ORIGINAL ARTICLE

Veterinary Research Forum. 2021; 12 (3) 339 - 348
doi: 10.30466/vrf.2019.104640.2491
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Kidney tissue engineering using a well-preserved acellular rat kidney scaffold and mesenchymal stem cells

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Article Info

Article history:

Received: 03 March 2019
Accepted: 14 September 2019
Available online: 15 September 2021

Keywords:

Decellularization
Extracellular matrix scaffold
Kidney
Mesenchymal stem cells
Recellularization

Abstract

The aim of this study was to acquire an effective method for preparation of rat decellularized kidney scaffolds capable of supporting proliferation and differentiation of human adipose tissue derived mesenchymal stem cells (AD-MSCs) into kidney cells. We compared two detergents, the sodium dodecyl sulfate (SDS) and triton X-100 for decellularization. The efficiency of these methods was assessed by Hematoxylin and Eosin (H&E), 4', 6 diamidino-2-phenylindole and immunohistochemistry (IHC) staining. In the next step, AD-MSCs were seeded into the SDS-treated scaffolds and assessed after three weeks of culture. Proliferation and differentiation of AD-MSCs into kidney-specific cell types were then analyzed by H&E and IHC staining. The histological examinations revealed that SDS was more efficient in removing kidney cells at all-time points compared to triton X-100. Also, in the SDS-treated sections the native extracellular matrix was more preserved than the triton-treated samples. Laminin was completely preserved during decellularization procedure using SDS. Cell attachment in the renal scaffold was observed after recellularization. Furthermore, differentiation of AD-MSCs into epithelial and endothelial cells was confirmed by expression of Na-K ATPase and vascular endothelial growth factor receptor 2 (VEGFR-2) in seeded rat renal scaffolds, respectively. Our findings illustrated that SDS was more effective for decellularization of rat kidney compared to triton X-100. We presented an optimized method for decellularization and recellularization of rat kidneys to create functional renal natural scaffolds. These natural scaffolds supported the growth of AD-MSCs and could also induce differentiation of these cells into epithelial and endothelial cells.

Introduction

The incidence of end-stage renal diseases (ESRDs) appears to be increasing in the world. Dialysis and renal transplantation are possible treatments for patients with ESRD.[1] Renal transplantation is considered as one of the most effective treatments, which can recover renal function,[2] and greatly improves life quality.[3] However, renal transplantation still faces a basic challenge: an inadequate supply of viable donor kidney for the growing demand of transplants.[4] Progress in tissue engineering and regenerative medicine can cut the gap between limited supply of organs and increasing demands.[5] Extracellular matrix (ECM) scaffold can be prepared by decellularization of native tissues.[5,6] Several decellularization processes including physical methods like osmotic shock and freeze-thaw, and chemical methods such as treatment with SDS or triton X-100, as well as enzymatic regimens have been used to reach the ECM-derived scaffolds.[7-9] Natural renal scaffolds should be free of all native cells, but preserve biological activity, tensile strength, and the primary proteins of the ECM (e.g. laminins, collagens, and

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fibronectins).[7] Moreover, these acellular renal scaffolds should support the kidney three-dimensional architecture of vasculature and capillary network.[10,11] Thus, when stem cells are seeded on renal ECM scaffolds, which are a complex of the proteins and biomolecules,[12] decellularized scaffolds play important roles in the cell adhesion, cell signaling,[13] proliferation, migration, and differentiation of stem cells into kidney-specific cell types.[13] Until now, decellularization methods have been used for a variety of tissues including blood vessels,[14,15] valves,[16] urinary bladder,[17] liver,[18] intestine,[19] trachea,[20] and kidney.[11,21] Due to differences in tissue mass, function, structure and biomechanical characteristics, each organ or tissue requires specific processing and investigation.[6,22] Several decellularization methods have been proposed for rat kidney and renal ECM scaffolds were produced.[23] The goal of the present study was to compare two detergents, the non-ionic triton X-100 and the ionic SDS, for decellularization of rat kidney and to optimize an effective decellularization method for possible kidney engineering. Following this, human AD-MSCs were seeded into the rat kidney scaffolds and their survival and differentiation were assessed after 1, 2 and 3 weeks of culture.

Materials and Methods

Chemicals. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/ streptomycin were purchased from Gibco (Paisley, Scotland). SDS, triton X-100, phosphate buffered saline (PBS), formalin and diaminobenzidine (DAB) were prepared from Sigma-Aldrich, (St. Louis, USA). Paraffin, Hematoxylin and Eosin (H&E) and 4', 6 diamidino-2-phenylindole (DAPI), were purchased from Merck Company (Darmstadt, Germany).

Organ preparation. In this study, rat kidneys were collected from 10 male Wistar rats (weighing 250-300 g, six weeks old) which were obtained from the Animal House of the Medical School, Mashhad University of Medical Sciences. All experiments were approved by the Animal Research Ethical Committee of Mashhad University of Medical Sciences (project code: MUMS 941327). Kidneys were divided into transverse sections of 10.00 × 10.00 × 2.00 mm pieces using a scalpel, and washed with normal saline.[24] All the experiments were performed under sterile conditions. The prepared sections included both cortex and medulla.

Decellularization of rat kidney. Kidney sections were embedded in decellularization solution of either 1.00% (v/v) SDS or 1.00% (v/v) triton X-100 diluted in distilled water at 4.00 ˚C in a shaking incubator (200 rpm). Decellularization solution was changed 8 hr after first tissue harvesting and then every 48 hr until tissues were transparent. Decellularized kidney sections were analyzed after 2, 5, 10 and 14 days of soaking in decellularization solutions to find ideal time point for decellularization. Afterward, the sections were washed twice with PBS to remove the detergents completely.[24] Finally, rat renal ECM scaffolds were fixed in 10.00% formalin and softly embedded in paraffin.[25]

Acellular kidney scaffolds characterization. In the current study, fresh, SDS-decellularized, and triton X-100-decellularized paraffin-embedded sections were analyzed by H&E, DAPI, and immunohistochemistry (IHC) staining.

Histological examinations. Both native kidney tissue and acellular paraffin-embedded tissue sections were cut into 5.00 µm sections and stained with H&E. Then slides were examined under optical microscope (Olympus, Tokyo, Japan) for detection of cell nuclei elimination and ECM architecture assessment.[26] To determine residual DNA and the degree of cell removal in samples, DAPI staining was used according to the routine protocol, followed by their analysis under a fluorescent microscope (Olympus).

Immunohistochemical analysis. To check the integrity and preservation of ECM architecture in rat kidney scaffolds prepared with the better detergent, between SDS and triton X-100, expression of laminin as the most important basement membrane protein was examined by immunohistochemistry.[23] For immunohistochemical staining, the 5.00 µm thick sections were deparaffinized, rehydrated and incubated with proteinase K. After endogenous peroxidase activity blocking with methanol containing 3.00% H_2O_2, tissue sections were incubated in 10.00% normal goat serum. Sections were then covered with primary antibody to laminin (dilution 1:50, rabbit polyclonal anti-laminin; Abcam, London, UK) and left overnight in a humid chamber at 4.00 ˚C. The next day, after washing with PBS, sections were incubated with HRP-conjugated secondary antibody (dilution 1:500, goat polyclonal secondary antibody to rabbit IgG; Abcam) for 2 hr at room temperature. Finally, the sections were developed using diaminobenzidine (DAB) as a chromogen. The slides were subsequently counterstained with hematoxylin and were observed under a light microscope.[27] The staining intensity was scored by three examiners blindly (0 = no staining, 1 = weak, 2 = moderate, and 3 = strong).[28]

Recellularization of kidney scaffolds with hAD-MSCs and histological examinations. The AD-MSCs were derived and characterized as previously described.[29] For sterilization, SDS-treated scaffolds were washed with distilled water and soaked in sterile PBS solution for 1 hr, followed by placing them in 70.00% ethanol for 15 min. Finally, decellularized kidney scaffolds were placed into wells of 24 well plates and soaked in culture medium (1.00 mL) with 10.00% FBS and 1.00% penicillin/streptomycin for 24 hr.[30] Then, cell seeding was conducted by dripping 0.05 mL cell suspension with the cell density of 2.00×10^5 cells per scaffold. One hour after seeding, 1.00 mL DMEM was added into each well, which were then incubated at 37.00 ˚C and 5.00% CO_2 for three weeks. Changing the

medium was performed every 48 hr. Unseeded scaffolds were used as controls and cell-seeded renal scaffolds were analyzed after 7, 14, and 21 days of culture with H&E and IHC staining. The H&E staining was performed to determine cellular attachment, survival and migration within the scaffolds. Also, three slides were selected at each day, and the numbers of cells were counted from part of the slides randomly (40×). After data collection, statistical analysis was used to determine the significance of time on the number of cells.[30] Furthermore, differentiation of AD-MSCs towards epithelial and endothelial cells of the kidney, was determined by staining with primary antibodies against sodium/potassium adenosine triphosphatase (Na/K-ATPase dilution 1:50; rabbit polyclonal anti- Na-K ATPase, Abcam) and vascular endothelial growth factor receptor-2 (VEGF-R2, dilution 1:20; rabbit polyclonal anti-VEGF-R2, Abcam).[31] The staining intensity was scored by three examiners blindly (0 = no staining, 1 = weak, 2 = moderate, and 3 = strong).[28]

Statistical analysis. The data were expressed as means ± SD. Kruskal–Wallis and Mann–Whitney non-parametric statistical tests were used to compare differences between immunostaining scores. The difference between means of parametric data was statistically analyzed using repeated measures analysis followed by Bonferroni test. Differences were considered statistically significant when $p < 0.05$.

Results

Histological and immunohistochemical analysis of the scaffolds. The gross observation of the decellularized tissues showed that the color of SDS-treated sections turned white and became translucent more efficiently and faster than triton-treated sections. Based on H&E, comparison of the decellularized and native kidney tissues revealed a successful elimination of cell nuclei with better extra-cellular matrix preservation in SDS-treated samples compared to the triton-treated scaffolds over the same time frame (Fig. 1). The DAPI staining similarly indicated that residual DNA absence in the SDS-treated scaffolds was greater than triton-treated scaffolds compared to fresh kidney sections (Fig. 2). This results showed that SDS was a better detergent for preparation of rat kidney scaffolds as determined by H&E. Microscopic analyses of H&E and DAPI staining revealed that treatment of sections with SDS showed limited decellularization after 2 days, while SDS treatment after 5 days or more, resulted in full decellularization with minimal changes in tissue volume and maintained the architecture of glomeruli, vessels, tubules and integrity of the extracellular matrix (Fig. 1). Furthermore, no cell nuclei or remaining DNA could be detected in these scaffolds after DAPI staining (Fig. 2). Eosinophilic prevalence structures in these scaffolds indicated that the tissue was largely composed of collagen.

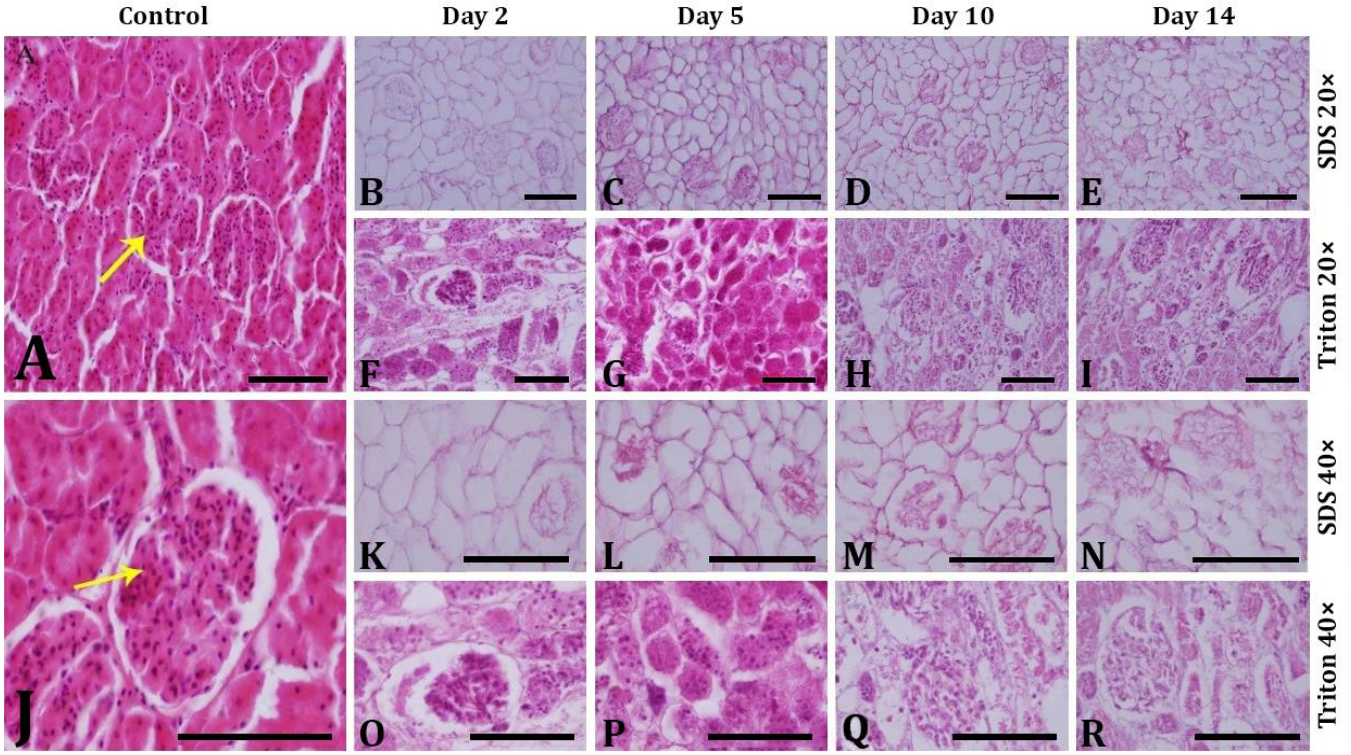

Fig. 1. Comparing the elimination of cell nuclei and extracellular matrix preservation in the native and decellularized kidney tissues: H&E staining of **A)** control (Scale bar= 200 µm), and **B to E)** samples treated with SDS at 2, 5, 10 and 14 days (Scale bar= 200 µm) and **F to I)** Triton X-100 after 2, 5, 10 and 14 days of decellularization (Scale bar= 200 µm). **G)** Control (Scale bar= 200 µm), **K to N)** samples treated with SDS on 2, 5, 10 and 14 days (Scale bar= 200 µm) and **O to R)** Triton X-100 on 2, 5, 10 and 14 days (Scale bar= 1000 µm).

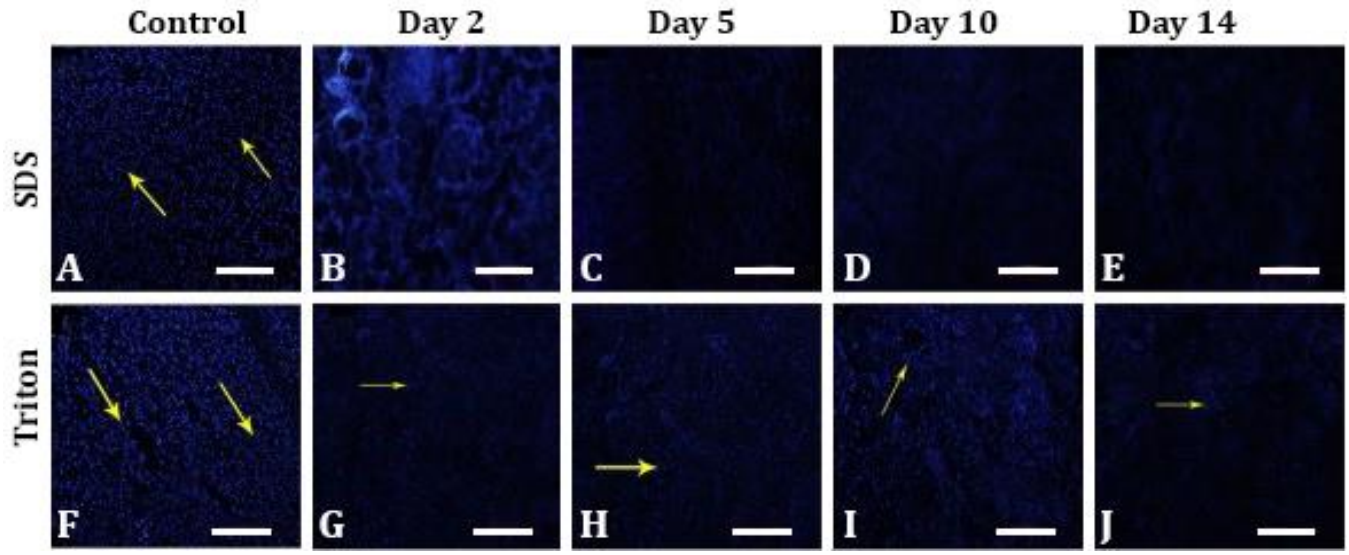

Fig. 2. DAPI staining of SDS-treated scaffolds and triton-treated scaffolds compared to **A, F)** fresh kidney sections, **B to E)** kidney decellularized with SDS on 2, 5, 10 and 14 days and **G to J)** decellularization with triton X-100 on 2, 5, 10 and 14 days. Nuclei are shown with arrows, (Scale bars = 200 µm).

To assess the conservation degree of decellularized renal ECM matrix composition, immunohistochemical staining was performed for laminin on renal ECM prepared using the SDS protocol and native rat kidney tissue. The results of IHC staining revealed that contiguous laminin network and basement membrane integrity were completely preserved during decellularization procedure and laminin was distributed in tubules and glomerular basement membrane. Also, laminin displayed similar expression patterns in both native and decellularized kidneys, however, we observed a little decrease in laminin expression from day 5 towards day 14 (Fig. 3). According to these results and the integrity of the ECM, decellularization with 1.00% SDS led to fully decellularized bioscaffolds on day 5. Further, the results indicated that expression of laminin was significantly reduced in decellularized bioscaffolds on day 10 and 14 in comparison with native rat kidney tissue ($p < 0.05$), (Fig. 4A).

Recellularization of kidney scaffolds with hAD-MSCs. In order to assess these scaffolds suitability for tissue engineering, AD-MSCs were seeded on SDS-treated scaffolds to check their attachment and differentiation potential. Histological examination indicated seeded cells presence and thus non-toxicity of the SDS-treated scaffolds on day 7. Furthermore, AD-MSCs could adhere and penetrate into rat renal scaffolds 7 days after culture (Fig. 5). By day 14, H&E staining results showed an increase in proliferation, the number of adhered cells and their tendency to migrate into the SDS-treated scaffolds compared to day 7 (Fig. 5). In some areas, cells formed a bilayer pattern and produced an epithelium-like structure. However, the number of AD-MSCs observed on or within the scaffold was decreased by time as seen in Figure 5 on day 21. Moreover, histological studies showed that the structure of seeded scaffolds with AD-MSCs was reconstructed compared to the renal ECM scaffolds which

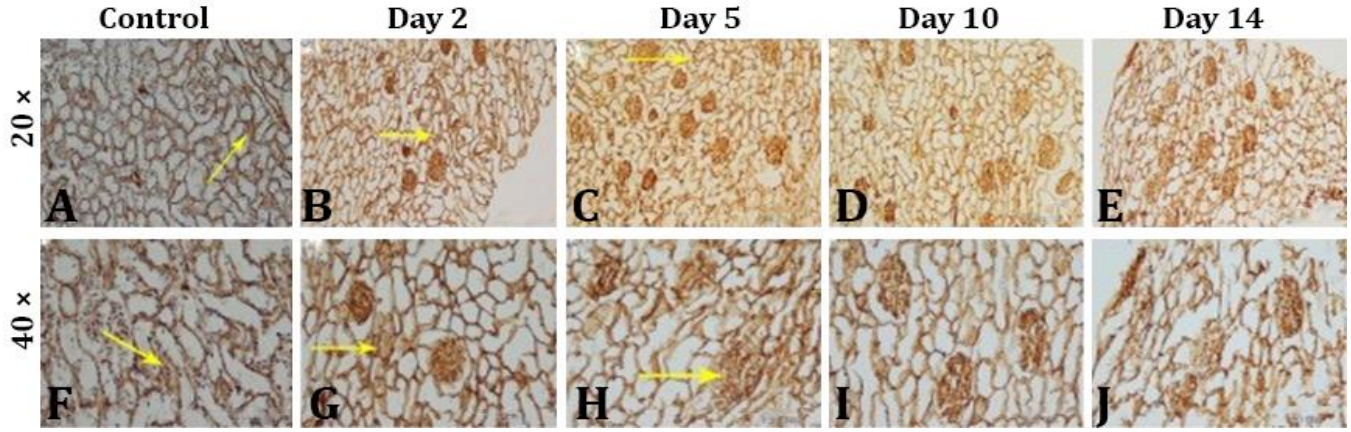

Fig. 3. Immunohistochemical staining of control and decellularized kidney sections with SDS for laminin. **A**) Native kidney, **B to E** (20 ×) and **F to J** (40 ×) kidney decellularized with SDS on 2, 5, 10 and 14 days. Immunoreactivity is shown with arrows.

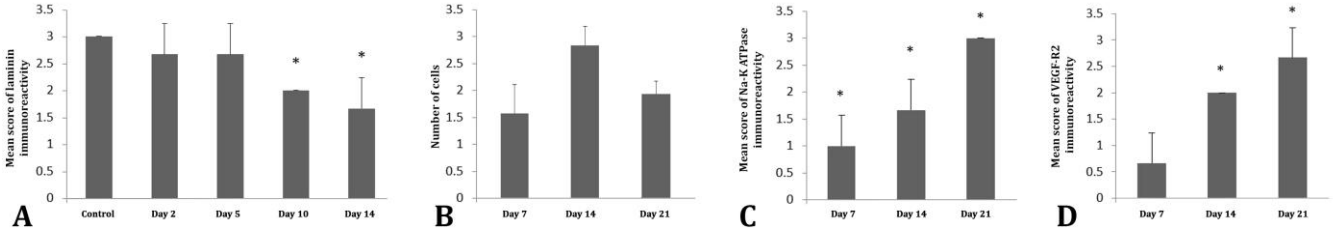

Fig. 4. A) Laminin immunoreactivity in kidney decellularized with SDS on 2, 5, 10 and 14 days. *$p < 0.05$: Significant difference was observed in decellularized kidney sections with SDS compared to native kidney; **B)** Average number of cells in each scaffold in recellularization rat kidneys with hAD-MSCs after 7, 14 and 21 days. There was no significant difference between the cellular densities in seeded SDS-treated scaffolds with AD-MSCs at days 7, 14, and 21; **C)** Na-K ATPase and **D)** VEGF-R2 immunoreactivity on 7, 14, and 21 days after recellularization of rat kidney scaffolds with hAD-MSCs. *$p < 0.05$: Significant difference was observed in seeded rat renal SDS-treated scaffolds with AD-MSCs compared to non-seeded scaffolds on the same days. Data are provided as mean ± standard deviation.

were harvested without cells on day 7. Most importantly, the highest regeneration was observed on day 14 compared to days 7 and 21. In the resultant graph of cell counting and density it is evident that the number of cells was increased on days 14, and 21 in comparison with day 7, however, this increase was not significant. Also, no significant difference was observed between the cell densities at day 14 compared to day 21 ($p < 0.05$), (Fig. 4B).

Immunohistochemical analysis of recellularized kidney scaffolds. Na-K ATPase expression, an ionic pump in the plasma membrane of cells, was evaluated with IHC staining to analyze differentiation of AD-MSCs seeded on SDS-treated scaffolds towards kidney epithelial cells. The results of IHC staining showed that Na-K ATPase was expressed in seeded rat renal scaffolds compared to non seeded scaffolds at different times of cell seeding. As shown in Figure 6, the adherent cells on recellularized kidney ECM scaffolds expressed higher levels of Na-K ATPase on day 14 compared to day 7 after hAD-MSCs seeding. The results of IHC staining showed that Na-K ATPase expression was increased on seeded scaffolds on day 21 compared to day 14. Furthermore, its expression levels were higher in seeded scaffolds than non-seeded scaffolds on day 21.

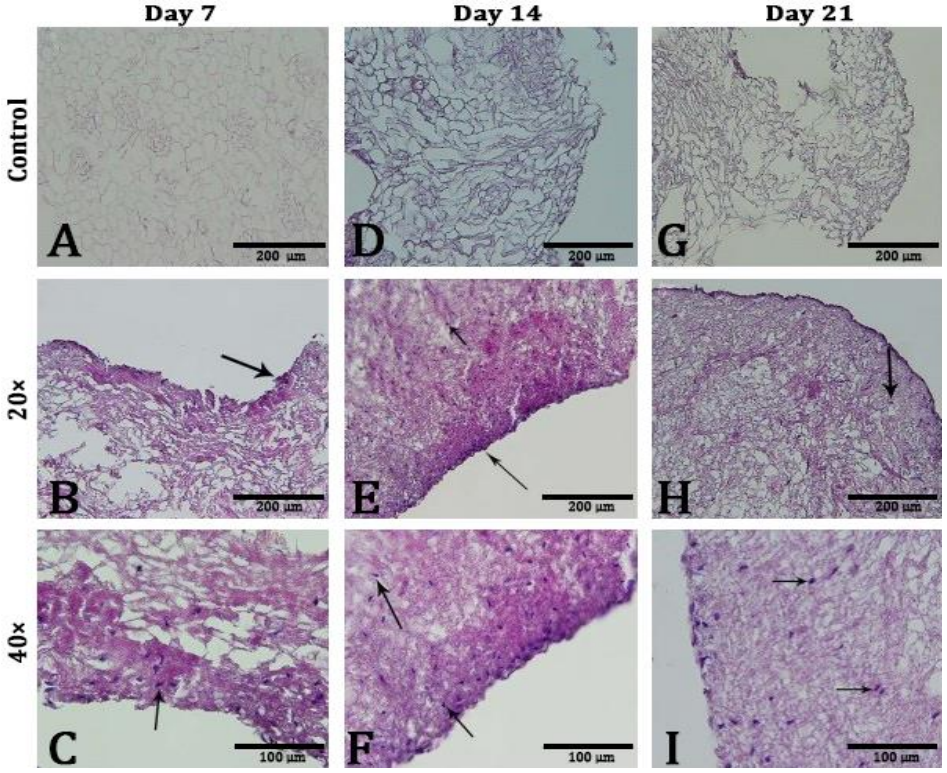

Fig. 5. Morphologic patterns of AD-MSCs seeded on acellular rat kidney scaffolds. **A, D, G)** non-seeded scaffolds after 7, 14 and 21 days. **B to I)** evaluation of AD-MSCs attachment and maintenance on acellular kidney scaffolds after 7, 14 and 21 days of culture. AD-MSCs are shown with arrows, (H&E staining).

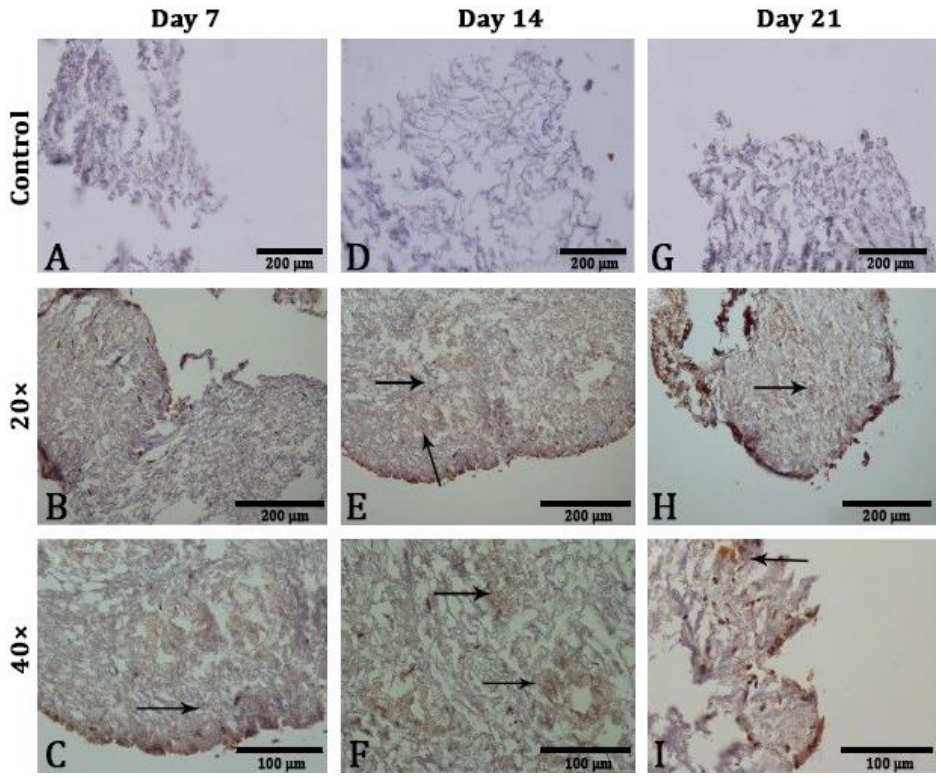

Fig. 6. Immunohistochemical analysis of recellularized rat kidneys with hAD-MSCs for Na-K ATPase. IHC staining of **A, D, G)** non seeded scaffolds after 7, 14 and 21 days. Positive immunoreactivity of **B to I)** seeded scaffolds with hAD-MSCs for Na-K ATPase. Immunoreactivity is shown with arrows.

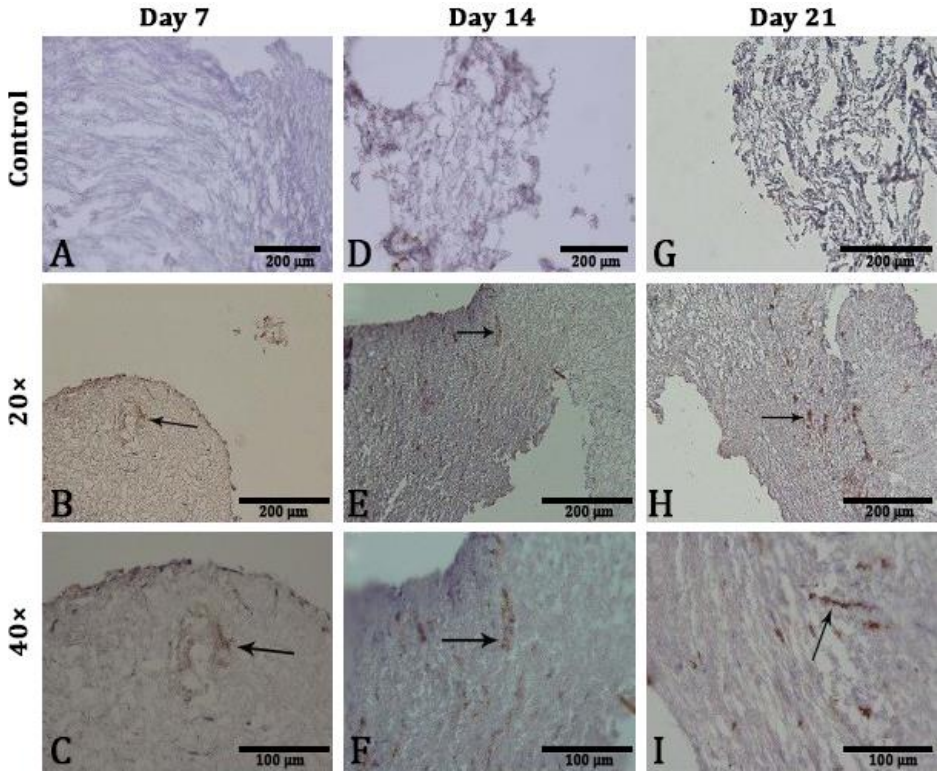

Fig. 7. Immunohistochemical analysis of recellularized rat kidneys with hAd-MSCs for VEGF-R2. IHC staining of non-seeded scaffolds after 7, 14 and 21 days **(A, D, G)**. Positive immunoreactivity of seeded scaffolds with hAd-MSCs for VEGF-R2 **(B to I)**.

Results of IHC staining indicated that VEGF-R2 was expressed in rat renal scaffold 7 days after cell seeding compared to non-seeded scaffolds (Figs. 7A-7C), and its expression in cells within the vasculature and glomerular capillaries showed a significant increase on day 14 compared to the acellular scaffold at the same time and rat renal scaffold on day 7 after cell seeding (Figs. 7D-7F).

Despite the decrease in the number of cells on recellularized kidney ECM scaffolds prepared with SDS, 21 days after Ad-MSC seeding in comparison to renal cell-seeded scaffolds on day 14, IHC staining illustrated higher VEGF-R2 expression levels on day 21 (Figs. 7G-7I). However, overall expressions of Na-K ATPase and VEGF-R2 were lower in seeded rat renal SDS-treated scaffolds compared to native rat kidney tissue. Moreover, the results showed that the expression of Na-K ATPase was significantly enhanced 7, 14, and 21 days after cell seeding in comparison with non-seeded scaffolds on the same days. The expression of VEGF-R2 was significantly enhanced 14, and 21 days after cell seeding in comparison with non-seeded scaffolds on the same days ($p < 0.05$), (Figs. 4C and 4D).

Discussion

Organ shortage for transplantation has dramatically increased in recent years and investigators have been prompted to discover novel solutions to overcome this problem.[2] Using the scaffolds derived from biological tissues can be a platform technology for regenerative medicine.[32] Accordingly, it is necessary to acquire an effective method for preparation of a decellularized kidney scaffold which can support proliferation and differentiation of various stem cells into kidney cells.[12] Even though various methods of decellularization for kidney in different species have been accomplished in several research studies,[3,32,33] there are fewer reports comparing different chemical detergents for producing ideal decellularized ECM architecture to support cell attachment and recellularization.[22,25] Here, in the present study, transverse sections of mature rat kidneys were required to use the minimum number of animals. Here, we aimed to compare different methods for preparation of a natural rat scaffold. To do so, two commonly ionic (SDS) and non-ionic (triton X-100) detergents with different physical and chemical attributes were used for decellularization. SDS is an ionic detergent with the ability to entirely denature proteins by disrupting protein–protein interactions while retaining the structure and composition of the ECM.[2,24] SDS is more powerful than triton X-100 in removing the entire cellular materials from solid tissues such as kidney.[5,25] Moreover, SDS achieves quick cell disruption and nuclear elimination compared to triton X-100 that achieves these slowly.[11]

On the other hand, triton X-100, a non-ionic detergent, disconnects lipid-protein and lipid-lipid interactions, however, not protein-protein interactions and thereby leads to separation of the cells from each other and release of the cytoplasmic materials as a result of cell membrane lysis.[34,35] There is a debate on suitability of these two popular detergents for preparation of kidney scaffolds. SDS has been stated as an effective detergent for kidney decellularization by Nakayama *et al.*,[25] Sullivan *et al.*[22] and Ross *et al.*[23], however, some studies have reported triton X-100 as a more suitable detergent.[24,36] In this study, the results of H&E staining could indicate that cell nuclei and cellular compartments of rat kidneys could be perfectly eliminated with SDS-based solutions in comparison with triton-based solutions. Moreover, maintenance of vascular integrity and native tissue architecture of renal ECM scaffolds in SDS-treated sections were more effective than triton-based solutions. DAPI staining results also demonstrated more residual DNA in the triton-treated scaffolds when compared to intact kidney tissue sections. Inadequate decellularization and cellular remains are the most important reason for acute immune response and rejection of tissue engineered scaffolds. Thus, validation of complete decellularization is necessary.[37] These results are bolstered by Sullivan *et al*. who compared the ionic and non-ionic detergents for porcine kidneys to recognize the best method for kidney decellularization. They illustrated that 0.50% SDS was a more effective detergent for decellularization of porcine kidneys compared to triton X-100.[22] Moreover, Orlando *et al*. reported that renal ECM scaffolds using SDS treatment were successfully produced from human kidneys. Furthermore, these scaffolds preserved renal ECM structure (glomeruli, tubules, vessels) and the biochemical properties.[38] Bonandrini *et al*. presented efficient production of an acellular ECM scaffold from rat kidney using only SDS instead of SDS and triton X-100 mixture. These scaffolds maintained integrity of ECM structure, glomerular capillaries and tubular membranes.[39] Fischer *et al*. reported that SDS and sodium deoxycholate (SDC) represented best cell removal efficacy, while triton X-100 insufficiently decellularized the porcine kidney pieces.[40] In the present work, SDS was reported as an appropriate detergent for providing rat renal ECM scaffolds in agreement with earlier studies on this topic. In addition to histological examinations, IHC was carried out to confirm the maintenance of ECM components in renal ECM scaffolds prepared using SDS protocol. Laminin is one of the most important components of the basement membrane which is involved in cell viability, migration, and differentiation.[33,41] In agreement with previous studies,[23,25,39] the IHC results determined complete decellularization of rat kidney tissue without loss of laminin expression. Based on laminin expression, the best time for recellularization of renal SDS-treated scaffolds was determined as five days after cell removal. The

challenge of recellularization should be addressed as the next step towards creating a viable tissue. For this purpose, AD-MSCs were seeded on the SDS-treated scaffolds, and cell survival and differentiation into kidney-specific cell types were assessed for up to 21 days. Use of AD-MSCs was preferred due to their high plasticity and differentiation potential towards many different cell types. Our results were indicative of proper attachment and growth of AD-MSCs on rat renal SDS-treated scaffolds. Attachment and a notable increase in the number of AD-MSCs were remarked by histological studies after 7, 14 and 21 days of scaffold recellularization. However, H&E staining showed a decrease in cell density on SDS-treated scaffolds on day 21 after cell seeding compared to day 14. MSCs destroy ECM proteins through secretion of proteases and open a new path for migration in extracellular matrix which is probable cause of scaffold destruction in the place of cell existence. In response to substrate, migrating cells activate various kinds of enzymes including proteases and metalloproteinases or increase their expression. After 21 days cultivation of AD-MSCs on scaffold of rat kidney, some of the cells that were not connected to basal membrane of matrix may experience apoptosis.[30,42] Moreover, reduction of the cell number on day 21 of recellularization could indicate cells progression towards apoptosis. These results showed that SDS-treated scaffolds present a suitable 3D microenvironment required for Ad-MSC proliferation and differentiation.[11,39] Ross *et al*. stated the first study on renal scaffolds repopulation in 2009. They successfully recellularized renal scaffolds created by SDS protocol with murine ES cells and presented cellular growth within the tubular structures and glomerular network.[23] In addition, as shown by Bonandrini *et al*. mouse embryonic stem (mES) cells were infused into kidney scaffolds and contributed in the glomerular capillaries and vascular network.[39] Our results were also consistent with the results reported by Guan *et al*.[11] Who reported that acellular renal scaffolds produced with 0.5% SDS were recellularized with mES cells.

In the present study, immunohistochemistry technique was also used to assess Na-K ATPase and VEGF-R2 expression in cultured AD-MSCs on renal SDS-treated scaffolds. The Na-K ATPase is an integral membrane protein which produces electrochemical gradients.[43] VEGF-R2 a receptor for VEGF-A, is known as the primary marker for endothelial cell growth which plays a role in proliferation, maintenance and migration of endothelial cells.[44] Nakayama *et al*. reported that human embryonic stem cells (hESCs) seeded on rhesus monkey kidney scaffolds were able to produce tubular structures. The recellularized constructs expressed renal markers including Acupoinine-2 and PAX2 after 8 or 16 days of culture.[34] As shown by Bonandrini *et al*. infused murine embryonic stem cells through the renal artery scaffold expressed CD31 and Tie-2, markers of endothelial lineage, after 24 and 72 hr.[39] In this study, IHC staining demonstrated that both VEGF-R2 and Na-K ATPase expressions were increased on AD-MSCs seeded on renal SDS-treated scaffolds. Higher Na-K ATPase and VEGFR expression were observed on day 21 after culture. However, expression of both markers was higher on native human kidney tissue compared to the seeded rat renal scaffolds after 7, 14, and 21 days from cell seeding. Eventually, the results of this study showed that these natural scaffolds not only supported the proliferation and growth of AD-MSCs but they were also good for differentiation of adherent cells into kidney cell types, endothelial cells of vascular structures, and glomerular capillaries. Our results demonstrated an effective decellularization technique with SDS capable of removing all cellular components from rat kidneys, and creating an intact three dimensional ECM architecture which can preserve tubular structure, glomerular capillaries, and vasculature. These natural SDS-treated scaffolds supported the growth of AD-MSCs and could induce their differentiation into epithelial and endothelial cells. Further experiments are required to assess the suitability of this method for preparation of whole organ kidney scaffolds and also testing them in animal models.

There are a few limitations to this study that need to be addressed in further studies. In this project, it was better to examine expression of more specific markers for kidney cells differentiation such as prominin-1 (CD133), CD10, PAX8 and PAX2. Therefore, the immunohistochemical evidence of the seeded rat renal SDS-treated scaffolds needs to be evaluated in further studies.

Acknowledgments

This study was part of PhD student thesis and financially supported by Mashhad University of Medical Sciences, Mashhad, Iran.

Conflicts of interest

The authors have no financial conflicts of interest related to this study.

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