with an Olympus DP5 camera attachment. For degenerative-necrotic changes as well as hyperplastic changes, mean averages and standard errors were taken by counting 10 different fields at 400× magnification. After skin wounds were evaluated, the organs of interest were evaluated semi-quantitatively in terms of vascular and inflammatory changes as well as fibrosis and pigment accumulation. Average scores obtained from 10 field counts were categorized as 0%: negative, 10-30%: few changes, 30-45%: mild changes, 45-60%: mild to moderate changes, 60-75%: moderate changes, and 75-100%: strong changes.

Statistical Analysis: For statistical analysis of degeneration and hyperplasia, the differences between the groups (parametric distribution) were evaluated using GraphPad Prism 8.4.2 (GraphPad Software, USA; www.graphpad.com) and one-way analysis of variance (ANOVA) by comparing effects for each row (organ type) and column (name of group). Values of P<0.05 were considered statistically significant at 95% confidence intervals. Analysis of biochemical parameters was conducted with SPSS 18.0 (PASW Inc., USA). The normality of all data was assessed by the Shapiro-Wilk test. The levels of urea as a nonparametric variable were tested using the Kruskal-Wallis test to determine which of the three groups differed from the others, followed by the Mann-Whitney *U* test with Bonferroni adjustment (P<0.001). The other data (parametric distribution) were analyzed by one-way ANOVA testing. Duncan's multiple range test was conducted when *F* values were significant (P<0.05).

Results

Biochemical Parameters: The effects of S-AgNPs and functional PET-g-HEMA nanofibers on plasma enzyme profile and urea, total protein, and albumin levels are presented in Table 1. In comparison with the G1 group, plasma AST, ALT, and GGT activities were significantly decreased in G2 and G3 (P<0.001). Plasma urea levels were significantly decreased in G2 compared to G3 (P<0.05). Neither S-AgNPs nor PET-g-HEMA caused changes in plasma CK or LDH activities or total protein, albumin, or globulin levels (P>0.05).

Oxidative stress: The effects of S-AgNPs and PET-g-HEMA nanofibers on oxidative stress are presented in Table 2. Plasma TOS and NO levels were not statistically significant differences between control and experimental groups (P>0.05), while plasma TAS levels were found to be higher in G1 compared to G2 and G3 (P≤0.05).

Table 1. Biochemical parameters of the groups (x ± Sx).

Parameters	Group-1	Group-2	Group-3	P
AST (U/L)	146.00 ±11.55[a]	91.57±6.11[b]	94.00±6.63[b]	<0.001
ALT (U/L)	76.38±5.16[a]	45.29±1.02[b]	45.00±3.48[b]	<0.001
GGT(U/L)	4.77±025[a]	3.02±0.21[b]	3.31±0.29[b]	<0.001
CK (U/L)	586.63±53.55	425.14±33.77	474.86±65.37	>0.05
LDH (U/L)	1959.75±226.76	1633.71±170.32	1738.14±265.28	>0.05
Total protein (g/dl)	4.81±0.09	4.73±0.13	4.68±0.06	>0.05
Albumin (g/dl)	3.41±0.04	3.50±0.05	3.53±0.06	>0.05
Globulin (g/dl)	1.4±0.11	1.23±0.11	1.15±0.06	>0.05
Urea(g/dl)	54[ab]	51[b]	54[a]	<0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Table 2. TOS, TAS and NO levels of the groups (x ± Sx).

Parameters	Group-1	Group-2	Group-3	P
TOS (μmol/L)	12.55 ±1.14	11.87 ±1.40	10.79±1.21	>0.05
TAS (mmol Trolax Equiv/L)	1.19±0.03[a]	1.13±0.01[b]	1.13±0.01[b]	<0.05
NO (μmol/L)	12.35±0.60	10.41±0.76	12.89±1.13	>0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Histopathological Findings

Liver: In the G1 group, hepatic cords were dissociated and hepatocytes included different sizes of vacuoles in the cytoplasm as well as displaying karyopyknosis and karyolysis. In some areas, necrosis was also encountered in lobules. Kupffer cells were hyperplastic and active in many microscopic fields. Sinusoids and vessels were mildly hyperemic in some high-powered fields. In G2, these degenerative and necrotic changes were diminished within some foci in lobules. Hyperplastic Kupffer cells were increased; however, the increase in number was not as great as that in G1. Sinusoid and vessels were conspicuously hyperemic in many fields. In G3, a few degenerations were observed in lobules. Kupffer cells were neither hyperplastic nor active, and the numbers of cells were in the normal range in lobules. Hyperemic sinusoids and vessels were not dense as in the other groups (Figure 1, a-c).
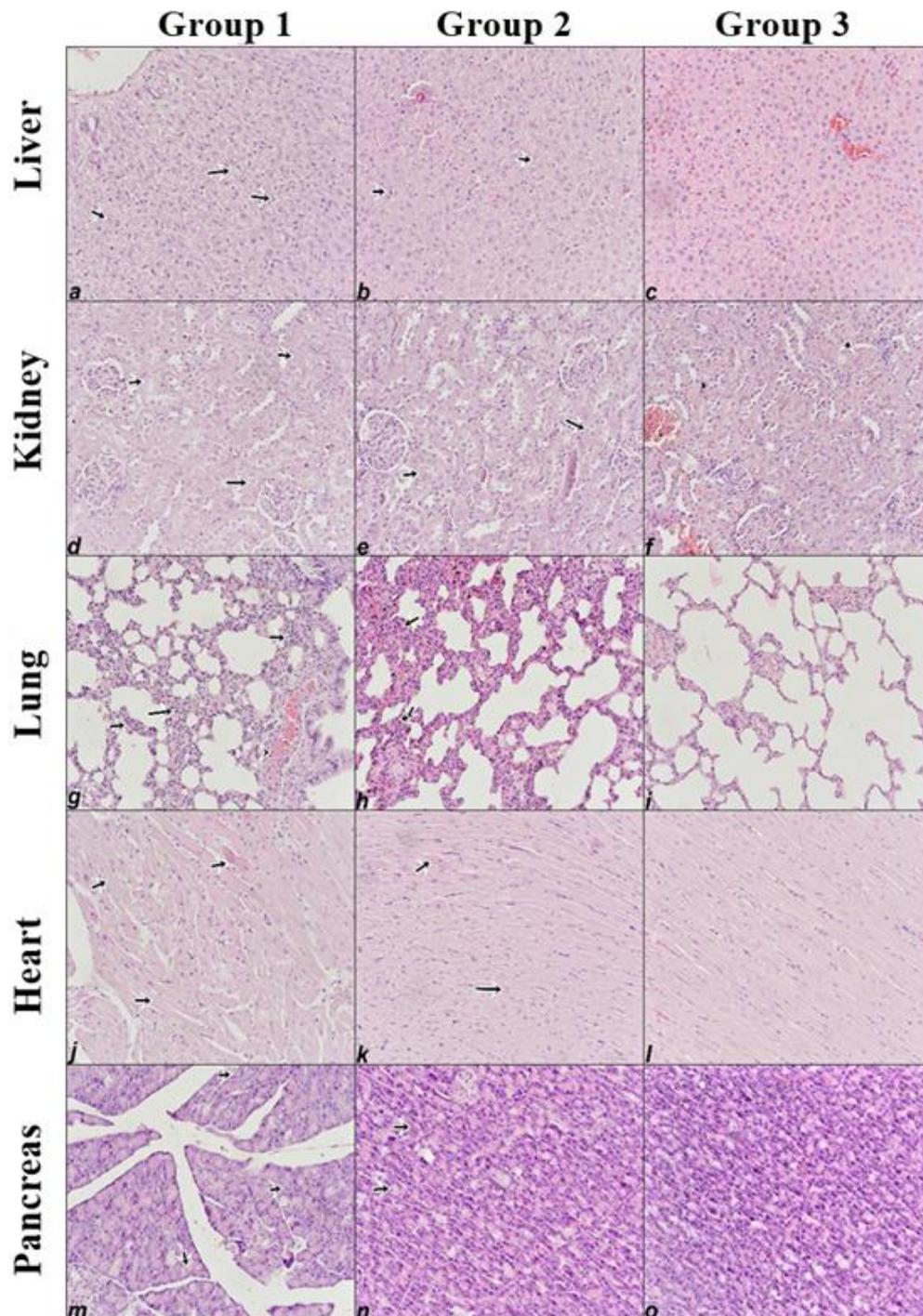

Figure 1. Acute cell swelling to severe hydropic degeneration (arrows) in hepatocytes (a-c), in cortical tubul epitheliums (d-f). Subacute inflammatory cell infiltration in interstitum (arrows) in lung (g-i), paranchymal degeneration and necrosis in cardiac muscles (arrows) in heart (j-l), cell swelling in pancreas (m-o), x100, Group I, II, and III. H&E staining.

Kidney: In G1, cortical tubules were degenerative in general. The degenerative changes were due to acute cell swelling, as seen in many high-powered fields. Glomeruli were normal in appearance. There were no inflammatory or vascular reactions and protein droplets or deposition in either the cortical or medullar region. In G2, there were a few degenerations in the cortical tubule epithelium. In G3, acute cell swellings were decreased in a few fields. No other findings were seen (Figure 1, d-f).

Lung: In all groups, a few pneumocytes were degenerative per field. The capillaries and vessels were hyperemic. Edema was also observed in many fields. At the interstitium, neutrophils, lymphocytes, and monocytes infiltrated into the lumina of alveoli in many fields. In particular, inflammatory and vascular changes were dense in G1. However, in the other groups, there were relatively fewer vascular and edematous changes. Inflammatory cell infiltrations were common in many fields. Lymphocytes were predominantly observed compared to other cell types (Figure 1, g-i).

Heart: In all groups, parenchymal degeneration involving cytoplasmic shrinkage and karyopyknosis was seen in some areas in addition to mild vascular changes except in G2 (Figure 1, j-l).

Pancreas: In G1, acute cell swelling was observed in many high-powered fields. In G2, there were a few degenerations. In G3, there were no degenerative, vascular, or inflammatory changes. Mild hyperemic changes were only observed in G2 (Figure 1, m-o).

Degenerative and necrotic changes in each group are given in Table 3. Hyperplastic changes on the basis of experimental groups, as shown in Table 4. Semi-quantitative scores were also calculated according to vascular and humoral changes, inflammatory cells, fibrosis, and pigment accumulation for each group, as shown in Table 5. The 8 animals in each group constituted the sample size. The group effect was responsible for 31.25% of the variance and the contribution of group effect to the results was evaluated as F=88.32, DFn=2, DFd=105. The organ effect was responsible for 27.59% of total variance (F=38.99, DFn=4, DFd=105). Both effects may thus be considered as extremely significant for the results (P<0.0001).

Table 3. Degenerative and necrotic changes on the basis of experimental groups (Mean±Standard Error).

Organs	Group-1	Group-2	Group-3	P
Liver	18±1.86[a]	5.5±0.34[b]	1.8±0.44[c]	<0.05
Kidney	10±1.73[a]	2.4±1.89[b]	0.6±0.33[b]	<0.05
Lung	0.8±0.44	0.4±0.22	0.1±0.1	>0.05
Heart	0.5±0.22	0.7±0.21	0.4±0.22	>0.05
Pancreas	9.9±1.22[a]	0.6±0.33[b]	0.4±0.22[b]	<0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Table 4. Hyperplastic changes on the basis of experimental groups (Mean±Standard Error).

Organs	Group-1	Group-2	Group-3	P
Liver (Kupffer' cell)	30±0.52[a]	28±1.13[a]	11±0.21[b]	<0.05
Lung (BALT)	1±0.12	1±0.19	1±0.12	>0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Table 5. Semiquantitative score in vascular and humoral changes, inflammatory cells, fibrosis, pigment accumulation on the basis of experimental groups.

Findings	Vascular and humoral changes			Inflammatory changes				Fibrosis	Pigment accumulation
Groups	*Hyperemia*	*Haemorrhage*	*Edema*	*Neutrophil*	*Lymphocyte*	*Macrophage*	*Plasma cell*		
Group-1									
Liver	-/+	-	-	-/+	+	-/+	-	-	-
Kidney	-	-	-	-	-	-	-	-	+++
Lung	++	-	++	++	+++	++	-	-	-
Heart	-/+	-	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-	-	-	-
Group-2									
Liver	+/++	-	-	-	-	-	-	-	-
Kidney	+	-	-	-	-	-	-	-	-
Lung	-	-	-	++	+++	++	-	-	++
Heart	-	-	-	-	-	-	-	-	-
Pancreas	-/+	-	-	-	-	-	-	-	-
Group-3									
Liver	-/+	-	-	-	-/+	-	-	-	-
Kidney	++	-	-	-	-	-	-	-	-
Lung	-	-	+	++	++	-	-	-	-
Heart	-/+	-	-	-	+	-	-	-	-
Pancreas	-	-	-	-	-	-	-	-	-

-: negative, -/+: a few positive, +: mild positive, +/++: mild to moderate positive ++: moderate positive, +++: strong positive.

Discussion and Conclusion

Severe burns can lead to multiple organ dysfunction and death as a result of the disruption of the body's homeostatic balance (7). Burn treatment is difficult, uncomfortable, and expensive for patients. Due to the antimicrobial effects of silver nanoparticles (AgNPs), they can prevent bacterial infection in wounds and accelerate wound healing (35). This study was carried out to determine the effects of burn treatment on changes in some biochemical parameters, oxidative stress, and histopathology in animals. The levels of these parameters are crucial in the physician's understanding of how the body is responding to the burn injury and the response to different possible treatments. The expected changes in the internal organs have been supported by the detection of liver and kidney function markers (28). Normally, aminotransferase enzymes are found in the liver and other tissues, possessing functions in energy metabolism, including transamination of aminocytes. However, in the event of cellular damage, AST and ALT leak into the circulation, and their activities in the blood increase (58). Khalil et al. (28) found that serum AST, ALT, and ALP activities increased while total protein and albumin levels decreased and bilirubin levels did not change in three burnt cattle. Anandani (5) found an increase in blood urea levels in people treated for burns. Jeschke et al. (24) stated that serum AST and ALT activities increased in rats with experimental burns. Şehirli et al. (54) stated that serum LDH activity increased in rats held in 90 °C water for 10 seconds and betaine administration decreased LDH activity. AgNPs are known to have many beneficial properties for wound management including antibacterial, anti-inflammatory, and healing properties (45). Adeyemi and Adewumi (2) investigated the biochemical effects of AgNPs in Wistar rats, administering AgNPs to the rats orally at doses of 100, 1000, and 5000 mg/kg for 7, 14, and 21 days. They observed that serum ALT activity had decreased at the 14th and 21st days with all three doses, as well as AST activity at the 7th day. Sulaiman et al. (53) showed that serum AST and ALP activities decreased in rats given 10, 50, 100 mg/kg AgNPs orally for 30 days, while ALT activity and total protein and urea levels decreased in rats given AgNPs at 50 and 100 mg/kg. It has been reported that oral administration of 30, 125, 300, and 700 mg/kg AgNPs to rats for 28 days did not affect serum ALP, ALT, or AST activities and total protein and albumin levels, and these enzymes remained in liver cells as under normal conditions (46). As a result of the antimicrobial efficacy of AgNPs, they can mitigate bacterial infection and accelerate the process of wound healing (35). In our study, the administration of AgNPs decreased the activities of AST and ALT, supporting the findings of the study conducted by Adeyemi and Adewumi (2). The decrease in AST activities caused by

the nanoparticles was probably due to inactivation emerging from the affinity of AgNPs for thiol (eSH) groups, thereby causing changes in the functional state of proteins (3). Abbas et al. (1) reported that AgNPs inactivated amino transaminases by acting as inhibitors. The inactivation of enzymes or proteins may affect reactions in key metabolic processes with dire consequences for cellular integrity. Consistent with the findings of Pourhamzed et al. (46), no difference was observed in the present study between the groups in total protein or albumin levels. Similar to the findings of Sulaiman et al. (53), in this study, administration of AgNPs significantly decreased serum urea values.

Oxidative stress is the disruption of the balance between oxidants and antioxidants in favor of the oxidant system, which leads to cellular damage in the organism as a result of lipid peroxidation due to the release of free radicals/reactive oxygen products (9, 56). If the defense mechanisms of the organism (antioxidant mechanisms) are insufficient against those free radicals, oxidative damage develops in the cells and cell functions are significantly impaired. Antioxidants are known as natural or synthetic substances that can prevent or delay cell damage caused by oxidants (9).

Damage to the skin causes the formation of reactive oxygen products, reductions in various enzymatic and non-enzymatic free radical scavengers, and lipid peroxidation, affecting the healing process (18, 40). Reactive oxygen species impair the wound healing process due to their detrimental effects on cells and tissues (4). In various studies (7, 11, 30, 55, 57), topical applications of products with free radical scavenging properties were shown to be significantly effective in wound healing and protecting tissues from oxidative damage. Bahadır et al. (7) reported that minocycline treatment administered to animals burnt with exposure to a 90 °C water bath decreased TOS levels in comparison to the untreated burn group. Although the sham and burn groups did not differ in terms of TAC levels, minocycline treatment increased the TAC levels of the burnt animals in comparison to the sham group. Şener et al. (55) induced burn wounds by exposing the backs of rats to a 90 °C water bath for 10 seconds, revealing that while significant increases were observed in kidney tissue malondialdehyde (MDA) levels in both 6-hour and 24-hour burn groups, glutathione (GSH) levels decreased in both burn groups. However, following the administration of 2-mercaptoethane sulfonate, GSH levels significantly increased and MDA levels decreased. Hence, they concluded that 2-mercaptoethane sulfonate has protective effects against thermally induced oxidative kidney damage. Cell damage by free radical attack is typical of burn injuries. Burn injuries are known to trigger the formation and release of oxidative free radicals and pro-

inflammatory mediators that mainly contribute to lipid peroxidation (44, 51).

In a previous study conducted with rats, scald burns were induced by pouring 90 °C water onto a patch of shaved dorsum 20 mm² in size. The rats were euthanized on the 21st day and levels of thiobarbituric acid reactive substances (TBARS) and GSH were measured in tissues and evaluated as markers of oxidative stress. A significant increase in TBARS and a decrease in GSH levels were found in the burn injury group. The application of an isoquercetin-based cream brought those increased levels closer to normal values again and tissue biochemical studies indicated a possible role of free radical scavenging of isoquercetin in wound healing. However, since burn injuries are considered acute dermatological problems representing multiple oxidative changes in a short period of time, the isoquercetin-based formulation was recommended for use in the treatment of burn injuries as early as possible to suppress free radical generation and oxidative stress (11). Bedlovičová et al. (9) reported that, in addition to the application of AgNPs in various fields, there have been many studies on the antioxidant properties of AgNPs in the last decade. For example, AgNPs are used in the treatment of Alzheimer's disease and cancer due to their antioxidant properties. The antioxidant properties of nanoparticles depend on the chemical composition of the chosen extracts. If the extracts are rich in phenolic compounds, the nanoparticles exhibit high levels of scavenging activity (9).

Kumandaş et al. (30) conducted a study to compare the effects of black seed oil and zinc-silver cream on wound healing by evaluating oxidative stress parameters in a rat wound model. They reported that the highest increase in plasma MDA levels was seen in the control group, while the lowest increase was in the silver-treated group. The results of that study showed that topical application of silver cream inhibits lipid peroxidation by increasing antioxidant activity. In the present study, the lowest plasma CAT level was observed in the zinc-silver group. This may have been due to anti-inflammatory effects. In our study, TAS values were higher in the G2 and G3 groups compared to G1 (P<0.05). The highest plasma TOS level was observed in the control group. Increases in plasma TAS levels may be a compensatory mechanism responding to the deteriorating oxidant-antioxidant balance. Kumandaş et al. (30) found that the application of zinc-silver cream to rats with dorsal wounds increased the animals' serum NO levels.

The production of NO is required for normal wound healing. While nitric oxide synthase inhibitors delay wound healing, the administration of NO accelerates wound healing (12, 59). Studies have shown that NO has a trophic effect on wound healing and plays an important role in collagen accumulation (10, 48).

Burn trauma may lead to multiple organ failure in addition to skin damage (38). Considering the microenvironment, increasing oxidative stress inevitably changes histological processes. A change in association with the histoarchitecture occurs in animals given thermal burns. After the burn trauma at skin level, cytokines and inflammatory cells, predominantly neutrophil leukocytes, disturb the microvascular dynamics at both the burn site and remote organs (20, 49). It is postulated that the increase in edema formation causes damage in the cells due to the release of several cellular enzymes. After longer exposure to thermal burns, a degenerative series can occur in the liver within the first week in proportion to the severity of the thermal injury. For instance, after the release of cellular enzymes, hepatocytes can move into necrosis and apoptosis within a short time because cellular homeostasis is disturbed more during degeneration processes (8, 24, 39). Likewise, based on pathogenetic knowledge, acute renal failure due to damage to tubular cells has been identified in the kidneys (21, 23). The cardiovascular system can also be affected by thermal injuries. As a result of decreased blood flow (i.e. oxygenation capacity), the tissue is not fed properly (37). An experimental study was performed regarding burn trauma modeling in rats and treatment with silver-containing wound dressings. Skin-protective silver particles were found mainly in the liver, kidneys, and spleen, as well as the brain, testes, lungs, heart, and muscle tissues (43). In another experimental study, the heart, liver, kidneys, spleen, lungs, and brain were evaluated histopathologically after 14 days in a rat burn model. Although the level of hepatotoxicity due to silver particles and Agicoat was higher, no pathological findings were identified in the brain, kidneys, spleen, heart, or lungs (26).

No findings in association with the spleen, lungs, and pancreas were noted after burn trauma in the present model. However, we obtained some notable histopathological results. We detected mainly vascular and humoral changes and inflammatory changes in all groups. However, the liver, heart, and lungs were more affected in the G1 group. Both vascular changes and edema, as well as inflammatory cell infiltrations were seen in those organs. In contrast, in the other groups, the distant organs were less affected by thermal injuries although liver injuries were seen. Severe hyperemia was also seen, although there were no inflammatory changes. The kidneys, heart, and pancreas were affected to a lesser extent. These findings all show us that the thermal injury group was more affected compared to the wound dressing group. Because of the imbalance in the microcirculation and deterioration in the cellular environment, degeneration and other alterations were seen more often

and that is why the liver, kidneys, heart, and lungs were more affected by such changes. In contrast, these organs were less affected in the other groups. Decreases in microvascular disturbances and inflammation confirmed that the nanoparticles were effective in treating side effects for organs. In particular, no inflammatory activity was seen in these groups. Only hyperemia and edema were observed in the lung tissues of both groups. Due to disturbances in the microcirculatory and microfluidic balance, cellular compositions were affected. Degeneration and necrosis occurred in all groups. However, lesions were seen to a lesser extent in G2 and G3, respectively. The most prominent differences were encountered in the liver and kidneys in terms of degeneration, while degeneration in the pancreas was also found to be more extensive in G1 compared to the other groups. Alterations in heart and lung tissues occurred to a lesser degree. Thus, we conclude that the liver and kidneys were the most affected organs in terms of vascularization and alterations, supporting the results of previously published studies. Accordingly, wound dressing materials containing s-AgNP-coated PET-g-HEMA nanofibers can be developed for commercial products such as SILVERCEL due to the occurrence of fewer lesions in organs, reduced enzymatic parameters such as blood ALT and AST, and high TAS values.

Financial Support

Conflict of interest

The authors declared that there is no conflict of interest.

Author Contributions

YŞ, ZGG, and MEA conceived and planned the experiments. YŞ carried out the experiments. YŞ, ZGG, MEA and MÇ contributed to sample preparation. YŞ, ZGG, MEA and MÇ contributed to the interpretation of the results. YŞ, MEA and MÇ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was carried out after the animal experiment was approved by Kırıkkale University Local Ethics Committee (Decision number: 2020-42).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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