Contribution of Autologous Omentum Transposition to the Regeneration of Renal Injuries in the Rat Model

Tayfun Bilgic (tbilgic77@gmail.com)
Acibadem Mehmet Ali Aydinlar Universitesi https://orcid.org/0000-0002-7564-3663
Umit Ince
Acibadem Mehmet Ali Aydinlar Universitesi
Fehmi Narter
Acibadem Mehmet Ali Aydinlar Universitesi

Research

Keywords: Omentum, renal trauma, rat, regeneration

Posted Date: December 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54600/v3

Abstract

Aim: After renal trauma, surgical treatment is vital, but sometimes there may be loss of function due to fibrosis. We aimed to evaluate the repair effect of transpositioned autologous omentum on injured renal tissue in a rat model.

Methods: A total of 30 female Wistar Albino rats were included and they were randomly separated into a sham group and four study groups. Iatrogenic renal injuries were repaired using a surgical technique (primary repair 1 and 2 groups) or transpositioned autologous omentum (omentum repair 1 and 2 groups). In all groups, blood samples were taken preoperatively and on the 7^{th} postoperative day in all groups and also on the 18^{th} postoperative day in the control and two study groups. All rats were sacrificed on the 7^{th} or 18^{th} day postoperatively and their right kidneys were taken for histopathological evaluation.

Results: There was a trend toward decrease in urea and creatinine levels in all the groups. There was no significant correlation between urea and creatinine levels and histological finding scores. The omentum repair group had significantly lower inflammation and granulation scores compared with the primary repair and sham groups. There was a significant and positive correlation between inflammation and granulation and fibrosis scores. There was a significant and negative correlation between healing completion score and either inflammation and granulation scores. There were also positive correlations between histological findings in the kidney specimen and surrounding tissues.

Conclusion: The use of the autologous omentum tissue for repair of kidney injury had attenuation effects on inflammation and granulation compared with primary repair. These results imply that use of omentum tissue to facilitate healing of kidney injury may theoretically lead to a more effective healing process and reduced fibrosis and tissue and function loss. These potentially beneficial effects of autologous omentum tissue should be investigated in further well-designed experimental and clinical studies.

Introduction

Renal trauma can occur associated with various mechanisms. Etiological factors are generally described as blunt kidney injuries (80-90%) and penetrating kidney injuries (10%). The main sign of renal trauma is hematuria which does not always happen (1). The main purpose of treatment in kidney trauma is to ensure that the kidney functions return to normal as soon as possible (2). Renal injuries are classified according to severity into 5 grades. Especially, grade 4-5 injuries in the renal tissue are candidate for surgical treatment. Major kidney injuries caused with blunt trauma are treated with conservative management (2,3). The other preferences of treatment are open or endoscopic surgical procedures such as laparoscopic/robot-assisted or open partial/total nephrectomy, nephrorraphy, autotransplantation, and embolization.

It is important to accelerate wound healing in renal traumas requiring surgical treatment in order to decrease morbidity and mortality rates. Wound healing includes three dynamic phases, namely:

inflammation, proliferation, and remodeling (4). Angiogenesis, inflamation, cellular proliferation, collagenization, granulation, and epithelialization are important processes in the remodeling of tissue (5). There are many molecules which have a role in tissue regeneration such as vascular endothelial growth factor (VEGF) and nitric oxide (NO) (6). Synthetic or autologous materials such as fat tissue, omentum, meshes and fascias can be used for regeneration of injured kidney tissue (7).

The omentum is a quite vascular and fatty structure and includes many growth and angiogenic factors in the progenitor cells (8,9). Therefore, it can migrate to damaged tissues and aid in the regeneration process (10,11). It has been used for many surgical procedures in the treatment of bone fractures, spine injuries, ischemic heart diseases, and hepatic injuries (12). Progenitor stem cells have high proliferation and differentiation capabilities. However, they have a very short life span in the tissue if they are injected into a target. In many studies, the omentum has been used to wrap the injured tissue and has been shown to be useful (13). According to the results of a previous study, progression to chronic kidney failure has been shown to slow down after subtotal nephrectomy when omentum was used to cover the kidneys (11). This is a unique contribution in the context of nephron-sparing.

We aimed to evaluate the repair effect of transpositioned autologous omentum on the injured renal tissues in a rat model. We measured renal injury biomarkers (creatinine and urea) and made a histopathological evaluation to determine the degree of injury and repair and also assessed the correlation of biomarkers with histopathological findings.

Material And Methods

A total of 30 female Wistar Albino rats were included, which were 5 to 6 months old and weighed 250 to 300 grams. They were randomly separated into a control and four intervention groups (6 rats per group). In all groups, blood samples were taken preoperatively and on the 7^{th} postoperative day for creatinine and urea analyses. Additional blood samples were obtained on the 18^{th} postoperative day for the same analyses in the control group and two intervention groups (group 2 and group 4). A sham operation was performed to the rats in the control group. All rats in the control group and groups 2 and 4 were sacrificed on the 18^{th} postoperative day and right kidneys were taken for histopathological evaluation. The rats in the groups 1 and 3 were sacrificed on the 7^{th} postoperative day and right kidneys were taken for histopathological evaluation. In all of the intervention groups (groups 1-4) an 8 mm diameter and 4 mm deep parenchymal damage was created with a Stiefel biopsy forceps on the front surface of the right kidneys according to the well described Stiefel biopsy technique in the literature. In the groups 1 and 2 (primary repair groups), kidney injuries were primary repaired with interrupted atraumatic matrix suture technique (Ethicon VICRYL Rapid 8-0, fastest absorbable, synthetic, braided, composed of a copolymer made from 90% glycolide and 10% L-lactide, absorption time 7-10 days). In groups 3 and 4 (kidney omentum repair groups), transpositioned autologous omentum was used without primary sutures on the injured renal tissue for repair. We preferred the time of sacrifice as the 18^{th} postoperative day, according to the study of Miguel et al. (14) which reported that posttraumatic necrosis in the tissue disappeared on

the 18th day. In that study, after this period, collagen maturation took place in the renal capsule and connective tissue at the edges of the wound were contracted (14). The summary of the procedures performed in the study groups are outlined in Figure 1.

The subjects were kept in standardized laboratory conditions of 20–24 C^0, 50–60% relative humidity, controlled light (day-night cycle of 12 h: 8/20 h), fed on standardized rodent food, and given filtered and chlorinated water. The animals were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). All rats were protected against the postoperative infections with an antibiotic (Cefazolin 15 mg/kg, SC).

The only exclusion criterion for this study was the death of the rats before the end of the study.

Tissue sampling and histopathological examination

All kidney samples were fixed in a 10% formaldehyde solution. Kidney tissues were embedded in paraffin and 5 µm tissue sections were obtained for hematoxylin-eosin (H&E) and Masson's Trichrome (MTC) staining protocol for collagen fibers. In addition to macroscopic view, histopathological evaluation consisted of granulation, inflammation, fibrosis, foreign body reaction, and healing in the injured kidney and surrounding tissue (omentum). All components were scored between 0 and 5 according to density of the changes in the tissue (normal:0, rare:1, mild:2, modest:3, common:4, and excessive:5). Macroscopic evaluation consisted only of macroscopic view of the kidney to review the surface of the kidney in terms of presence of abnormal structures and it was performed with a quantitative/semi-quantitative analysis. The degree of granulation was evaluated in the glomeruli and parenchymal tissue and by reviewing these structures in terms of edema, inflammatory cells, angiogenesis, and fibroblasts. Inflammation was evaluated by reviewing the tissues regarding acute inflammatory cells, macrophages, and lymphocytes. The degree of fibrosis (connective tissue evaluation) was evaluated by the presence of fibroblasts and their density. Foreign body reaction was evaluated with the following: necrosis, erythrocytes, and chronic inflammation findings. Healing was determined by the findings of regeneration and normalization in the tissues. The cut sections were examined for completeness and one representative section of each kidney was selected for tissue processing. The histological damage was examined under a light microscope by a pathologist who was blind to the study design (sham vs. renal regeneration). All pathological slides were scanned using a digital pathology system (3D Histech company, P250 – Flash III Dijital Scanner, 20X) and microscopic photos were taken using a software (3D Histech company, CaseViewer program, .tiff format and 300 dpi).

The combined morphologic score was calculated for the renal tissue as follows: macroscopy score/6 + granulation score/6 + inflammation score/6 + connective tissue score/6 + foreign body score/6 + healing score/6. The combined morphologic score was also calculated for the surrounding tissues as follows: granulation score/5 + inflammation score/5 + connective tissue score/5 + foreign body score/5 + healing score/5.

Biochemical analysis

The concentrations of creatinine and urea in the serum were determined by an enzymatic assay (Roche Diagnostics GmbH, Mannheim, Germany). Serum samples for the measurement were collected and stored at -80^0C until the analysis was carried out. All laboratory investigators were blind to each rat's clinical information.

Statistical analysis

It was estimated that 5 groups comprising 6 rats per group would be required to detect 3 units of improvement (with 1.5 units as standard deviation) as a significant effect in a wound healing model, assuming a power of 80% and a confidence level of 95%. SPSS 21.0 (IBM Corp., Armonk, NY) was used for statistical analysis. The descriptive statistics for categorical variables were given as the numbers and percentages. Ordinal variables were presented as medians and continuous variables were presented as means $\pm$ standard deviations (SD). Kolmogorov-Smirnov test was used to assess normality of the variables. Ordinal variables were compared using the Kruskal Wallis and the Mann-Whitney U tests. Nonparametric dependent variables were compared using the Wilcoxon test. Spearman's test was used for correlation analyses. Statistical significance was defined as $p < 0.05$.

Results

Results of biochemical analysis are shown in Figures 2 and 3; and combined scores of morphologic evaluation in the renal and surrounding tissue are shown in Table 1 and Figure 4. Examples of histopathological changes are shown in Figures 5-9.

Table 1. Comparison of combined histological scores of the study groups

			N	Mean	Std. Deviation	95% Confidence Interval for Mean		P-value
						Lower Bound	Upper Bound	
Combined morphologic score for renal tissue	Sham day 18		6	2.233	0.151	2.075	2.391	
		First KR day 7	6	3.567	0.234	3.321	3.812	<0.001
		Second KR day 18	6	3.000	0.537	2.437	3.563	0.002
		First KOR day 7	6	2.300	0.245	2.043	2.557	0.996
		Second KOR day 18	6	2.367	0.266	2.088	2.646	0.947
	First KR day 7		6	3.567	0.234	3.321	3.812	
		Second KR day 18	6	3.000	0.537	2.437	3.563	0.034
		First KOR day 7	6	2.300	0.245	2.043	2.557	<0.001
		Second KOR day 18	6	2.367	0.266	2.088	2.646	<0.001
	Second KR day 18		6	3.000	0.537	2.437	3.563	
		First KOR day 7	6	2.300	0.245	2.043	2.557	0.006
		Second KOR day 18	6	2.367	0.266	2.088	2.646	0.014
	First KOR day 7		6	2.300	0.245	2.043	2.557	
		Second KOR day 18	6	2.367	0.266	2.088	2.646	0.996
		Total	30	2.693	0.601	2.469	2.918	<0.001
Combined morphologic score for surrounding tissue	Sham day 18		6	1.100	0.276	0.811	1.389	
		First KR day 7	6	2.367	0.151	2.209	2.525	<0.001
		Second KR day 18	6	2.333	0.207	2.117	2.550	<0.001
		First KOR day 7	6	2.000	0.506	1.469	2.531	<0.001
		Second KOR day 18	6	1.667	0.163	1.495	1.838	0.019
	First KR day 7		6	2.367	0.151	2.209	2.525	
		Second KR day 18	6	2.333	0.207	2.117	2.550	1.000
		First KOR day 7	6	2.000	0.506	1.469	2.531	0.220
		Second KOR day 18	6	1.667	0.163	1.495	1.838	0.003
	Second KR day 18		6	2.333	0.207	2.117	2.550	
		First KOR day 7	6	2.000	0.506	1.469	2.531	0.303
		Second KOR day 18	6	1.667	0.163	1.495	1.838	0.004
	First KOR day 7		6	2.000	0.506	1.469	2.531	
		Second KOR day 18	6	1.667	0.163	1.495	1.838	0.303

		Total	30	1.893	0.550	1.688	2.099	<0.001

KOR: omentum repair groups, KR: primary repair groups

Figures 2 and 3 outline the urea and creatinine levels of the study groups. The mean creatinine level decreased significantly from day 1 to day 7 in the sham group (p=0.038). The mean creatinine and urea levels significantly decreased from day 1 to day 7 in the primary repair group (p=0.005 and p<0.001, respectively). In the primary repair 2 group, mean creatinine level decreased significantly from day 1 to day 7 (p=0.011). There was no significant change in urea or creatinine levels in the omentum repair group 1. On the other hand, mean urea level significantly decreased from day to day 7 in the omentum repair 2 group (p=0.005). There was no other significant change in urea or creatinine levels within the study groups.

There was no significant difference in kidney macroscopic evaluation or kidney connective tissue scores between the study groups (p=0.56 and p=0.19, respectively). On the other hand, there were significant differences in granulation degree (p<0.001), inflammation degree (p<0.001), foreign body reaction (p=0.001), and completion score for healing process in the kidney specimen (p=0.004). There were also significant differences between granulation degree (p=0.009), inflammation degree (p=0.013), fibrosis degree (p=0.010), and foreign body reaction (p<0.001) in the surrounding tissue.

The primary repair 1 and 2 groups had significantly higher median granulation and inflammation scores in the kidney specimen compared with the sham and omentum repair groups. The omentum repair groups had similar granulation and inflammation scores with the sham group. The foreign body reaction score in the kidney specimen was significantly higher in the primary repair groups compared with the sham group. The completion score for healing process in the kidney specimen was significantly higher in the omentum repair groups compared with the primary repair groups. Figure 10 outlines granulation, inflammation, and completion of healing scores in kidney specimens in the study groups.

The sham group had a significantly lower median foreign body score in the surrounding tissue compared with all other study groups and significantly lower connective tissue reaction scores in the surrounding tissues compared with the primary repair groups and omentum repair 2 group. The primary repair groups had significantly lower median granulation and inflammation scores in the surrounding tissues compared with the omentum repair 2 group. The median histological scores in the surrounding tissues were similar in the remaining comparisons between the study groups.

There were no significant correlations between urea or creatinine levels and histological finding scores.

The granulation degree in kidney specimen had positive moderate or strong correlations with granulation degree (r=0.478, p=0.008), inflammation degree (r=0.591, p=0.001), fibrosis degree (r=0.394, p=0.031), and foreign body reaction in the surrounding tissue (r=0.635, p<0.001). Inflammation degree in the kidney specimen had positive moderate or strong correlations with granulation degree (r=0.512, p=0.004), inflammation degree (r=0.507, p=0.004), fibrosis degree (r=0.434, p=0.017), and foreign body reaction in the surrounding tissue (r=0.660, p<0.001). Fibrosis degree in the kidney specimen

had a moderate and positive correlation with fibrosis degree in the surrounding tissue (r=0.429, p=0.018). Foreign body reaction in the kidney specimen was strongly and positively correlated with fibrosis degree in (r=0.829, p<0.001) and foreign body reaction in the surrounding tissue (r=0.813, p<0.001). Healing process completion score in the kidney had negative moderate or strong correlations with granulation degree (r=-0.625, p=0.001) and foreign body reaction in the surrounding tissue (r=-0.425, p=0.039).

Inflammation degree in the surrounding tissue had positive moderate or strong correlations with granulation degree (r=0.490, p=0.006), fibrosis degree (r=0.397, p=0.030), and foreign body reaction in the surrounding tissue (r=0.431, p=0.017). Fibrosis degree in the surrounding tissue had positive moderate or strong correlations with inflammation degree (r=0.397, p=0.030) and foreign body reaction in the surrounding tissue (r=0.708, p<0.001).

Granulation degree in the kidney specimen was strongly and positively correlated with inflammation degree (r=0.824, p<0.001) and foreign body reaction in the kidney specimen (r=0.872, p<0.001); and a strong and negative correlation with healing process completion score in the kidney (r=-0.627, p=0.001). Inflammation degree in the kidney specimen was strongly and positively correlated with foreign body reaction in the kidney specimen (r=0.731, p=0.001) and strongly and negatively correlated with healing process completion score in the kidney specimen (r=-0.608, p=0.002).

Discussion

The prevalence of renal trauma ranges between 0.3%-3.25% in the literature and the most common causes are blunt trauma followed by penetrating trauma. The most commonly used renal trauma classification is that of the American Association for the Surgery of Trauma (AAST) which ranges between grades 1-5 (15). Currently, except for the hemodynamically unstable grade 4-5 renal trauma, renal injuries are followed up with a conservative approach. Surgical intervention is also considered in case of significant vital changes related with renal injury.

Partial/total nephrectomy or nephrorrhaphy can be preferred according to the type or degree of injury. Usually transperitoneal surgical approach is more preferable because this route provides some advantages such as the early control of large veins and arteries. Surgery for a renal trauma comprises control of the bleeding by sutures, watertight closure of the collecting system, and closure of parenchymal injuries. Even preserving thirty percent of kidney capacity can provide adequate kidney functions. The renal capsule should be preserved at all possible cases for a successful repair (16). Sometimes, if renal capsule is not available, a pedicle flap of omentum, free peritoneal graft, free fat graft, or polyglycolic acid mesh can be used for coverage of a large defect. In the technique, omentum is placed on the injured tissue and superficially sutured with monofilament absorbable sutures (17-19).

The omentum has long been known to have the capacity to migrate to injured organs such as bones, spinal cords, heart, liver, and pancreas and facilitate their healing. Many studies have shown that a reduction in total nephron capacity may cause kidney failure in the future, thus maximum protection of kidney tissue should be the main purpose. Some suture material and surgical techniques can be harmful

to the kidney tissue. For this reason, alternative techniques have been developed to better protect the kidney tissue especially in case of large tissue lose. One of them is to use the omentum or fatty tissue for repairing of renal injury.

The mesenchymal stem cells (MSCs) can be obtained from adipose tissue, peripheral blood or bone marrow. Another alternative source for repairing of injured tissue is the omentum. It is a very vascular structure and suitable to use to facilitate repair in case of injury as it contains a large number of growth and angiogenic factors and progenitor cells for regeneration (20). MSCs were first isolated from adipose tissue in 2001 by Zuk et al. (19). It is well-known that MSCs have the abilities of multipotency, self-renewing, proliferation, regeneration, and differentiation (20). Of note, MSCs can accelerate tissue repair by direct migration to the injured sites (21,22). Alternatively, MSCs may be administered locally or systemically for treatment. It is widely agreed that transplanted MSCs can directly reconstruct impaired organs. They have some specific features as endocrine (growth factors, chemokines, and cytokines with paracrine and autocrine activities), immunomodulatory (T-cells, dendritic cells, and natural killer cells), and anti-inflammatory effects (23). These factors suppress the local immune system, inhibit fibrosis and apoptosis, enhance angiogenesis, and stimulate proliferation and differentiation. Firstly, Iwai et al. discovered that local injection of adipose tissue derived MSCs facilitated attenuation of fibrosis (24).

Normal wound healing process includes endothelial injury, myofibroblast activation, macrophage migration, inflammatory signal stimulation, immune activation, matrix deposition, and remodeling. Especially in the first 24-28 hours, many molecular reactions occur in the tissue. Fibroblasts are very crucial members at the inflammation process. Moreover, functional microcirculatory bed has been shown to be of critical importance in the prevention of epithelial loss and fibrosis (25). Fibrosis is one of the most common and refractory pathological processes. Fibrosis is a redundant accumulation of extracellular matrix (ECM) in tissues by collagen reaction and at the end of the recovery process a thick fibrotic neocapsule can occur. On the other hand, MSCs can directly release HGF and BMP-7, which are important inhibitors of fibrosis. MSCs have been shown to exert anti-fibrotic effects in animal models by matrix metalloproteinases (26). Unlike synthetic meshes, autologous MSCs are immune compatible and this is an advantage in the remodeling process.

In the present study, granulation and inflammation scores in the kidney specimen were significantly lower in the omentum repair groups compared with the sham and primary repair groups. This finding suggests that omentum attenuated granulation and inflammation related with kidney injury. Transpositioned autologous omentum may be effective by reducing macrophage infiltration as well as reducing fibrosis.

In many studies, the histological damage of the kidneys has been evaluated in tissues with the EGTI scoring system (27). This scoring system consists of histological damage in 4 individual components: endothelial, glomerular, tubular, and interstitial (EGTI Scoring system) and is scored from 0 to 4. This scoring is performed in the renal cortex, especially for glomerular units. Therefore, we preferred

to use a new scoring system for histopathological evaluation, so that it was possible to evaluate different components of the regeneration on the whole kidney tissue.

There was a trend towards decrease in urea and creatinine levels in the study groups. Also there was no correlation between urea and creatinine levels and histological finding scores. These findings can be explained by the fact that we could not produce sufficient nephron damage with our trauma model. In the future, this model may be planned to be repeated with major kidney tissue damage. Contrary to our results, Garcia-Gomez et al. reported that the omentum was effective in treatment of kidney injuries. In the context of the use of omentum, progression to chronic kidney disease could be reduced in that rat model (12). But in that study, kidney injuries were larger (5/6 subtotal nephrectomy).

According to the results of the present study, granulation and inflammation in kidney specimens were positively correlated with granulation, inflammation, fibrosis, and foreign body reaction in the surrounding tissue. Healing process completion in the kidney specimen was inversely correlated with granulation and foreign body reaction in the surrounding tissue. As expected, inflammation in the surrounding tissue was positively correlated with granulation, fibrosis, and foreign body reaction in the surrounding tissue. Moreover, fibrosis in the surrounding tissue was positively correlated with inflammation and foreign body reaction. Therefore, one can consider that inflammation and granulation may lead to fibrosis and interventions to reduce inflammation and granulation after injury may aid in prevention of fibrosis and permanent tissue damage.

Granulation in the kidney specimen was strongly and positively correlated with inflammation and foreign body reaction in the kidney specimen and strongly and negatively correlated with healing process completion score in the kidney specimen. Moreover, inflammation in the kidney specimen were positively correlated with granulation and foreign body reaction in the kidney specimen, and negatively correlated with healing process completion score in the kidney specimen. Therefore, we can speculate that inflammation and granulation after injury are also related with a reduced healing capacity and measures to reduce may also aid in acceleration of healing.

Among the limitations of this study are the fact that only blood creatinine and urea levels were used for biochemical evaluation of renal injury and we did not measure the urine concentrations due to the technical inadequacy of urine collection in the rats. Moreover, we were able to evaluate histopathological analysis only qualitatively. We also lacked a kidney injury group without primary repair or omentum repair. The use of such a group might improve the quality of evaluation of the effect of primary repair and omentum repair in comparison. Lastly, the injury model used in this study did not cause an increase in urea or creatinine levels. Therefore, performing a similar study to see the effect of primary repair and omentum repair after a larger kidney injury model would provide a better of the effect of these interventions.

Conclusion

We aimed to determine the repair capacity of omentum tissue on renal injury in a rat model. According to our results, the use of the autologous omentum tissue for repair of kidney injury had attenuation effects on inflammation and granulation compared with primary repair. These results imply that use of omentum tissue to facilitate healing of kidney injury may theoretically lead to a more effective healing process and reduced fibrosis and tissue and function loss. These potentially beneficial effects of autologous omentum tissue should be investigated in further well-designed experimental and clinical studies.

Declarations

Ethics approval and consent to participate

The study was approved by the Turkish Medicines and Medical Devices Agency and the Local Ethical Committee on Animal Experiments (Acıbadem Mehmet Ali Aydınlar University, HDK-2018/47).

Consent for publication

Not applicable.

Availability of data and materials

We agree that the materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the Acıbadem Mehmet Ali Aydınlar University under Grant 2018-03-05.

Authors' contributions

All authors contributed to the design and performance of this study and in writing and critical revision of the final manuscript.

Acknowledgements

None

Authors' information

References

1. Erlich T and Kitrey ND. Renal trauma: the current best practice. Ther Adv Urol 2018, Vol. 10 (10) 295–303, DOI: 10.1177/ 1756287218785828.
2. Danuser H, Wille S, Zoscher G, Studer U. How to treat blunt kidney ruptures: primary open surgery or conservative treatment with deferred surgery when necessary? Eur Urol 2001;39(1):9-14.
3. Moudouni SM, Hadj Slimen M, Manunta A, Patard JJ, Guiraud PH, Guille F, et al. Management of majör blunt renal lacerations: is a nonoperative approach indicated? Eur Urol 2001;40: 409-14.
4. Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M, Roy S. Oxygen, oxidants, and antioxidants in wound healing. Ann NY Acad Sci. 2002; 57: 239–49
5. Khanna S, Roy S, Bagchi D, Bagchi M, Sen CK. Upregulation of oxidant-induced VEGF expression in cultured keratinocytes by a grape seed proanthocyanidin extract. Free Radical Biol Med. 2001;31: 38–42.
6. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci. 2004; 9: 283–9.
7. Santucci RA, Wessells H, Bartsch G, Descotes J, Heyns CF, McAninch JW, Nash P, Schmidlin F. Evaluation and management of renal injuries: consensus statement of the renal trauma subcommittee. BJU International: 93, 937–954, 2004 doi:10.1111/j.1464-410X.2004.04820.x
8. Litbarg NO, Gudehithlu KP, Sethupathi P, Arruda JAL, Dunea G, SinghAK: Activated omentum becomes rich in factors that promote healingand tissue regeneration. Cell Tissue Res 328: 487–497, 2007.
9. Singh AK, Patel J, Litbarg NO, Gudehithlu KP, Sethupathi P, ArrudaJAL, Dunea G: Stromal cells cultured from omentum express pluripotent markers, produce high amounts of VEGF, and engraft to injuredCell Tissue Res 332: 81–88, 2008
10. Liebermann-Meffert D: The greater omentum. Anatomy, embryology, and surgical applications. Surg Clin North Am 80: 275–293, xii, 2000
11. Vernik J, Singh AK: Omentum: Power to heal and regenerate. Int J Artif Organs 30: 95–99, 2007.
12. Garcia-Gomez I, Pancholi N, Patel J, Gudehithlu KP, Sethupathi P, et al. Activated Omentum Slows Progression of CKD J Am Soc Nephrol 25: (6):1270-81. doi: 10.1681/ASN. 2013040387.
13. Di Nicola V. Omentum a powerful biological source in regenerative surgery Regen Ther. 2019 Dec 1; 11: 182–191.
14. Lloris-Carsı JM, Barrios C, Prieto-Moure B, Miguel J, et al. The effect of biological sealants and adhesive treatments on matrix metalloproteinase expression during renal injury healing. PLoS One 2017 May 11;12(5): e0177665. doi: 10.1371/journal.pone.0177665. eCollection 2017.
15. Metro MJ, McAninch Surgical Exploration of the Injured Kidney: Current Indications and Techniques. Int Braz J Urol Mar-Apr 2003;29(2):98-105. doi: 10.1590/s1677-55382003000200002.
16. Santucci RA, McAninch JW. Diagnosis and management of renal trauma: past, present, and future. J Am Coll Surg 2000; 191: 443–5.

17. Delany HM, Ivatury RR, Blau SA et al. Use of biodegradable (PGA) fabric for repair of solid organ injury: a combined institution experience. Injury 1993; 24: 585–9.
18. ZhuangQ, Ma R, Yin Y, Lan T, Yu M, Ming Mesenchymal Stem Cells in Renal Fibrosis: The Flame of Cytotherapy. Stem Cells Int. 2019 Jan 13;2019: 8387350. doi: 10.1155/2019/8387350. eCollection 2019
19. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Engineering, vol. 7, no. 2, pp. 211–228, 2001.
20. Charbord P.Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther 21:1045–1056, 2010.
21. Togel F, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. Am J Physiol Renal Physiol 292: F1626–F1635, 2007.
22. Zhao JJ, Liu JL, Liu L, Jia HY. Protection of mesenchymal stem cells on acute kidney injury. Mol Med Rep 9:91–96, 2014.
23. Baer PC, Geiger Mesenchymal stem cell interactions with growth factors on kidney repair. Curr Opin Nephrol Hypertens, 2010 Jan;19(1):1-6. doi: 10.1097/MNH.0b013e328333062c.
24. IwaiS, Sakonju I, Okano S, Teratani T, Kasahara N, et al. Impact of Ex Vivo Administration of Mesenchymal Stem Cells on the Function of Kidney Grafts From Cardiac Death Donors in Rat Transplant Proc. 2014 Jun;46(5):1578-84. doi: 10.1016/j.transproceed.2013.12.068.
25. Reinders MEJ, Fibbe WE, Rabelink TJ. Multipotent mesenchymal stromal cell therapy in renal disease and kidney transplantation Nephrol Dial Transplant (2009) 1 of 8. doi: 10.1093/ndt/ gfp 552
26. MiasC, Lairez O, Trouche E, Roncalli J, Calise D, Mesenchymal Stem Cells Promote Matrix Metalloproteinase Secretion by Cardiac Fibroblasts and Reduce Cardiac Ventricular Fibrosis After Myocardial Infarction Stem Cells, 2009 Nov;27(11):2734-43. doi: 10.1002/stem.169
27. Khalid U, Chavez GP, Nesargikar P, et al. Kidney ischemia reperfusion injury in the rat: the EGTI scoring system as a valid and reliable tool for histological assessment. J Histol Histopathol J 2016; 3: 1-7.

Tables

Table 1: Summary of the procedures performed in the control and intervention groups

Groups	Postoperative 7th day	Postoperative 18th day
Control group (Sham)	Blood samples obtained	Blood samples obtained + sacrificed
Group 1 (primary repair 1)	Blood samples obtained + sacrificed	NA
Group 2 (primary repair 2)	Blood samples obtained	Blood samples obtained + sacrificed
Group 3 (omentum repair 1)	Blood samples obtained + sacrificed	NA
Group 4 (omentum repair 2)	Blood samples obtained	Blood samples obtained + sacrificed

NA: Not available

Table 2: Descriptive statistics of blood creatinine and urea values of the study groups.

Table 3: Score of histopathological evaluation in kidney tissue for control and study groups.

Table 4: Scoring evaluation of histopathological findings in the kidney for the control and study groups

Table 4: Scoring evaluation of histopathological findings in the surrounding tissue for the control and study groups

Study groups and time of analysis	n	Mean	Std. deviation	Minimum	Maximum
Sham group urea sampled on day 1	6	43,667	5,279	36	51
Sham group creatinine sampled on day 1	6	0,273	0,041	0,23	0,35
Sham group urea sampled on day 7	6	38	3,742	32	43
Sham group creatinine sampled on day 7	6	0,228	0,063	0,17	0,33
Sham group urea sampled on day 18	6	36,167	2,401	34	40
Sham group creatinine sampled on day 18	6	0,273	0,033	0,24	0,33
Kidney primary repair 1 urea on day 1	6	39,333	3,933	37	47
Kidney primary repair 1 creatinine on day 1	6	0,358	0,043	0,3	0,42
Kidney primary repair 1 urea on day 7	6	31,333	3,077	28	36
Kidney primary repair 1 creatinine on day 7	6	0,27	0,022	0,24	0,3
Kidney primary repair 2 urea on day 1	6	43,333	5,574	37	51
Kidney primary repair 2 creatinine on day 1	6	0,288	0,039	0,24	0,36
Kidney primary repair 2 urea on day 7	6	37,167	3,312	33	41
Kidney primary repair 2 creatinine on day 7	6	0,2	0,027	0,17	0,24
Kidney primary repair 2 urea on day 18	6	39,667	4,761	34	46
Kidney primary repair 2 creatinine on day 18	6	0,22	0,02	0,2	0,25
Kidney repair with omentum 1 urea on day 1	6	35,5	2,881	32	40
Kidney repair with omentum 1 creatinine on day 1	6	0,282	0,037	0,24	0,34
Kidney repair with omentum 1 urea on day 7	6	33,167	4,355	25	37
Kidney repair with omentum 1 creatinine on day 7	6	0,29	0,033	0,24	0,33
Kidney repair with omentum 2 urea on day 1	6	43,167	5,269	35	51
Kidney repair with omentum 2 creatinine on day 1	6	0,265	0,037	0,21	0,31
Kidney repair with omentum 2 urea on day 7	6	33	5,367	27	43
Kidney repair with omentum 2 creatinine on day 7	6	0,26	0,04	0,23	0,33
Kidney repair with omentum 2 urea on day 18	6	32,833	2,563	29	37
Kidney repair with omentum 2 creatinine on day 18	6	0,272	0,088	0,16	0,43

Study variables	n	Mean	Std. deviation	Minimum	Maximum
Urea level in blood	30	34,6333	4,47586	25,00	46,00
Creatinine level in blood	30	0,2783	,08408	0,16	,64
Macroscopy of the kidney specimen	30	4,7333	,52083	3,00	5,00
Granulation degree in kidney specimen	30	,9000	1,37339	0	4,00
Inflammation degree in kidney specimen	30	1,5000	1,40810	0	4,00
Fibrosis degree in kidney specimen	30	1,1667	,46113	0	2,00
Foreign body reaction in kidney specimen	18	,9444	,80237	0	2,00
Healing process completion in kidney	24	4,5000	,72232	3,00	5,00
Granulation degree in surrounding tissue	30	1,9667	,76489	1,00	3,00
Inflammation degree in surrounding tissue	30	2,7333	,73968	2,00	4,00
Fibrosis degree in surrounding tissue	30	1,7333	,58329	1,00	3,00
Foreign body reaction in surrounding tissue	30	3,0333	1,62912	0	5,00

Kidney (Right)		Macroscopy	Granulation	Inflammation	Connective Tissue Fbirosis	Foreign Body Rx	Healing
Control	1	5	0	0	1	0	NA
	2	5	0	0	1	0	NA
	3	5	0	1	1	0	NA
	4	5	0	0	0	0	NA
	5	5	0	1	1	0	NA
	6	5	0	0	1	0	NA
Group-1	1	4	3	3	1	2	4
	2	5	4	3	1	2	3
	3	5	3	3	1	1	4
	4	4	4	3	1	2	4
	5	5	3	4	1	2	5
	6	5	3	3	1	1	4
Group-2	1	5	1	2	1	1	5
	2	4	1	3	2	1	4
	3	3	0	2	1	1	3
	4	5	2	2	1	2	4
	5	5	1	4	2	1	3
	6	5	2	4	1	1	5
Group-3	1	5	0	0	2	NA	5
	2	5	0	1	1	NA	5
	3	4	0	0	1	NA	5
	4	5	0	1	2	NA	5
	5	4	0	0	1	NA	5
	6	5	0	1	1	NA	5
Group-4	1	5	0	1	1	NA	5
	2	4	0	0	1	NA	5
	3	5	0	0	2	NA	5
	4	5	0	0	1	NA	5
	5	5	0	1	1	NA	5
	6	5	0	2	2	NA	5

Surrounding Tissue		Granulation	Inflammation	Connective Tissue Fibrosis	Foreign Body Rx
Control	**1**	2	2	1	0
	2	2	2	1	0
	3	2	3	1	0
	4	1	2	1	0
	5	2	2	1	0
	6	3	4	1	0
Group-1	**1**	2	3	2	4
	2	3	3	2	4
	3	3	4	2	4
	4	2	3	2	4
	5	3	3	2	4
	6	2	4	2	4
Group-2	**1**	2	3	2	4
	2	3	3	2	4
	3	3	2	1	4
	4	2	3	2	5
	5	3	3	2	4
	6	2	4	2	5
Group-3	**1**	1	3	2	3
	2	2	4	3	4
	3	2	2	2	4
	4	3	3	3	4
	5	1	3	1	3
	6	1	2	1	3
Group-4	**1**	1	2	2	3
	2	1	2	1	3
	3	2	2	2	3
	4	1	2	2	4
	5	1	2	2	4
	6	1	2	2	3

Figures

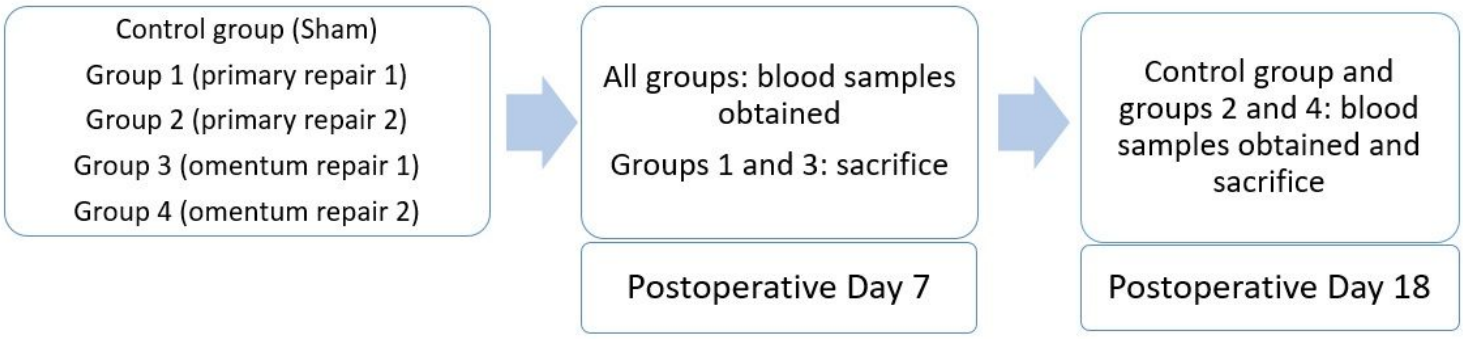

Figure 1

A schematic view of the study groups and timing of study interventions and investigations

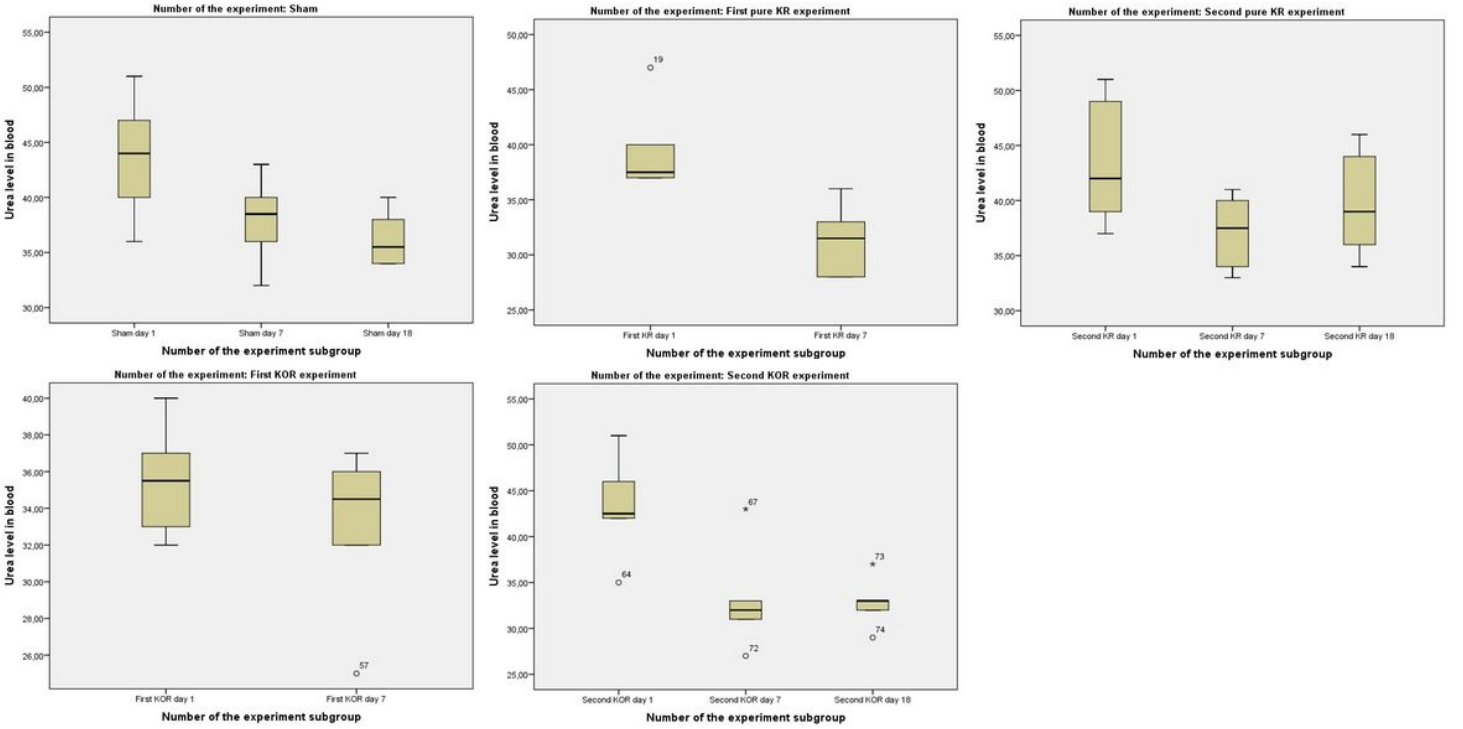

Figure 2

Boxplot graphics for urea levels of the study groups.

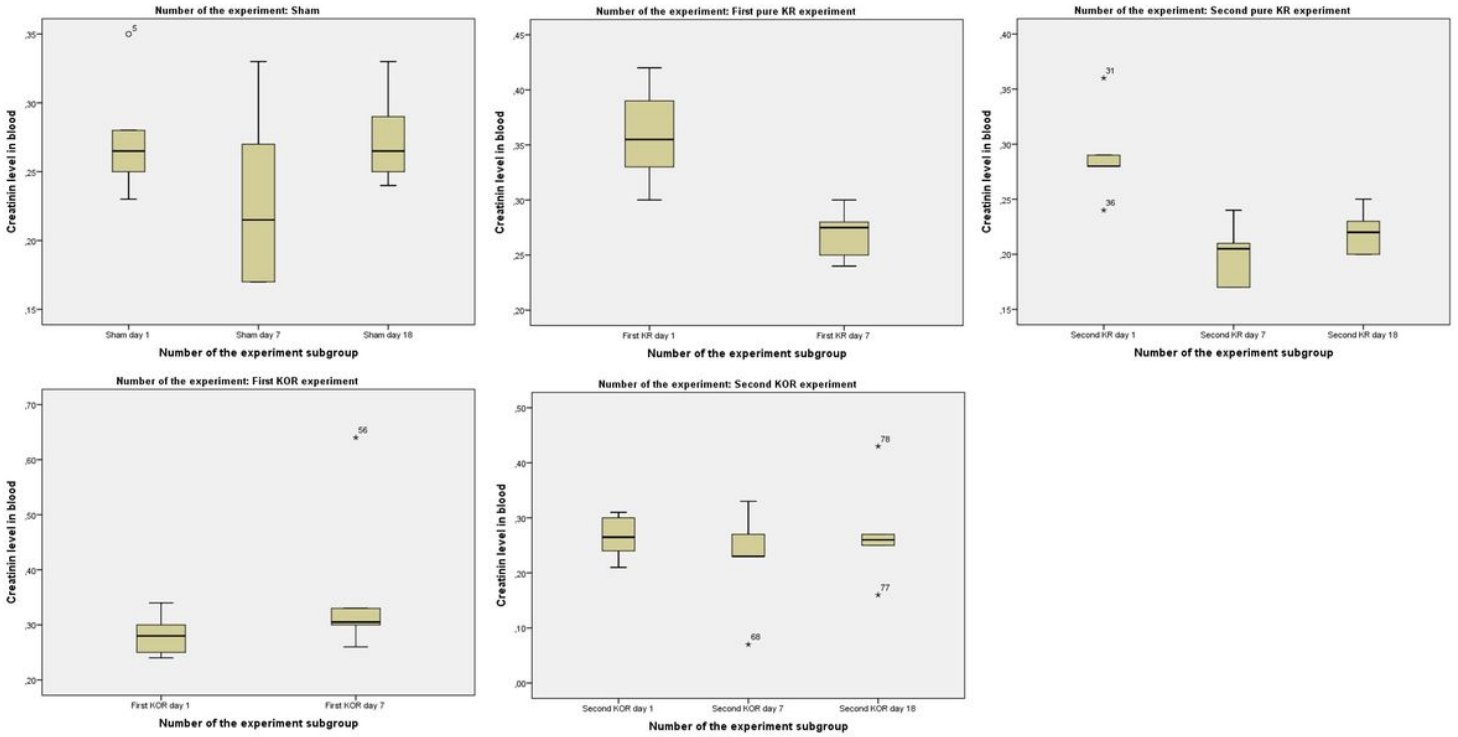

Figure 3

Boxplot graphics for creatinine levels of the study groups.

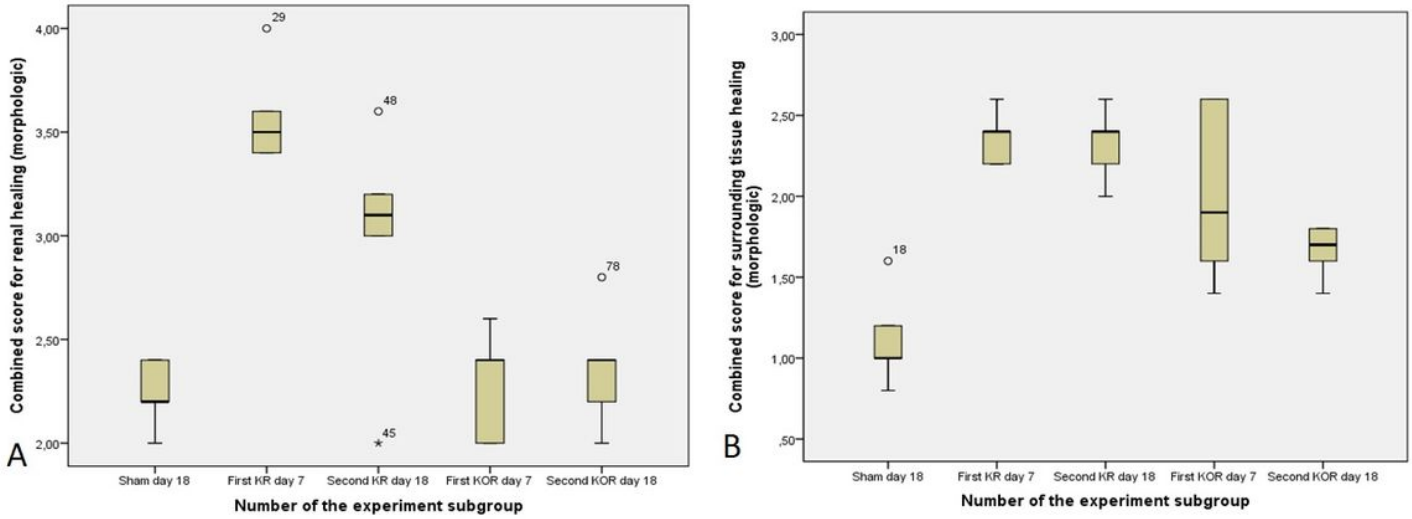

Figure 4

Combined morphologic score for renal healing (panel a) and surrounding tissue (panel b) of the study groups. KR: primary repair groups, KOR: omentum repair groups.

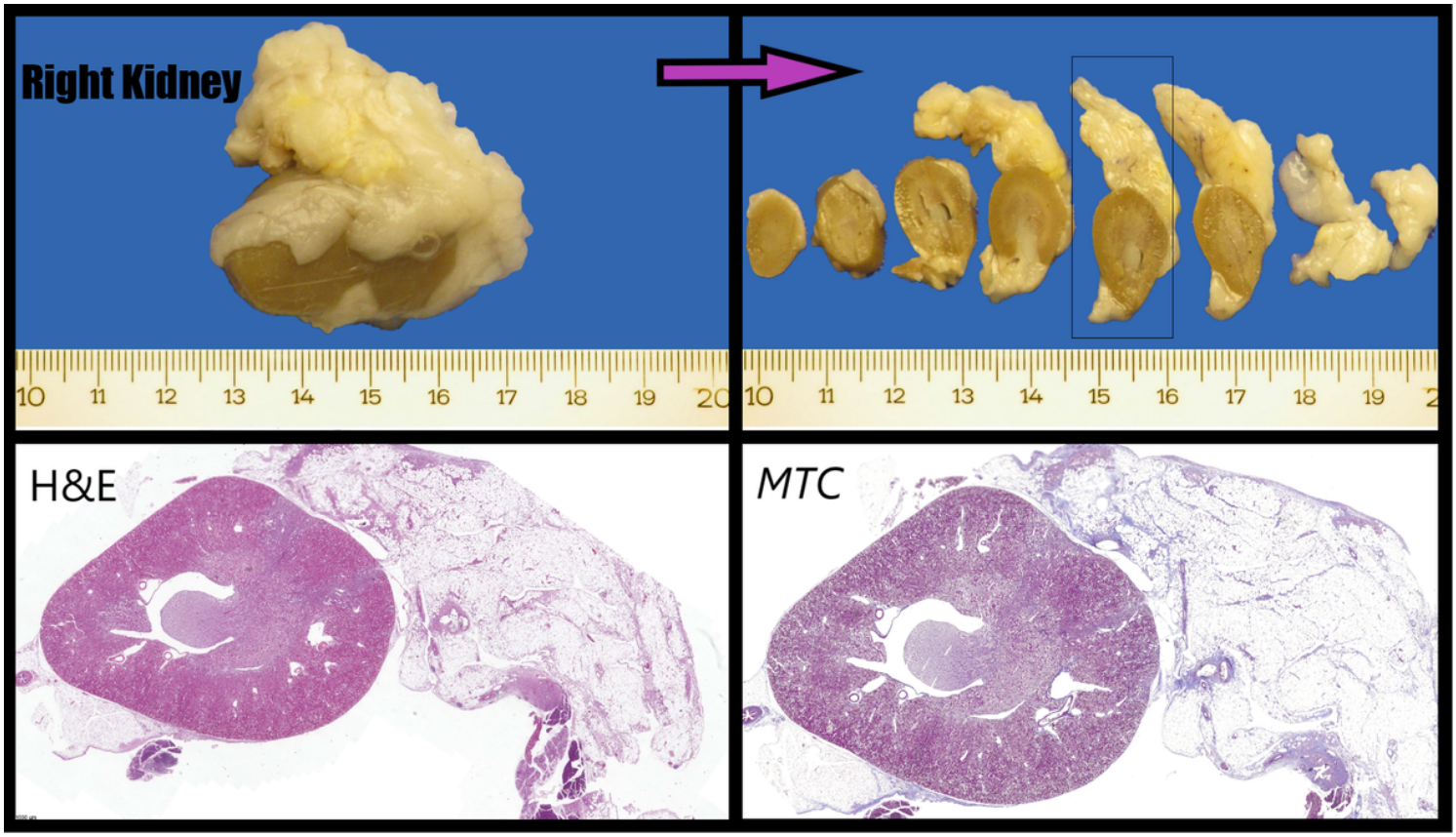

Figure 5

Macroscopic view of kidney and omentum. Left Bottom, Cross section of the whole kidney slice (4µ), Hematoxylin & Eosin staining (57.6X). Right Bottom, Cross section of the whole kidney slice (4µ), Masson Trichrom staining. (30X)

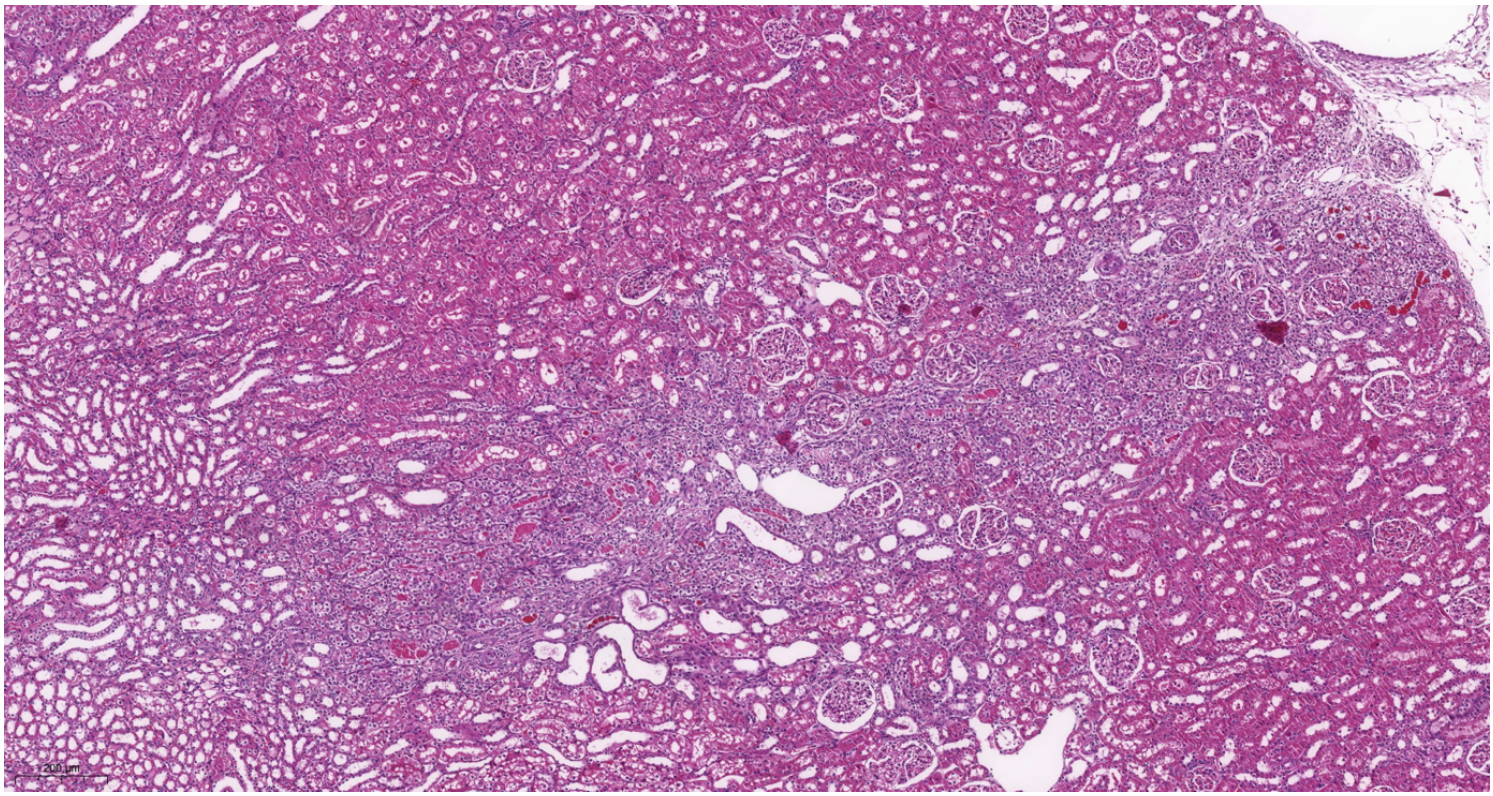

Figure 6

Kidney, parenchyma, trauma line– microscopically, Hematoxylin & Eosin staining (380X)

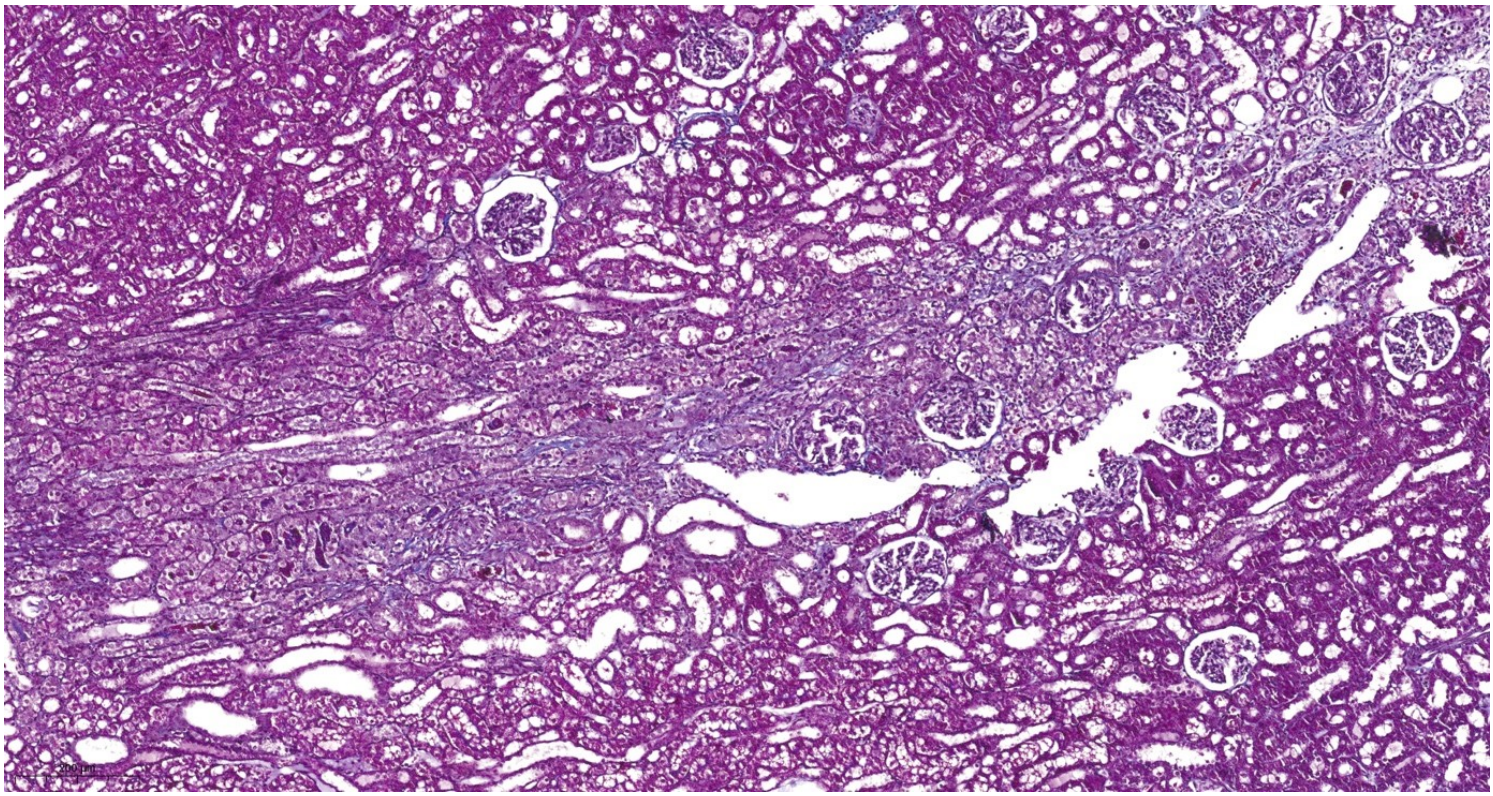

Figure 7

Kidney, parenchyma, trauma line– microscopically, Masson Trichrom staining. (256X)

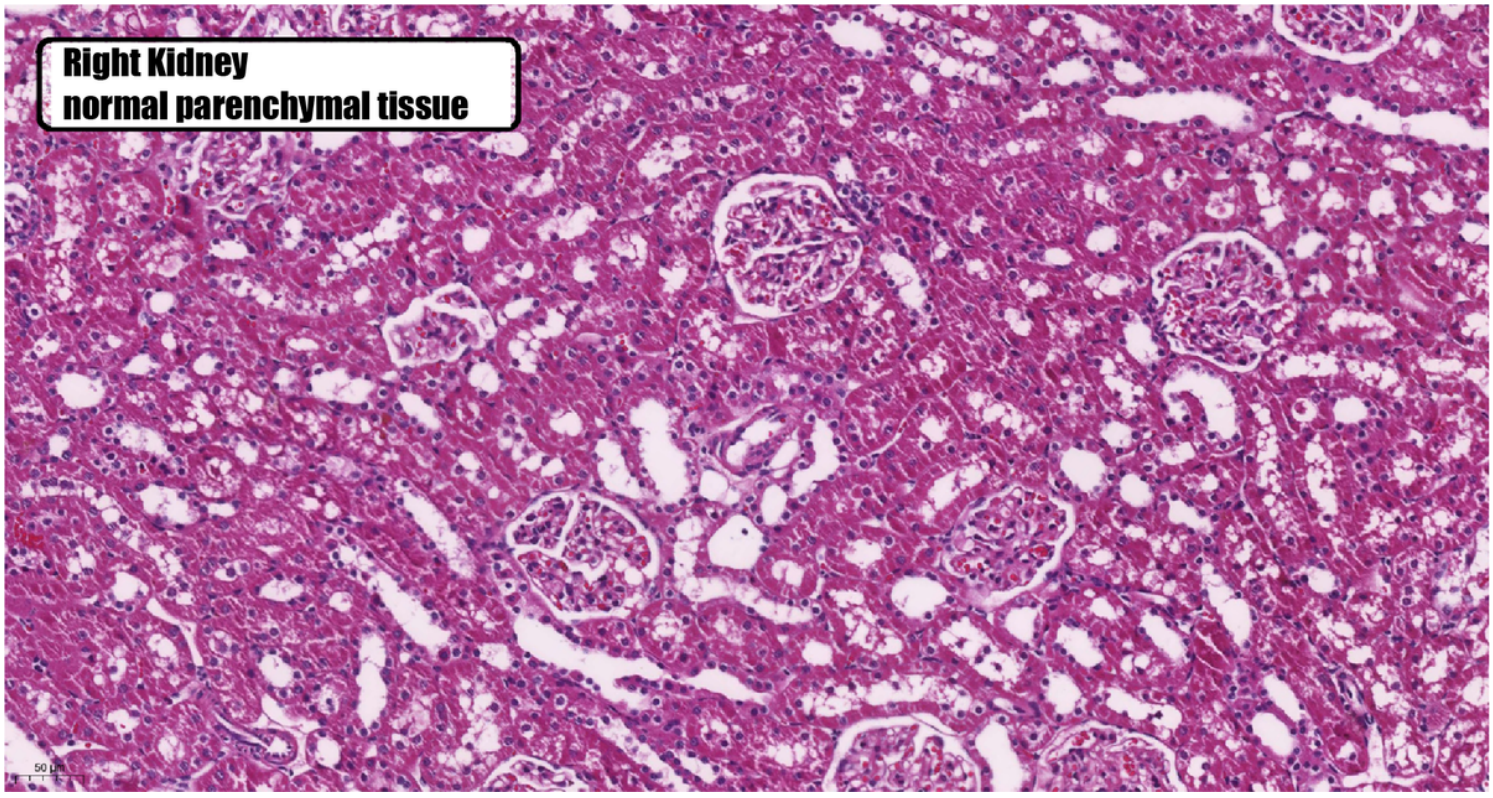

Figure 8

Kidney, parenchyma–microscopically, Hematoxylin & Eosin staining. (1120X)

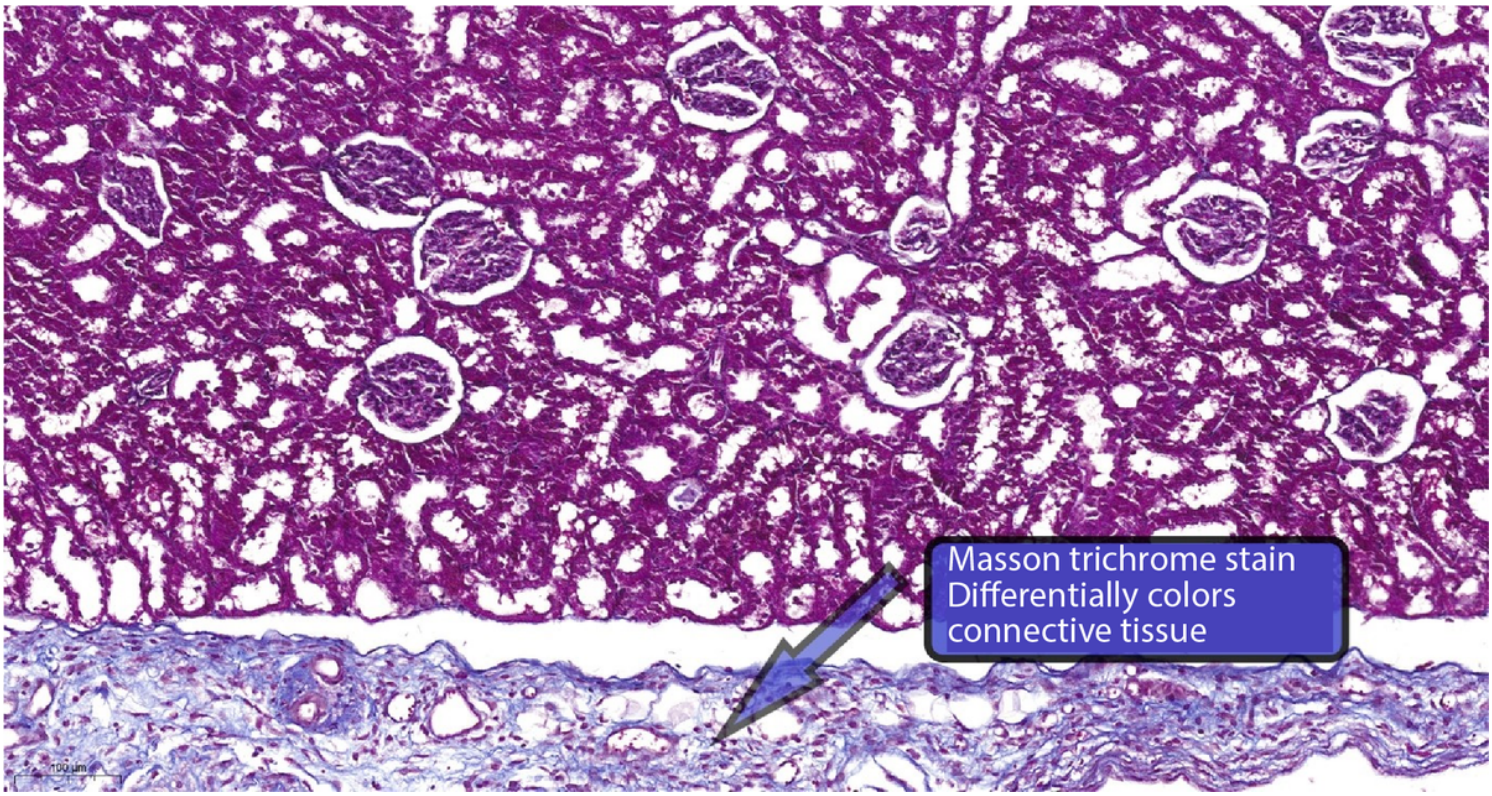

Figure 9

Kidney, parenchyma, surrounding area – microscopically, Masson Trichrom staining. (440X)

Figure 10

Mean granulation, inflammation, and completion of healing scores in kidney tissues in the study groups in comparison.