



# Comparison of Sodium Lauryl Ether Sulfate and Sodium Dodecyl Sulfate in the Intestinal Decellularization of Rats Using a Histological Method and Confocal Raman Microscopy

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## Abstract

**Background:** Sodium dodecyl sulfate (SDS) detergent is widely used in tissue decellularization to produce scaffolds for tissue engineering. Despite its strong decellularization, this substance has relatively high toxicity and causes changes in tissue composition. Sodium lauryl ether sulfate (SLES) is a new poly anionic detergent that is less toxic than SDS but weaker than it. The present study aimed to decellularize the intestinal tissue using SDS and SLES solutions, forming a cell scaffold, and examining scaffolds obtained from this tissue.

**Methods:** Eighteen male Sprague-Dawley rats were divided into three groups. The intestines of all rats were removed after anesthesia. In the first group (controls), rats' intestines were placed in a 10% formalin solution. In the second group, intestines were decellularized using an SLES solution. In the third group animals' intestines were decellularized using an SDS solution. To evaluate decellularization, samples were stained with hematoxylin-eosin staining and Alcian blue staining for glycosaminoglycans (GAGs), and Masson's trichrome for collagen fibers. A confocal Raman microscope was used to compare collagen, lipid, GAG, and genetic content.

**Results:** Hematoxylin-eosin staining showed that the nucleus and DNA were removed in the decellularized scaffolds by SDS or SLES. The SLES group, compared to the SDS group, showed fewer changes in the epithelial tissue, and muscle layers in both scaffolds were well preserved. The results of confocal Raman microscopy showed that tryptophan, lipid, glycogen, and protein were broken down by both detergents; however, the residual amount of glycogen was the same in both substances, but disulfide bonds of proteins, hydroxyproline, and lipids in the decellularized intestine with SLES were mostly preserved.

**Conclusion:** Both substances were suitable for intestinal decellularization and removed the overall structure of intestinal tissue, but SLES retained collagen and GAG content better than SDS.

**Keywords:** Tissue engineering, Intestinal failure, Tissue scaffolds, Decellularized extracellular matrix, Sodium dodecyl sulfate

**Citation:** Asadi-Yousefabad SH, Daneshi S, Pouretezari M, Tanideh N, Zamani Rarani M, Kargar-Abarghouei E, et al. Comparison of sodium lauryl ether sulfate and sodium dodecyl sulfate in the intestinal decellularization of rats using a histological method and confocal raman microscopy. *Journal of Kerman University of Medical Sciences*. 2023;30(2):86–91. doi:10.34172/jkmu.2023.14

**Received:** June 8, 2022, **Accepted:** December 24, 2022, **ePublished:** April 25, 2023

## Introduction

Intestine failure (IF) develops due to the loss of physiological or anatomical function. The inability of the intestine to perform various natural secretory and absorption functions of essential and nutritious

supplements, water, and electrolytes leads to the need for artificial and injectable nutrition for the patient (1).

Gastrointestinal dysfunction reduces the quality of life and, ultimately, death. On the other hand, the risk of diseases such as malnutrition, liver disease, and also life-



threatening diseases such as sepsis increases following intravenous nutrition (1). Studies conducted by Lloyd et al showed that as a result of intestine failure, the survival rate of affected individuals decreased from 86% in the first year to 77% and 73% in the third and fifth years, respectively (2).

Transplantation is known as a common treatment that, despite many innovative developments in recent years, has limitations such as rejection of the transplanted organ by the host body, the high cost of procedures, the need for severe and long-term suppression of the immune system, low survival rate in the first five years, and also the need for matched donors (3,4). However, there has been a shortage of donors and an increase in patients requiring new organs in recent years, with about 100 000 patients being added to the waiting list every year in the United States alone. All of these factors affect the long-term success of small bowel transplants. Therefore, the development of new treatment strategies for intestine failure is an area that needs further consideration (5). Tissue engineering is a new approach that can replace the lost tissues in the body.

Tissue engineering produces appropriate tissue based on the use of three factors: cell, scaffold, and growth factor (3). Cellular scaffolds support cellular functions, transfer growth hormones, and ultimately form a suitable environment for the production of tissues similar to the original tissue (6-8). There are several methods for creating cell scaffolds, of which the decellularization method is currently more suitable than other methods. Among the various methods for tissue decellularization and scaffolding, it is very important to choose a method that does not destroy the extracellular structure and content while destroying the cells entirely. Different methods, such as detergent solutions, enzymatic and mechanical methods, surfactants, and Triton X-100, are used to destroy tissue cells (9,10).

One of the materials used for decellularization is sodium dodecyl sulfate (SDS) which consists of a double-stranded structure with one end hydrophilic and the other end hydrophobic. This structure destroys the cell contents (including the cytoplasm and the nucleus and proteins) and leaves a decellularized scaffold. This substance is widely used for decellularization in various tissues such as the lung, heart, and kidneys (10-12). Sodium lauryl ether sulfate (SLES) is a new anionic detergent that has recently been used in studies to decellularize various tissues such as ovaries, kidneys, and lungs; despite being weaker, SLES is less toxic than SDS (13,14).

In the present study, to decellularize intestinal tissue using SDS and SLES solutions, we formed a cell-free scaffold and used a confocal Raman microscope with imagery and analysis of tissue with high resolution, contrast, and specificity. Tissue structures, like molecular

compositions, provide molecular interaction, and we examine and compare scaffolds remaining from tissues.

## Materials and Methods

### Animals

Eighteen healthy male Sprague-Dawley mice were used in this study with the following details: weighing  $20 \pm 200$  g and aged 12 to 14 weeks kept in controlled laboratory conditions (temperature  $25^{\circ}\text{C}$ , humidity  $\pm 55$ , 12 hours of light, proper ventilation, and free access to food and water). Mice were equally divided into three groups (six in each group).

- Group 1 (control): Animals whose intestines were removed after anesthesia and placed in 10% formalin solution.
- Group 2: Animals whose intestines were removed after anesthesia and decellularized using SLES solution (CAS No. 68585-34-2, 68891-38-3, RawChem SCM Co, China)
- Group 3: Animals whose intestines were removed after anesthesia and decellularized using SDS solution (CAS No. 151213, Sigma, Germany).

### Intestinal decellularization

Ketamine and xylazine were injected intramuscularly to anesthetize the rats based on their weight. Then, the abdominal wall was cut and 1 mL of heparin was injected into the inferior vena cava to prevent blood clotting. Next, a part of the intestine was separated and placed in a detergent solution (SDS or SLES) and remained in the shaker until the intestinal tissue became clear. Afterward, the decellularized intestines and the control samples were fixed with 10% formaldehyde for histological and confocal Raman microscopic evaluation (model: LabRam HR made by HORIBA, Japan; Located in the central laboratory of Shiraz University).

### Histological evaluation

The evaluation was performed to prepare microscopic sections of the samples by the usual method of preparing tissue sections. In this method, after formaldehyde (10%) fixation, various stages of tissue passage, including dehydration with increasing ethanol concentration, xylene clarification, and paraffin impregnation, were performed using a tissue processor. Then, after leaving the tissue processor, the samples were molded, and 5- $\mu\text{m}$ -thick sections were prepared using a microtome. Different staining methods were used to study histology.

Hematoxylin-eosin staining was used to observe the structure of the intestine and to investigate the presence of nuclei in the decellularized intestinal scaffold. Alcian blue staining was used to observe glycosaminoglycans (GAGs), and Masson's trichrome staining was used for collagen fibers.

### Evaluation with confocal Raman microscopy

First, samples were cut (5 mm by 5 mm with a thickness of 2 mm) and lyophilized by freeze-drying at  $-50^{\circ}\text{C}$ . For evaluation with confocal Raman microscopy, a laser with a power of 50 mW and a wavelength of 633 nm was used. For this purpose, the absorption range of 200 to  $1500\text{ cm}^{-2}$  with a resolution of  $4\text{ cm}^2$  was investigated.

### Results

Hematoxylin-eosin staining showed that the scaffolds de-cellulated using SDS or SLES were coreless and DNA-free. In the intestine decellularized with SLES, compared to the SDS detergent, there were fewer changes in the epithelial tissue, and the structure was well preserved. The muscle layer in both scaffolds was well preserved. Also, the serous layer in the intestine decellularization intestine with SLES remained better than that of SDS detergent (Figure 1).

### Comparing the results of Masson's trichrome staining of intestinal scaffolds prepared with SDS and SLES detergent

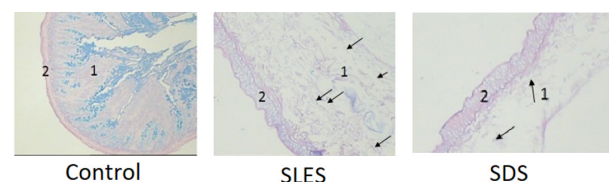
Using Masson's trichrome staining, it was observed that collagen fibers in intestinal connective tissue obtained by SLES were better preserved than SDS (Figure 2).

### Comparing the results of Alcian blue staining of intestinal scaffolds prepared with SDS and SLES detergents

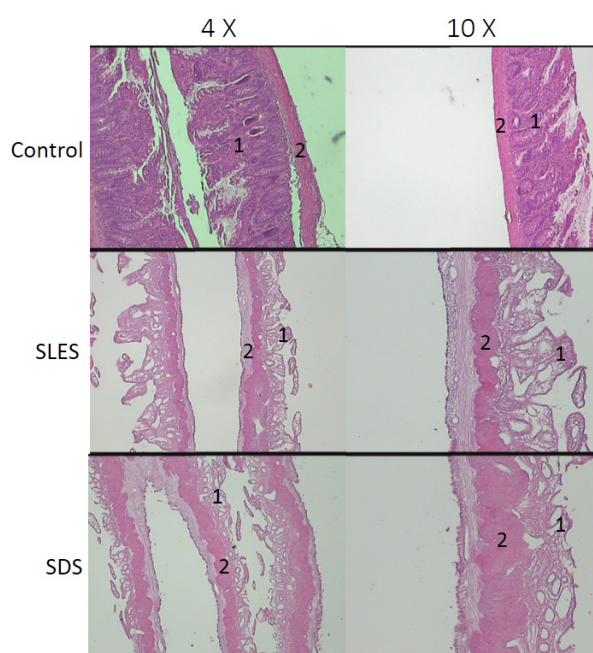
The results of Alcian blue staining showed that GAGs were well preserved in scaffolds prepared using both SDS and SLES detergents (Figure 3).

### Comparing the results of the evaluation of natural intestine and scaffolds prepared with SDS and SLES detergents using confocal Raman microscopy

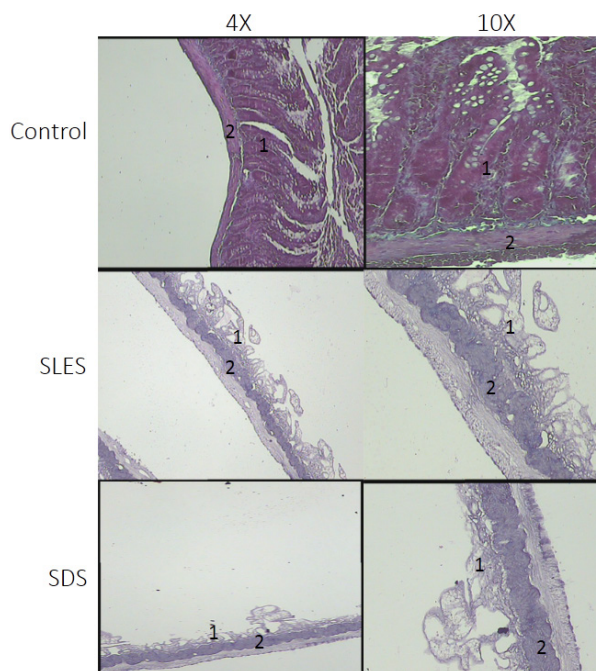
The results of a confocal Raman microscopy in the  $490\text{ cm}^{-1}$  spectrum showed that glycogen was similarly conserved by both spectra. In the  $524\text{ cm}^{-1}$  spectrum, which is related to the disulfide bonding of proteins, a greater amount was retained in the intestine decellularization with SLES. Findings from a confocal Raman microscope in the  $573\text{ cm}^{-1}$  range showed that tryptophan was eluted with both detergents. The  $1033\text{ cm}^{-1}$  spectrum showed the presence of phenylalanine, the predominant amino acid in type IV collagen, which has the same amount in the SLES-degenerated intestine as the extracellular matrix (ECM) obtained by SDS. The  $1062\text{ cm}^{-1}$  spectrum belongs to the OSO-3 molecular group, which is in the structure of GAGs and was retained more in the SDS-de-cellulated intestine. Findings from confocal Raman microscopy in the  $1140$  and  $-1185\text{ cm}^{-1}$  spectra showed that lipids, glycogen, and proteins were eluted with both detergents. The  $1206\text{ cm}^{-1}$  spectrum is related to hydroxyproline as the major amino acid of type 1 collagen, which remains mostly



**Figure 2.** Masson's trichrome staining. Collagen fibers are well defined with arrows and blue color. Number 1 indicates the epithelial tissue, and number 2 indicates the muscle layer



**Figure 1.** Hematoxylin-eosin (H&E) staining showed that the cells in the intestines were de-cellulated with both SLES and SDS detergents. Number 1 indicates the epithelial tissue, and number 2 indicates the muscle layer



**Figure 3.** Alcian blue staining. Acid carbohydrates with a dark purple color are well-shown that are the same in both the SLES and SDS groups. Number 1 indicates the epithelial tissue, and number 2 indicates the muscle layer

in the cell decellularization with SLES. The  $1266\text{ cm}^{-1}$  spectrum represents amide III and alpha-helix structure in collagen, and the  $1266\text{ cm}^{-1}$  spectrum represents amide III, fatty acid, and collagen, which remain the same in both scaffolds. The  $1746\text{ cm}^{-1}$  spectrum is related to lipids, which were retained more in the cell depletion with SLES (Figure 4).

## Discussion

In this study, rats were anesthetized with intramuscular injection of ketamine and xylazine. After removal of the rat intestine, heparin was injected into the inferior vena cava in order to prevent blood clots and thrombosis. In 2018, Wang et al stated that the use of heparin helps prevent blood clots after the implantation of decellularized scaffolds in the body (15).

Due to the binding and release capacity of heparin to growth factors, such as VEGF and bFGF, as well as the modulation of angiogenesis, along with its antithrombotic properties, heparin has been used in many different systems, including hydrogels, films, and electrospun

fibers (16).

Many studies have shown that the use of heparin in the *in vitro* environment, along with the culture of endothelial or smooth muscle cells, increases the mechanical properties, biocompatibility, and anti-thrombogenicity of these grafts and the potential of heparin to expand endothelialization and cell differentiation.

In this study, SDS and SLES were used for intestinal tissue decellularization. The structural difference between these molecules is attributed to the ethylene oxide (EO) unit, which is the distance between hydrophilic and hydrophobic parts in SLES (17).

As a strong anionic detergent, SDS can degrade proteins, damage phospholipid membranes, and develop cytotoxicity, and is useful for the rapid disruption of biological membranes. Since it causes a rapid disruption of the tissue structure and inhibits the activity of RNase and deoxyribonuclease (DNase), it is one of the main substances used to destroy nucleic acids. The performance of this material can be significantly affected by its purity.

SLES is a milder anionic detergent compared with SDS,

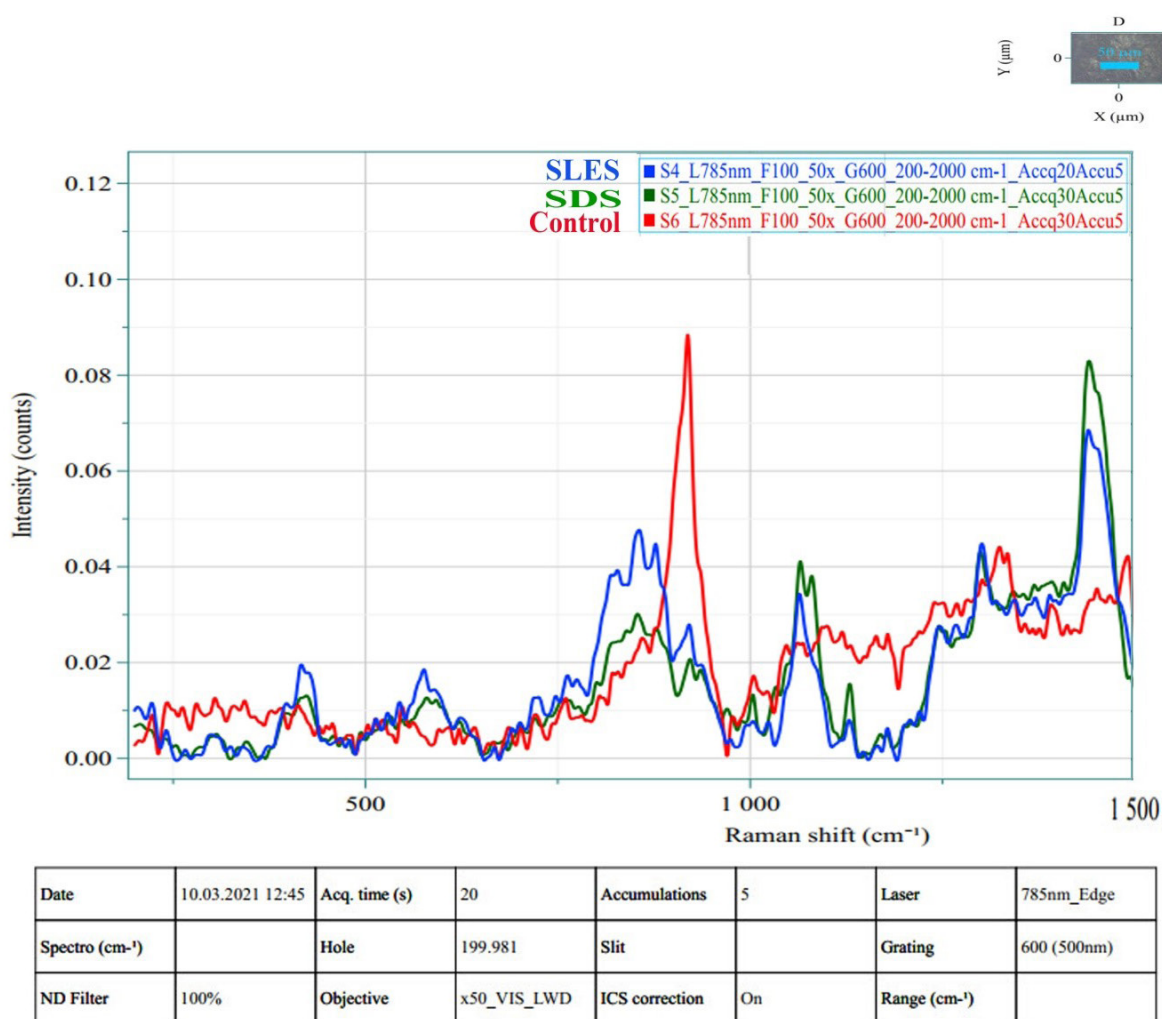


Figure 4. Raman spectra of control and decellularized intestine treatment using SDS and SLES

characterized by high dispersion, wide compatibility, and great biodegradability (14-18).

As previously mentioned, in SLES decellularized intestines, compared to SDS decellularized intestines, there were fewer changes observed in the epithelial tissue, and the structure was better preserved. The muscle layer and GAGs were well preserved in both scaffolds, but the serous layer and collagen fibers in SLES decellularized intestines were better preserved than in SDS decellularized intestines; it can have a detrimental effect on the structure and integrity of the ECM in some types of tissues (19).

The harmful effects of SDS on ECM composition, mechanical integrity, and structure may depend on the concentration of SDS and the duration of its use for tissue decellularization. For example, decellularization of the porcine aortic valve with SDS 0.1% showed that the ECM structure was better preserved than with trypsin or Triton X-100 (20). Decellularization of articular cartilage with 2% SDS solution for 8 hours also showed that it removes all GAG content, so it significantly alters the ECM (21). In addition, since removing the remaining SDS may be difficult, it may involve the host response during organ ligation (22).

Ren et al showed that in the decellularization of rat liver tissue using Triton X-100 and SDS, collagen was preserved in both treatments. However, after treatment with SDS and Triton X-100 compared to normal tissue, the reduction of elastin was measured using immunostaining, and results were about 20% and 60%, respectively; reduction of GAG content was analyzed using the Bicolor Blyscan Assay Ki, and results were about 10% and 50%, respectively; the decrease of hepatocyte growth factor was investigated via ELISA, and results were about 20% and 60%, respectively (23).

Kawasaki et al Showed that SLES significantly reduces inflammation and thrombogenesis in transplanted hearts and kidneys (24). In addition, SLES provided better preservation of proteoglycans, cytokines (such as FG), and ECM microstructure and basement membranes around vessels compared to SDS. These results showed that decellularized scaffolds with SLES could be used for the differentiation of stem cells and had higher efficiency than scaffolds produced with SDS (25).

In 2015, Kawasaki et al showed that SLES-decellularized tissues were more protected and less damaged than tissues treated with SDS. Mesenteric transplantation also showed that SLES, unlike SDS, did not cause significant inflammation. Platelet adhesion to tissues decellularized with SLES was significantly reduced. In general, they stated that SLES could replace older detergents such as SDS in the decellularization process to create re-transplantable organs (24).

## Conclusion

The results of the current study showed that

both detergents were suitable for intestinal cell decellularization. Both removed the overall structure of intestinal tissue, however, SLES retained collagen and GAG content better than SDS. According to our findings and previous studies, due to the cheaper price and lower toxicity characteristics of SLES, it can be more appropriate for intestinal decellularization compared to SDS.

## Competing Interests

The authors declare that there is no conflict of interest.

## Ethical Approval

The study was approved by the Ethics Committee of Hormozgan University of Medical Sciences (Ethical code: IR.HUMS.REC.1398.427).

## Funding

This project was funded by the Student Research Committee and the Medical University Research Council of Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

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