

Decellularization of kidney tissue: Comparison of sodium lauryl ether sulfate and sodium dodecyl sulfate for allotransplantation in rat

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Abstract

Chronic kidney diseases (CKD) and end stage renal disease (ESRD) are growing threats worldwide. Tissue engineering is a new hope to surpass the current limitations such as the shortage of donor. To do so, the first step would be fabrication of an intact decellularized kidney scaffold. In the current study, an automatic decellularization device was developed to perfuse and decellularize male rats' kidneys using both sodium lauryl ether sulfate (SLES) and sodium dodecyl sulfate (SDS) and to compare their efficacy in kidney decellularization and post-transplantation angiogenesis. After anesthesia, kidneys were perfused with either 1% SDS solution for 4 h or 1% SLES solution for 6 h. The decellularized scaffolds were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Masson's trichrome, and alcian blue to determine cell removal and glycogen, collagen and glycosaminoglycans (GAGs) contents, respectively. Moreover, scanning electron microscopy (SEM) was performed to evaluate the cell removal and preservation of microarchitecture of both SDS and SLES scaffolds. Additionally, DNA quantification assay was applied for all groups in order to measure residual DNA in the scaffolds and normal kidney. In order to demonstrate biocompatibility and bioactivity of the decellularized scaffolds, allotransplantation was performed in back muscle and angiogenesis was evaluated. Complete cell removal in both SLES and SDS groups was observed in SEM and DNA quantification assays. Moreover, the extracellular matrix (ECM) architecture of rat kidney in the SLES group was significantly preservation better than the SDS group was shown. The formation of blood capillaries and vessels were observed in the kidney allotransplantations in both SLES and SDS decellularized kidneys. In conclusion, we demonstrated that both SLES and SDS could be promising tools in kidney tissue engineering. The better preservation of ECM than SDS, introduces SLES as the solvent of choice for kidney decellularization.

Keywords

Decellularized scaffold; Acellular scaffold; Kidney; Rat; Sodium dodecyl sulfate; Sodium lauryl ether sulfate

1. Introduction

The kidney diseases have many diversity and complexity such as acute kidney injury (AKI), chronic kidney disease (CKD) and end-stage renal disease (ESRD) (1). Recently, following the increased prevalence of non-communicable metabolic diseases such as diabetes mellitus, hypertension and metabolic syndrome, ESRD is a rapidly growing cause for morbidity and mortality worldwide (1). In order to treat ESRD patients, renal replacement therapy methods such hemodialysis, peritoneal dialysis and renal transplantation have been introduced (2, 3). These treatments are expensive, time-consuming and not available to the majority of people. The majority of mortalities of ESRD are occurring in the developing countries of Asia and Africa with low and middle-income, which their people don't have enough accessibility to, or can't afford these treatment modalities (4). Additionally, the rising number of patients requiring renal replacement therapy alongside the shortage of acceptable donors are concerning issues (5).

To overcome these limitations, tissue engineering has been introduced as a promising new method to treat chronic kidney disease and ESRD patients (6). Several *in vitro* and *in vivo* studies in the field of kidney tissue engineering have demonstrated the efficacy of tissue engineering in the treatment of kidney diseases as a new therapeutic approach (7). Alongside with artificial kidney scaffolds, cell- and nucleic acids-free biological scaffolds are new approaches for renal transplantation (8). Using a decellularized kidney scaffold in order to seed patients' own stem cells would be a proper approach to some dilemma in the developing of the engineered kidney (9). A crucial step toward for producing a kidney with a natural function is to obtain an acellular kidney scaffold (10). therefore, a proper decellularizing agent, which is usually a detergent might be essential, to clean the cells and even their nucleic acids. These methods may provide an extracellular matrix (ECM) including their main proteins, functional and structural molecules which all of them might have an important role in cell filtration, attachment, differentiation and proliferation (11).

There are different decellularizing agents such as sodium dodecyl sulfate (SDS), surfactants, triton X-100, enzymatic and mechanical agents (12). The previous study in porcine heart tissue has shown that SDS may potentially damage ECM proteins (13). In addition, the residue of detergent in a scaffold may induce apoptosis, cellular dysfunction, and thrombosis (13). Another detergent for decellularization is sodium lauryl ether sulfate (SLES) that have been used to decellularize rat heart (14). The SLES is a weaker detergent in comparison to SDS (15). To best of our knowledge, there is no decellularizing study on kidney tissue using SLES. Since it has been reported that SLES had good results on heart, lung and bone tissues (14-16), and sensitivity and complexity of kidney tissue which is composed of abundant vasculature, it was assumed that SLES might be a better detergent in comparison to SDS in preserving ECM. In the present study, we compared the efficacy of these two detergents (SDS and SLES) for kidney tissue decellularization and their effects on post-transplantation angiogenesis.

2. Materials and Methods

2.1. Animals preparation

This experimental study was approved by Bushehr University of Medical Sciences Ethics Committee (Ethical Code number approval for experiments on animals: IR.BPUMS.REC.1398.105). Male Sprague-Dawley (200 – 250 g) were purchased from animal laboratory of Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences. All animal experiments were performed in Bushehr University of Medical Sciences. The animals were housed under controlled temperature of $23\pm1^{\circ}\text{C}$, $55\pm5\%$ relative humidity, and 12-

hour light/dark cycles. They had free access to standard food and water during the experimental periods. Rats were randomly divided into three groups: normal group as the control group, SDS group rats were decellularized with SDS, and SLES group rats were decellularized with SLES. In the SDS and SLES groups, rats were used for perfusion studies, histology, electron microscopy, DNA quantification and subcutaneous implantation. The perfusion studies and subcutaneous transplantation were not performed in the normal group.

2.2. Kidney decellularization

At the first, the rats were anesthetized with ketamine (200 mg/kg, Alfasan Co. Netherland) and xylazine (10 mg/kg, Alfasan Co. Netherland). A longitudinal abdominal incision was made and the left kidney, aorta, vena cava and ureter were identified. Heparin (Caspian Tamin Co., Iran) was injected into inferior vena cava to prevent blood clotting during the operation. Renal artery was cannulated and perfused with 0.1 M phosphate buffer saline (PBS) for 2 h at a flow rate of 2 mL/min to remove blood from the kidney using an automatic decellularization system (Percia ADS, PersiaVista R&D Co., Iran) and the renal vein was cut. The detergents, 1% SDS (Parstous Biotech Co., Iran) for 4 h or 1% SLES (Kimia Tehran Acid Co., Iran) for 6 h were perfused at a flow rate of 1 mL/min, separately in each group. Then PBS was perfused another 2 h to remove the remaining detergents in the scaffold. The physiological pressure was maintained within 62 to 107 mmHg (assumed to be rat kidney pressure) by setting the device flow rate between 1 to 2 mL/min through the entire duration of the decellularization process.

2.3. Histological evaluation

The acellular SDS and SLES scaffolds and normal kidneys were fixed in 10% paraformaldehyde, dehydrated using ascending concentration of alcohol, embedded in xylene and paraffin and 5 μ m sections were obtained. Hematoxylin and eosin (H&E), Masson's trichrome for collagen detection, alcian blue for glycosaminoglycans (GAGs) analysis, and periodic acid–Schiff (PAS) for glycogen substances staining were performed with standard protocols (17-19). The slices of the SDS and SLES decellularized scaffolds were observed by light microscope (Olympus, Japan) and images captured by phone camera (Samsung A8 2017, Korea) and microscope-phone adaptor (PerciaVista Co., Iran).

2.4. Image analysis

Image analysis was performed by ImageJ software (Fiji-ImageJ x64, US National Institutes of Health). In details, using “Image type” option in “Image” panel, images were converted to 8-bit. Then images scale was set using “Set scale” option in “Analyze” panel. The non-stained area in different staining was selected and measured manually by “Threshold” algorithm. The selected non-stained area of images was calculated by “Analyze particles” option in analyze panel. Finally, the data of non-stained area was extracted from “ROI manager” panel and saved as an excel file with CSV format.

2.5. Scanning electron microscopy (SEM)

SEM was performed based on a method previously used by Kashi et al. (20). Briefly, to assess microarchitecture of acellular scaffolds, the samples were fixed in 2.5% glutaraldehyde for 24 h at 4°C and then dehydrated in an increasingly graded ethanol series (50%, 70%, 90%, and 100%). Then samples were transferred into a 1:2 solutions of hexamethyldisilazane (HMDS) to 100% ethanol then into a 2:1 solution of HMDS to 100% ethanol and finally into a 100% HMDS solution

overnight to air-dry in a fume hood, respectively. Finally, samples were covered with a thin layer of gold using Q150R-ES sputter coater (Quorum Technologies, London, UK) and imaged by a VEGA3 microscope (TESCAN, Czech Republic).

2.6. DNA quantification

DNA quantification was performed using a commercial kit (TritaGene Co., Iran). The decellularized scaffold and the normal kidney were sliced into four pieces and one part was weighted and used for the test. At the first step, samples washed with PBS and then were homogenized using ultrasonication method and 750 μ L PBS was added to this homogenized solution. The solution was centrifuged for 30 secs at a rate of 9000 rpm. Then the supernatant PBS was removed and care was taken not to scatter and remove the sediment particles. First, 25 μ L of cell lysis buffer 1 was added and was vortexed for 30 sec. Then 600 μ L of cell lysis buffer 2 was added to the mixture and was vortexed. The solution was centrifuged for 90 secs at a rate of 9000 rpm. The upper portion removed. Then the precipitant was washed with washing solutions. Another centrifuge was performed for 90 secs at a rate of 9000 rpm and then again upper portion of solution was removed. Finally, 40 μ L of distilled water was added. The quantity and quality of extracted DNA was measured using nanodrop (DeNovix Co., USA).

2.7. *In vivo* study of decellularized scaffolds

2.7.1. Animal model of transplantation

Six male Sprague-Dawley rats ($n=3$) with 3-month-old were purchased from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences. They were kept in optimized condition including temperature of $23\pm 2^{\circ}\text{C}$, 12h light/dark control and free access to food and water for one week. The rats were housed in separate cages. The cages were cleaned every 5 days. All surgical procedures were performed under general anesthesia by intraperitoneal injection of ketamine-xylazine (1 mg/kg BW 10% ketamine and 0.25 mg/kg BW 2% xylazine). The hair of the back between their shoulders was completely shaved and sterilized by povidone-iodine (Sahand Co., Iran). The skin and fascia were cut about 1 cm in length and then the back muscle was cut using fine watchmakers' forceps to make a hole 3–5-mm deep. The 0.5 cm pieces of each SDS and SLES decellularized scaffolds were used for the SDS and SLES groups, inserted into back muscle and sutured by absorbable sutures (Pezeshkyaran Co., Iran). Finally, the skin was sutured and the animals were kept in standard condition for one month.

2.7.2 Evaluation of transplanted scaffolds

After one month, the animals were sacrificed and the SDS and SLES decellularized transplanted scaffolds were separated from muscles and fixed by 10% formaldehyde. The scaffolds were processed by tissue processor (Didsabz Co., Iran) and paraffin embedded blocks were cut into 7 μ m using microtome (Didsabz Co., Iran). Slices were dewaxed and rehydrated in xylene and descending percentage of ethanol (100%, 90%, and 70% ethanol, respectively) and were stained with H&E dye (Arian Cellul Sepehr Co., Iran). Then, the slices were dehydrated by ascending percentage of ethanol and were cleared by xylene. Finally, the slices were mounted by Shandon™ Consul-Mount™ glue (Thermo Fisher Scientific Inc. Germany) and covered by glass coverslip. In the SDS and SLES slices, the angiogenesis and cell infiltration were qualitatively evaluated using light microscope (Olympus, Japan) and images captured by phone camera (Samsung A8 2017, Korea) and microscope-phone adaptor (PerciaVista Co., Iran).

2.8. Statistical analysis

The data were analyzed using IBM SPSS Statistics 26 software (SPSS for Windows, version 26, SPSS Inc, Chicago, Illinois, USA). The statistical analyses were performed using one-way ANOVA and *post hoc* Tukey test. Quantitative data were expressed as the mean value \pm standard error of mean (SEM). Additionally, *p* values of less than 0.05 was considered statistically significant. Graphs drawing was performed by GraphPad Prism software (v7.0a, GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Macroscopically, SLES and SDS decellularized kidney

The scaffolds changed macroscopically during decellularization process. The color of the SDS and SLES kidney scaffolds became pale and finally turned to white and became semi-translucent (Figure 1). The tubules were recognizable in decellularized scaffolds. Total kidney shape was preserved after decellularization procedure without any malformation. Macroscopically, there could be no difference seen between the SDS and SLES kidney scaffolds after decellularization.

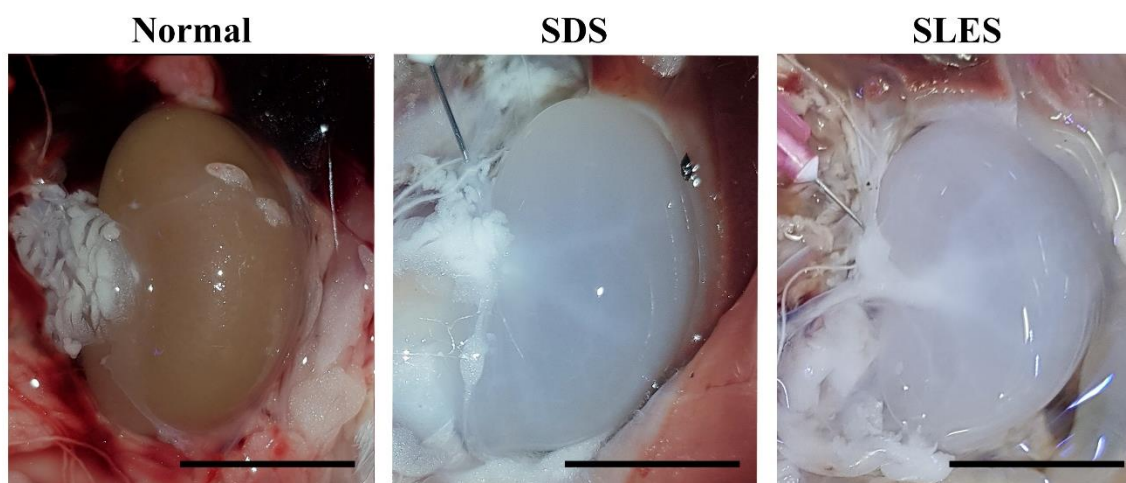


Figure 1. Macroscopic imaging of kidney tissue and decellularized scaffolds using sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) in rat. Scale bars are 1 cm.

3.2. Microscopically, SLES preserved ECM of kidney better than SDS

Histological analysis of the stained tissues showed that cells were successfully removed in both decellularized scaffolds, compared with the normal kidney (Figure 2). Despite of the removing of cells in two scaffolds, the architecture of blood vessels, renal tubules and Bowman capsules were well preserved. The histological images of the decellularized kidneys showed that the renal tubules structures were more preserved with the SLES than SDS scaffolds (Figure 2). In histological evaluation, the amount of non-stained areas in the SDS and SLES scaffolds with all staining methods were higher than the control kidney, which indicate the decellularization of kidney ($p < 0.001$, Figure 3). The H&E staining of cortex and medulla of rat kidney showed that the SDS and SLES kidney scaffolds had higher percentage of non-stained area than the normal kidney scaffolds ($p < 0.001$, Figure 3A). Moreover, there was a higher percentage of non-stained area in the SDS scaffold than the SLES scaffold in cortex, which was not similar to renal medulla ($p < 0.001$).

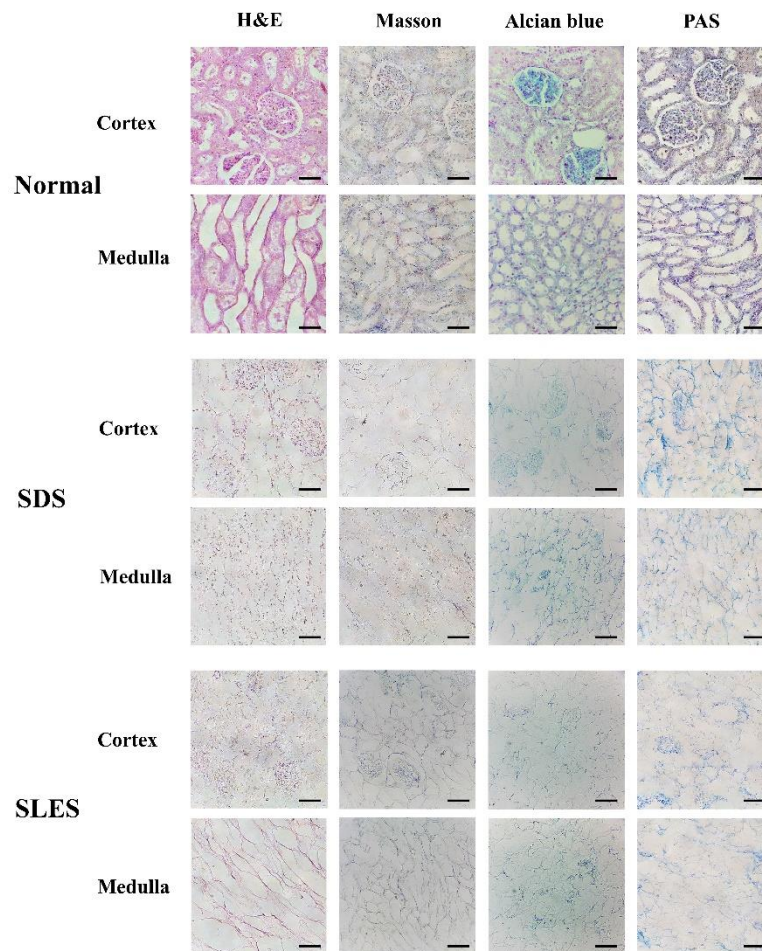


Figure 2. Histologic images of normal kidney and decellularized kidney using sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) in rat. Hematoxylin and eosin (H&E), Masson's trichrome for collagen analysis (Masson), alcian blue for glycosaminoglycans (GAGs) detection, periodic acid-Schiff (PAS) for glycogen substances staining were performed. Scale bars are 50 μ m.

The histological image analysis of Masson's trichrome showed a significant higher percentage of non-stained areas in the SDS scaffolds than the SLES scaffolds in kidney cortex ($p < 0.001$, Figure 3B). In other words, the collagen and other ECM fibers in the SLES scaffold was higher than the SDS scaffold. In cortical assessment, the non-stained areas of the SDS and SLES scaffolds were higher than the control kidney, like the H&E staining ($p < 0.001$). In contrast to cortex, in the medulla, the image analysis of Masson's trichrome demonstrated that there was no significant difference between the SDS and SLES scaffolds in non-stained area. In addition, in line

with cortex analysis, the non-stained areas of the SDS and SLES scaffolds were higher than the control kidney ($p<0.001$).

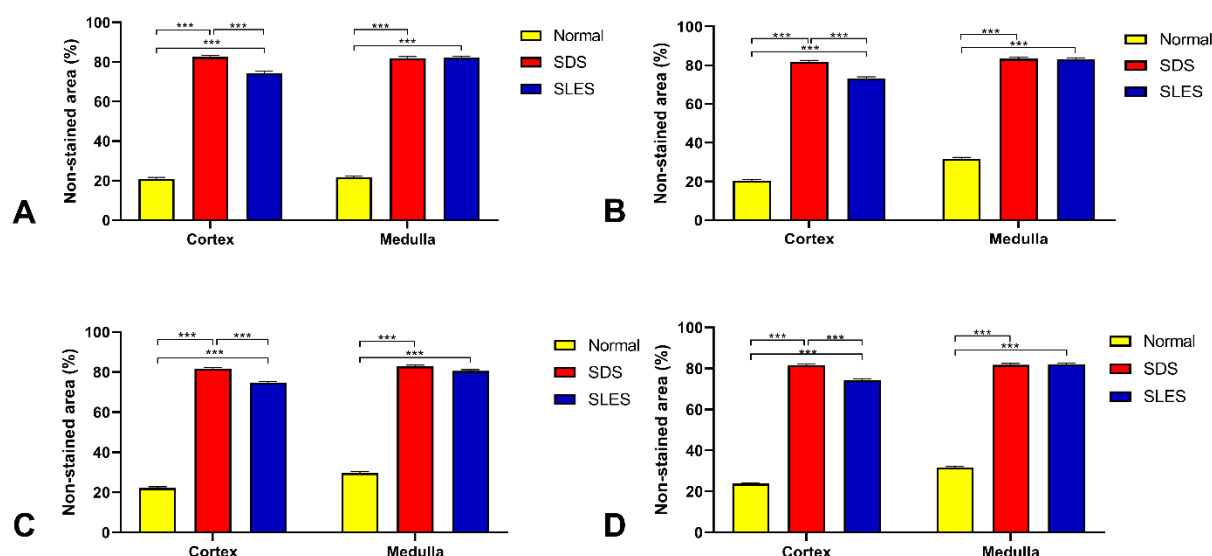


Figure 3. Histologic analysis of normal kidney and decellularized kidney using sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) in rat. A) Image analysis of hematoxylin and eosin staining. B) Image analysis of Masson's trichrome staining. C) Image analysis of alcian blue staining. D) Image analysis of periodic acid-Schiff (PAS) staining. Lines above the columns show significant differences between the groups (*** $p<0.001$).

The alcian blue staining was performed to stain GAGs. The result of image analysis showed that in cortex and medulla of kidney of the scaffolds, the non-stained areas percentage of the SDS and SLES kidney scaffolds were higher than the normal kidney scaffolds ($p<0.001$, Figure 3C). In cortex, the non-stained area percentage of SDS kidney scaffold was higher than the SLES kidney scaffold ($p<0.001$), in contrast with the medulla of kidney scaffolds which in there was no significant difference in GAGs amount in the SDS and SLES scaffolds.

Moreover, the PAS staining was used to detect and evaluate the glycogen content of the scaffolds. The PAS staining analysis showed that in both cortex and medulla, the non-stained areas percentage in the SDS and SLES scaffolds were higher than normal scaffold, like the previous staining ($p<0.001$, Figure 3D).

3.3. The ECM of scaffolds of SLES was better preserved than SDS

The SEM evaluation of normal and decellularized scaffolds showed that the scaffolds were well decellularized (Figure 4A). The microarchitecture of renal tissue scaffolds was well preserved. Furthermore, the three-dimensional spaces of cleared various cells remained unscathed. Additionally, in higher magnification, the ECM structure was appeared to be intact (Figure 4B). The structure and direction of scaffold's fibers like collagen was also intact. In addition, the microarchitecture integrity of tissue in the SDS scaffolds was lesser than the SLES scaffolds (Figure 4).

3.4. SLES and SDS removed the DNA content of the kidney scaffolds

The DNA content of normal kidney (149.8 ± 54.2 ng/mg of dry tissue weight) was higher than the SDS and SLES scaffolds ($p < 0.05$, Figure 4C). Moreover, the DNA evaluation of the scaffolds revealed that the DNA content of the SLES scaffolds was zero (0 ng/mg of dry tissue weight). Although there was no significant difference between the DNA content of the SDS and SLES scaffolds ($p > 0.05$), the DNA content of the SDS scaffolds was not zero (1.2 ± 0.2 ng/mg of dry tissue weight).

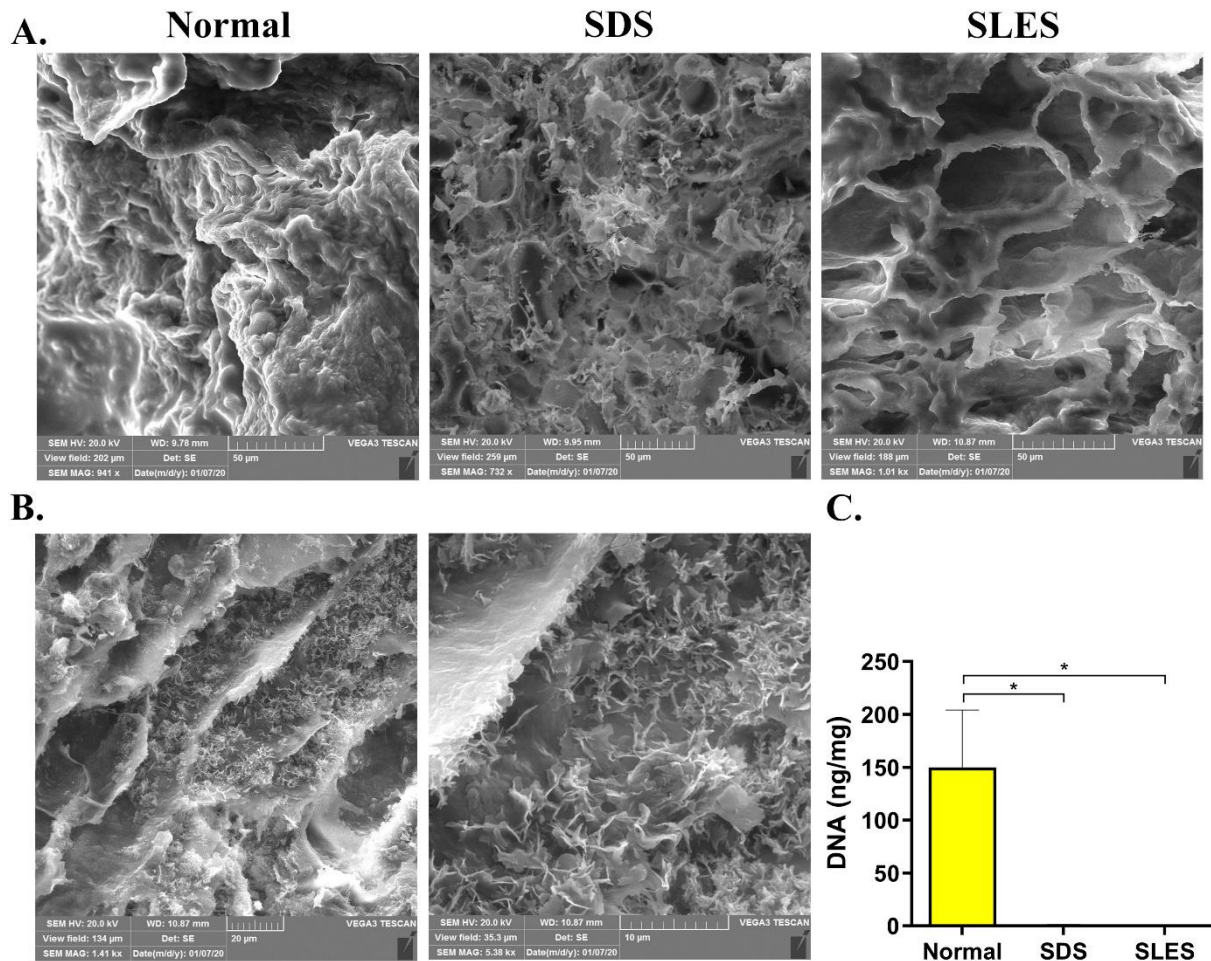


Figure 4. Scanning electron microscopy and DNA quantification analysis of normal kidney and decellularized kidney using sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) in rat. A) Scanning electron microscopy of normal, SDS and SLES kidney scaffolds with 50 nm magnification. B) Decellularized SLES scaffold at higher magnification of 20 and 10 nm. C) DNA quantification of intact kidney and SDS- and SLES-decellularized kidney scaffolds. Lines above the columns show significant differences between the groups ($*p < 0.05$).

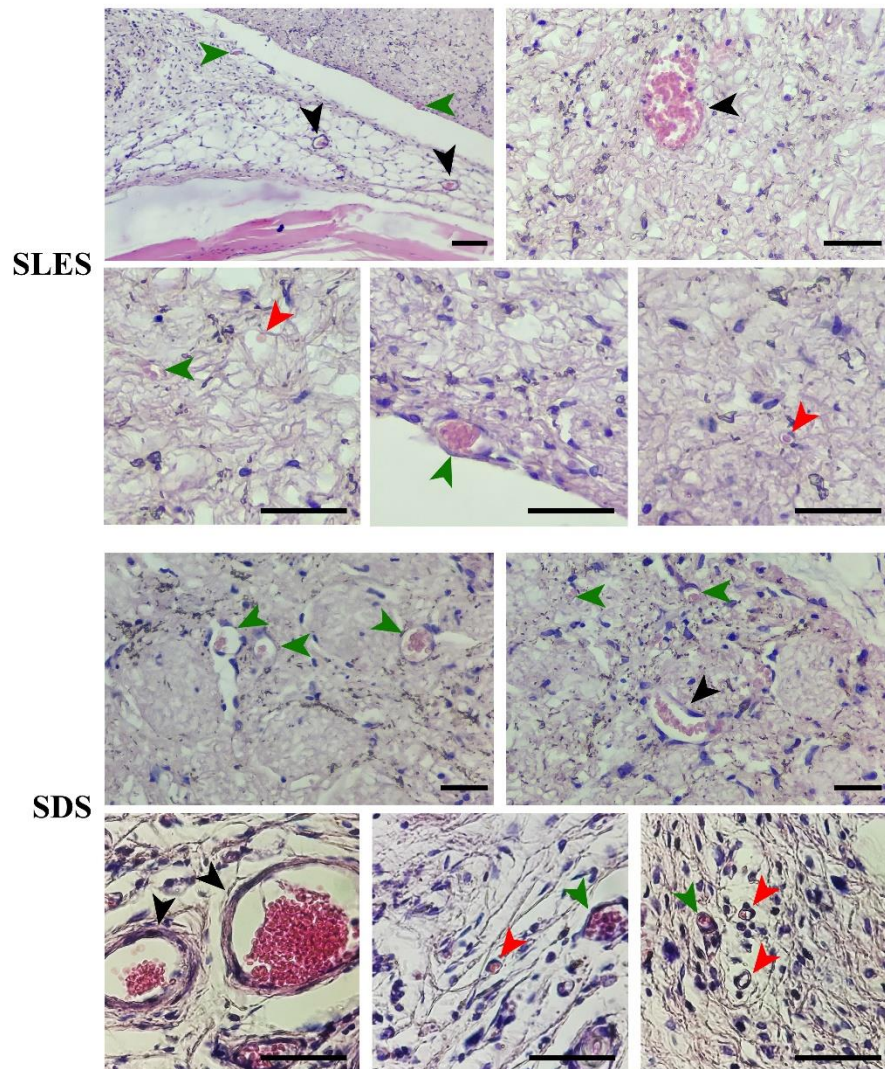


Figure 5. Post-allotransplantation vascularization of kidney scaffolds after decellularization using sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) in rat after 1 month. Angiogenesis and cell infiltration from recipient body has been showed. The black arrows represent large vessels, the green arrows represent small vessels and the red arrows are very small vessels (arterioles and capillaries). The scale bars in all images are 50 μ m.

3.5. SLES and SDS scaffolds were vascularized after allotransplantation

The SDS and SLES kidney scaffolds showed bioactivity and biocompatibility after allotransplantation in the rat back muscle. There was no scaffold rejection in the SDS and SLES scaffolds after the scaffolds allotransplantation. Additionally, there was no toxic effect of scaffolds on recipient body. After one month of scaffolds' transplantation, the transplanted the SDS and SLES scaffolds were evaluated. Although there was no evidence of rejection in the grafts, there was scarce infiltration of host cell into the grafts which some of them were leukocytes (Figure 5). Moreover, the angiogenesis process was evaluated after one month of the SDS and SLES scaffold

transplantation. The H&E staining revealed that new vascularization was taken place in the SDS and SLES scaffolds (Figure 5). The vascular tubular shape and red blood cells inside of these tubules indicated the neovascularization. In the SDS and SLES scaffolds, three types of vessels were observed; large vessels, small vessels, and very small vessels (arterioles or capillaries) (Figure 5). The very small vessels were distinguished from other vessels by their narrowing and the thin layer of their walls (Figure 5). The number of large vessels in the SDS and SLES scaffolds was qualitatively determined which was between 0 to 2 vessels per each field. Additionally, the number of small vessels was counted which was between 2 to 5 vessels per each field, and number of very small vessels was counted which was between 1 to 5 vessels per each field.

4. Discussion

Kidney tissue engineering is an emerging technology in regenerative medicine which has been targeted to overcome the kidney donation limitations for renal transplantation (6). To do so, at the first step, a proper scaffold needed for transplantation. The present study was conducted to decellularize kidney and finally produce a proper scaffold in rats. In decellularization procedure, especially in the vessels, an important part is the avoidance of clot formation (21, 22). As the kidney has very small vessels, especially arterioles and capillaries, even a small clot can obstruct vessels and impair its blood flow (23). Hence, obstruction of the liquid flow can interrupt the decellularization and cause partial decellularization; therefore, the first step in the present study targeted to prevent clot formation. Therefore, instantly after rat thoracotomy, heparin was injected to the inferior *vena cava*. Then in order to eliminate all blood cells, the tissue was perfused by PBS. After 2 h, the flow liquid which exited from the tissue turned from red into colorless, indicating RBC elimination. At the next step, the main decellularization step was performed. Other important aspects of decellularization procedure include the flow rate of decellularization and the percentage of detergents, which were different from each other in different studies (11, 15, 24, 25). Based on previous studies and also based on our pilot study, the flow rate and the percent of detergent were optimized. Then, the decellularization process was performed.

The kidney tissues successfully decellularized by SDS and SLES detergents and then, these detergents were compared to the normal kidney tissue. In several studies mammalian tissues were decellularized using SDS detergent with good results (9, 11, 25). In the present study, the concentration of 1% SDS solution was used, like the previous studies (9, 25). The SDS decellularization method had very good results in previous study of tissue decellularization (9, 11, 25). In recent years, the SLES reagent was also used for tissue decellularization (15, 24, 26). This new detergent had very good efficacy for tissue decellularization (15, 24). In order to compare SDS and SLES detergents for kidney decellularization, this study was performed. Firstly, the histological and image analysis was evaluated. The results of histological and image analysis showed that the SDS and SLES scaffolds had higher non-stained areas than the normal kidney, indicating decellularization process. The H&E staining demonstrated that no cells were remained in the SDS and SLES decellularized scaffolds. Additionally, Masson's trichrome staining showed that non-stained areas of the SDS and SLES scaffolds were $82.6 \pm 0.7\%$ and $78.2 \pm 0.7\%$, respectively which were higher than the normal kidney counterparts ($26.1 \pm 0.6\%$). This indicates that total number of proteins including collagen were reduced in both decellularized scaffolds, however they were not completely deleted.

Furthermore, the Masson's trichrome histology images showed that the structure of kidney scaffolds was preserved after decellularization. This indicates that the total protein structure of kidney in the SDS and SLES scaffolds was preserved. Moreover, two decellularizing agents, SDS

and SLES, are comparable with each other. In cortex of the kidney scaffolds, there was significant difference between non-stained areas of the SDS and SLES kidney scaffolds in both H&E and Masson's trichrome staining. These results demonstrate that in SLES scaffolds, the non-stained area was lower than the SDS scaffolds, indicating that more collagen was preserved in the SLES scaffolds than the SDS scaffolds. This finding may present better effect of SLES detergent in comparison to SDS detergent. In contrast, in medulla, the percentage of non-stained areas in the SLES and SDS scaffolds showed no difference.

The histology of alcian blue staining showed that the non-stained areas of the SDS and SLES scaffolds were higher than the normal kidney. As the alcian blue staining represents the content of GAGs and carbohydrates of tissue, such as mucosubstances (27), this result showed that most of the GAGs was removed during decellularization process; however, the amounts of GAGs didn't reached zero and some amount of GAGs were preserved in total structure of renal tissue. In addition, the GAGs' amount of the SLES scaffold in cortex was higher than the SDS scaffold which represents that more GAGs in the SLES scaffolds were preserved; indicating another proof for better yield of SLES detergent than SDS. This difference was not observed in medulla of decellularized scaffolds, like the previous staining methods.

Furthermore, the PAS staining of kidney tissues demonstrated that the glycogen substances of the SDS and SLES scaffolds were lower than the normal kidney. This indicates that the total amounts of glycogen were decreased after decellularization and most of them were removed by SDS and SLES detergents; however, the essential parts of them which exist in structural parts of kidney were preserved. Like the previous staining methods, the amount of non-stained area of the SLES scaffold was lower than the SDS scaffold in cortex of kidney scaffolds, indicating that more glycogen in the SLES scaffolds were preserved.

The findings of histological analysis of decellularized kidney showed that SDS and SLES successfully decellularized kidneys. Besides of decellularization, a key element of proper producing of scaffolds is their ECM molecules such as proteins like collagen and elastin, carbohydrates and GAGs (15). During decellularization of kidney by SDS and SLES detergents, some of these ECMs factors were removed either. According to the histological analysis, some amount of the ECMs factors were not totally eliminated and total structure of kidney was preserved. Additionally, glomerular and tubular basement membranes were well preserved in the histological staining. In line with the findings of the present study, previous study of SDS and SLES decellularization also demonstrated that the total amounts of ECMs factors reduced after decellularization, but the total structure of tissue and some of these ECMs factors remained intact (14). Additionally, the results of current study showed that in some cases, especially in cortex of the SLES scaffolds, the ECM was preserved better. The results of previous studies are in line with the current study and all of them indicates that SLES had better performance in tissue decellularization than SDS reagent (14, 15).

In order to prove the preservation of total structures of decellularized kidney, SEM analysis was performed. The SEM analysis showed that the microstructure of the SDS and SLES scaffolds were well preserved. Furthermore, the pores and three-dimensional spaces of previous cells of kidneys were preserved as well. In higher magnification, it was shown that total structure of ECMs' fibers of SLES scaffolds were more intact than SDS scaffolds. These fibers in the SLES scaffolds were more in line with each other than the SDS scaffolds and the total microstructure integrity of the SLES scaffolds were more preserved. In consistent with our results, a decellularization study of lung tissue demonstrated a better preservation of the SLES lung scaffolds microstructures than the SDS scaffolds (14).

The confirmatory assessment of total decellularization was performed by DNA quantification. The contents of DNA in the SLES scaffolds which was determined by DNA quantification was zero. Furthermore, the DNA contents of the SDS scaffolds were reduced, but not completely eliminated. Additionally, there was no significant difference between the DNA contents of the SLES and SDS scaffolds. However, the DNA contents of the SLES and SDS scaffolds were significantly differ from the normal kidney. These results showed that no nuclei were remained in the SLES scaffolds and these nuclei also reduced remarkably in the SDS scaffolds. Previous studies of tissue decellularization are in line with the results of the present study and they also demonstrated a reduction of total content of DNA after decellularization (14, 24, 28). However, to best of our knowledge, there is no study in literature to show that the DNA content of the SLES scaffolds totally eliminated and reached to zero level.

When the decellularization was confirmed, the biocompatibility and bioactivity of the SDS and SLES scaffolds evaluated in *in vivo* study. Firstly, no rejection happened after allotransplantation in all recipient rats, indicating biocompatibility of decellularized scaffolds by SDS and SLES. After one month of the transplantation, the scaffolds were evaluated qualitatively by light microscopy. The primary observation showed infiltration of the host cells into the decellularized grafts, which implies their bioactivity. However, in further evaluation, the angiogenesis was observed in the transplanted scaffolds. The vessels were recognized by their tubular shape and the presence of RBCs inside of these tubules. Additionally, most of these vessels had a layer of epithelial cells and in some cases muscular cells around them. The dimeter of these tubules and also the dimeter of layer around them were different. Based on these differences, the vessels divided into three groups; large vessels, small vessels and very small vessels including arterioles and capillaries. Totally, in the qualitative analysis, there was no significant difference between both scaffolds in their angiogenesis. In previous study of rat lung decellularization by SDS and SLES, the similar results of the current study has been shown with a higher amount of infiltrating cells and larger vessels in the SLES scaffolds in comparison to the SDS scaffolds (14). These findings may be related to vigorous detergent effect of SDS which makes more depletion of growth factors in the scaffolds (29).

5. Conclusions

Taken together, SLES detergent, as a new anionic detergent had a better performance for renal tissue decellularization. To best of our knowledge, this was the first kidney decellularization study which compared and optimized SDS and SLES detergents. The results demonstrated that the rat kidney tissue was entirely decellularized by SDS and SLES. However, the microarchitecture of the scaffolds remained intact and SLES was more efficient in this preservation process. Additionally, to best of our knowledge, this is the first *in vivo* analysis of the SDS and SLES decellularized kidney scaffolds which evaluated their biocompatibility and bioactivity in an *in vivo* model. The SDS and SLES scaffolds showed angiogenesis and there was no rejection of the SDS and SLES grafts. SLES is a promising detergent that could be a useful decellularization agent to produce acellular kidney scaffold much alike normal kidney scaffold. SLES detergent in comparison with SDS as a strong anionic detergent with higher biodegradation ability, had milder chemical properties and better preservation of ECM proteins and microarchitecture, the properties which later would be crucial for the cell seeding. In summary, SLES is an alternative detergent for SDS which can be used in renal tissue engineering confronting disadvantages related to SDS.

Acknowledgements

The authors wish to thank Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences; The Center of Marine Comparative and Experimental Medicine, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences; and Shiraz University of Medical Sciences for providing lab equipment in this project.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclose financial support for the research from the Bushehr University of Medical Sciences (grant no. 1279).

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