Original Article

Improved Healing of Colonic Anastomosis with Allotransplantation of Axillary Skin Fibroblasts in Rats

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Abstract
Objective: Colonic anastomosis is associated with serious complications leading to significant morbidity and mortality. Fibroblasts have recently been introduced as a practical alternative to stem cells because of their differentiation capacity, anti-inflammatory, and regenerative properties. The aim of this study was to evaluate the effects of intramural injection of fibroblasts on the healing of colonic anastomosis in rats.

Materials and Methods: Inbred mature male Wistar rats were used in this experimental study (n=36). Fibroblasts were isolated from the axillary skin of a donor rat. In the sham group, manipulation on descending colon was done during laparotomy. A 5 mm segment of the colon was resected, and end-to-end anastomosis was performed. In the control group, 0.5 ml of phosphate buffer saline (PBS) was injected into the colonic wall and in the treatment group, 1×10^6 fibroblasts were transplanted. Following euthanasia on day 7, intra-abdominal adhesion, leakage and peritonitis were evaluated by necropsy. Mechanical properties were assessed using bursting pressure and tensile tests. Inflammation, angiogenesis, and collagen deposition were examined histopathologically.

Results: The mean scores for adhesion and leakage were decreased in the treatment group versus control samples. Lower infiltration of inflammatory cells was observed in the treatment group (P=0.03). Angiogenesis and collagen deposition scores were significantly increased in the fibroblast transplanted group (P=0.03). Tensile mechanical properties of the colon were significantly increased in the treatment group compared to the control sample (P=0.01). There was no significant difference between the control and treatment groups in terms of bursting pressure (P=0.10). Positive weight changes were found in sham and treatment groups, but the control rats lost weight after 7 days.

Conclusion: The results suggested that allotransplantation of dermal fibroblasts could improve the necroscopic, histopathological, and biomechanical indices of colonic anastomosis repair in rats.

Keywords: Allogeneic Transplantation, Colorectal Surgery, Fibroblasts, Rats, Wound Healing

Introduction

Anastomosis following colonic resection is performed as a standard treatment for colon neoplastic lesions and chronic ulcerative colitis. Anastomosis is often associated with severe complications such as leakage, dehiscence, and infection (1). Reportedly, the incidence of anastomotic leak ranges from 0.5 to 30% (2). Sepsis and mortality are considered the most severe consequences of anastomotic leakage (3). It is believed that the mechanical stress for defecation and high luminal microbial load are the causes of these complications (4). Different anastomotic devices like staplers, various surgical techniques, and intensive preoperative care have been adopted to decrease the complications, however, fast and safe healing of the anastomosis is still a major concern for colorectal surgeons.

As an alternative strategy, cell-based therapy benefits transplanting multipotent cells to improve the healing process and decrease the rate of complication. Naturally, stromal cells migrate from bone marrow to the site of injury and based on the environmental signals, they differentiate into fibroblasts to deposit collagen and extracellular matrix proteins (5). The multilineage differentiation potentials and minimal immunogenicity of stromal cells are reported to result in the extensive application of these cells in cell-based regenerative medicine.

Although promising results were obtained following transplantation of mesenchymal stromal cells (MSCs) and significant improvement was observed in histological and mechanical properties of the anastomoses in several studies (6, 7), the invasive and painful procedure of bone marrow harvest, time-consuming and expensive multistep procedures for isolation, characterization and expansion can limit MSCs application.

Isolation of adipose-derived stromal cells (ASCs) is performed less invasively, and higher cell yield can be obtained compared to bone marrow. However, their short life span necessitates multiple passages to reach a therapeutic dose. Reportedly, ASCs rapidly undergo

replicative senescence after multiple *in vitro* passages (8). The cellular senescence reduces differentiation capacity and predisposes genomic instability and malignant transformation, thus, the application of ASCs in cell therapy could be challenging. Due to these limitations, the clinical application of MSCs is still being investigated to find the best cell type and method for isolation and expansion.

Fibroblasts are known as the resident mesenchymal stromal/stem cells in connective tissues (9), a key player in wound healing by producing extracellular matrix and collagen fibers (10). Also, fibroblasts enhance angiogenesis in the healing tissue through different growth factors (11). Fibroblasts transform into myofibroblasts that are responsible for wound contraction (12). It seems that MSCs could be practically replaced with fibroblasts due to their anti-inflammatory, regenerative and immune-modulatory properties. In addition, fibroblasts could be easily harvested in large quantities using a cutaneous punch biopsy. The expansion and culture of fibroblasts are markedly easier and require a shorter doubling time compared to MSCs (13). However, there are a few drawbacks in fibroblast cultures such as slow growth especially in their older populations, and susceptibility to mycoplasma contamination (14). Previous studies have shown that fibroblasts transplantation improved skin wound healing in a variety of animal models (15, 16).

To the best of the authors' knowledge, the effects of fibroblasts allotransplantation on colon anastomosis have not been studied, yet. In this study, allogeneic dermal fibroblasts were transplanted into the wall of the colon after surgical anastomosis, and necroscopic, histopathological, and mechanical aspects of repairs were studied.

Materials and Methods

All experimental protocols were performed based on the Iranian guidelines of animal welfare and approved by the Ethics Committee in Urmia University, Faculty of Veterinary Medicine, Urmia, Iran (IR-UU-AEC-3/1024/AD).

Study design

In this experimental study, 36 inbred adult male Wistar rats weighing 220.00 ± 30.00 g were obtained from the Laboratory Animal Center of the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The rats were housed in plastic cages in a group of two and fed standard commercial pellets and had free access to a bottle of water. The rats were randomly divided into three groups of sham, control, and treatment (n=12). The mean body weight of rats in each group was recorded as baseline values to compare to the post-operative weights at the end of the study. All chemicals for this analysis were purchased from Sigma-Aldrich (Darmstadt, Germany) unless otherwise stated.

Fibroblast isolation

To isolate dermal fibroblast, 1 cm^2 of skin was harvested from a randomly selected donor out of the sham rats. In this regard, the rat was anesthetized using intraperitoneal (IP) injection of 90 mg/kg ketamine hydrochloride (Alfasan, Woerden, Netherlands) and 5 mg/kg xylazine hydrochloride (Alfasan, Woerden, Netherlands). The right axillary region was prepared aseptically, and the skin sample was excised using a 10 scalpel blade and placed in phosphate buffer saline (pH=7.2, Gibco, Grand Island, USA). Then, the donor site was sutured using a 3-0 nylon (SPUA, Iran) with a simple interrupted pattern. The skin sample was cut into small pieces, then placed in 10 ml of DMEM/F12 medium supplemented with antibiotic/antimycotic agents (Gibco, USA), and digested enzymatically with medium containing 10% collagenase type II (Sigma-Aldrich, USA) for 90 minutes at 37˚C. Then, 10 ml of culture medium containing 10% fetal bovine serum (FBS, Gibco, USA) was added to stop digestion. Using a 70 μm cell strainer (BD Falcon™, BD Biosciences, USA), the tissue suspension was filtered. The resulting suspension was centrifuged at 700 g for 5 minutes. After 5 minutes, the supernatant was gently removed, and the pellet was re-suspended by pipetting in a complete culture medium. The pellet was then cultured in a cell culture flask (T25, SPL life Sciences, Seoul, Korea) containing DMEM, 15% FBS, penicillin (100 IU/ml, Sigma-Aldrich, USA), and streptomycin (100 μg/ml, Sigma-Aldrich, USA) and then it was placed in 37˚C and 5% CO_2 incubator. The fibroblast started to exit tissue fragments within 2-5 days and on day 14 the first subculture was performed. Non-adherent cells were discarded before medium replacement and subculture processes; therefore, morphological methods were used to confirm the fibroblast characteristics of the isolated cells. The isolated cells were large, adherent with lamellipodia that are well-known characteristics of skin fibroblasts (17). To characterize the fibroblasts, the cell migration rate, the pattern of migration, and *in vitro* hydroxyproline concentration were evaluated.

Cell migration

The pattern and rate of migration were evaluated through a scratch wound healing assay as previously described by Jonkman et al. (18). In this regard, 1×10^6 cells/well were seeded in a 6-well plate with 2 ml of DMEM until 90% confluence was reached. Then, a linear scratch wound was created in the monolayer with a sterile 200 μl plastic micropipette tip. Any cellular debris was removed by washing the plate with phosphate buffer saline (PBS, Sigma-Aldrich, USA). Then, 2 ml of fresh medium were added to the cultures which were then incubated at 37˚C inside an incubator with a 5% CO_2 humidified atmosphere for 24 hours. Cell migration was determined after 24 hours using an inverted microscope equipped with a digital camera. The wound width was measured at predetermined time points and average widths were recorded. The migration rate was calculated using the

below equation (19):

Migration rate (μm/hours)=W(t1)W(t2)/Δt
where, W(t1) is the initial wound width, W(t2) is the final wound width, and Δt is the duration of migration.

In vitro hydroxyproline assay

To determine hydroxyproline concentration, the cells were homogenized using KCl (150 mM, pH=7.40). The homogenate (0.5 ml) was digested in 1 ml of 6 N HCl at 120˚C for 8 hours. Then, to oxidize the free hydroxyproline, citrate/acetate buffer (50 μl, pH=6) and 1 ml of chloramine-T solution (282 mg of chloramine-T, 2.00 ml of n-propanol, 2 ml of H_2O, and 16 ml of citrate/acetate buffer) were added to 50 μl of samples and kept at room temperature for 20 minutes. 1 ml of Ehrlich's solution was added to each sample, and then the samples were placed in a water bath at 65˚C for 15 minutes. After cooling to room temperature, the sample absorbance was measured with a microplate reader (Stat Fax® 2100; Awareness Technology Inc., USA) at 550 nm. A concentration range of 0.00 to 10.00 μg/ml hydroxyproline standard was used to establish a standard curve (20).

Cell viability

Before transplantations, the cell viability was assessed by the trypan blue dye exclusion test. Briefly, the fibroblasts were trypsinized and centrifuged (200 g for 8 minutes). The pellet was suspended in DMEM and 20 μL of the cell suspension was mixed with 0.4% trypan blue solution in the ratio of 1:10. Live (colorless) and dead (stained blue) cells were counted in a Neubauer chamber to determine cell viability.

Surgery

Under general anesthesia, the caudal abdomen was prepared for aseptic surgery and opened through a ventral midline incision. Descending colon was carefully exteriorized and manipulated for 10 minutes in the sham group and then was returned to its anatomic position. The abdominal incision was then sutured using 3-0 nylon suture (SPUA ,Iran) in a simple continuous pattern. In control and treatment groups, to avoid intraabdominal contamination, saline-soaked gauze was used to isolate the colon. Then, a 5 mm segment of the descending colon was excised. Subsequently, end-to-end anastomosis was performed using 10 simple interrupted 6-0 nylon sutures (SPUA, Iran, Fig.1A). The anastomosis was tested for leakage by injecting 1 ml of sterile saline intraluminally while the colon was occluded proximal and distal to the anastomosis (Fig.1B). Additional sutures were placed if necessary. The rats in the control group were injected 0.5 ml PBS intramurally (into the colonic wall) at both sides of anastomosis. In the treatment group, 1×10^6 homologous dermal fibroblasts suspended in 0.5 ml PBS, as the carrier, were injected in the same fashion (Fig.1C). Then, the abdomen was closed as mentioned above.
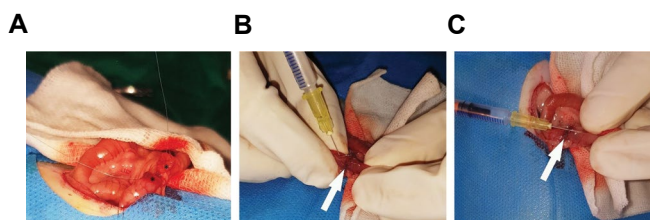

Fig.1: The surgical procedure of colonic anastomosis in rats. **A.** Asterisks show the transected ends of the descending colon and the first suture was passed through both ends. **B.** The white arrow shows the complete anastomosis which was followed by a leak test with the injection of normal saline into the lumen of the colon. **C.** Fibroblasts were injected intramurally (into the colonic wall) at both sides of the anastomotic site (white arrow) in the treatment group.

Sampling

On day 7, the rats were euthanized by anesthetic overdose (IP injection of 300 mg/kg ketamine hydrochloride and 30 mg/kg xylazine hydrochloride). Before necropsy, the rats' bodyweight was measured to evaluate the catabolic and the anabolic states postoperatively and was compared to the baseline values. The abdomen was re-opened and after gross examinations, the colon (n=6, including the anastomotic site) was harvested and then divided into two longitudinal halves using a scalpel blade. One half was tested for mechanical tensile test and the other half was evaluated by histopathology. Six other samples were harvested en bloc for bursting pressure.

Macroscopic evaluations

Any peri-anastomotic adhesion formation, abscess, and peritonitis were scored as described previously (21). Adhesion severity was classified as none: no adhesion (score=0), mild: adhesions formation between the anastomotic site and the greater omentum (score=1), moderate: adhesions between anastomotic site, greater omentum and small intestines (score=2), and severe: extensive adhesions (score=3). The scoring system of Wu et al. was used to evaluate the severity of leakage from the anastomosis, in which score 0 indicates no leakage, score 1 indicates mild leakage associated with abscess around the anastomotic site, score 2 represents moderate leakage and presence of intra-abdominal feces leading to peritonitis with or without abscess formation, and 3=death due to severe leakage (22).

Mechanical evaluation

The second half-strips from each group (n=6) were subjected to mechanical testing. Each sample was mounted on an STM-5 tensile machine (Santam Engineering Design Co., Tehran, Iran) supplied with a 20 kg load cell (Bongshin Loadcell Co. Ltd., Seoul, South Korea). The constant velocity of 20 mm/minutes was used for the tensile test until breakage. A load-displacement curve and the following mechanical properties were obtained: maximum load (N), load in yield point (N), and energy absorption (J). Figure 2 shows the diagrams of load-displacement curves of the experimental groups.
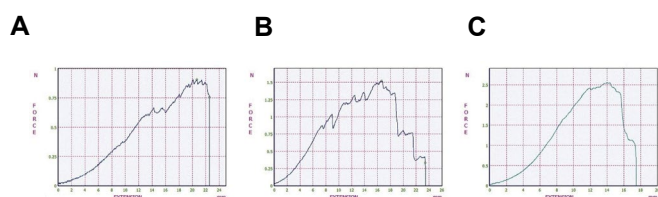
Fig.2: The load-displacement curve of colon samples during the tensile test under the constant velocity of 20 mm/minutes. **A.** Control group, **B.** Treatment group, and **C.** Sham group.

Bursting pressure

The bursting pressure test was done *ex vivo*. The anastomoses (n=6 from each group) were resected en bloc as well as a 15 mm segment of the intact colon on each side. After washing out the feces, the proximal end was ligated by a 2-0 Dexon suture (SPUA, Iran), and the distal end of the colon was secured to an intravenous catheter and attached to the bursting pressure measurement apparatus thorough a T-shaped three-way. The colon was placed in a water-filled container and a constant oxygen flow (1 L per minutes) was used to inflate it. A manometer was used to measure the bursting pressures. It was recorded when bubbles were observed at the anastomotic site, or a sudden pressure decrease was noted on the manometer.

Histopathological assessment

The longitudinal strips of the colon (n=6) from the control and treatment groups were formalin-fixed and paraffin-embedded. Five μm sections were stained with hematoxylin and eosin (H&E). Infiltration of inflammatory cells and neovascularization were scored as described in a previous study (23). Collagen content was scored according to modified Ehrlich & Hunt in Masson's trichrome (MTC) stained sections. For MTC stain, Masson Kit (HT15, Sigma-Aldrich, USA) was used. In brief, the sections were deparaffinized, rehydrated, and immersed in Bouin's solution at 56˚C for 15 minutes. The slides were washed using tap water for 5 minutes then stained in Weigert's hematoxylin for 5 minutes. After washing with water, the slides were stained in the Biebrich scarlet-acid fuchsin. Then, the slides were incubated for 5 minutes in the phosphotungstic-phosphomolybdic acid. The slides were stained using aniline blue for 5 minutes and finally were fixed in acetic acid for 2 minutes. Then the slides were rinsed in distilled water, dehydrated with methanol, and mounted.

Accordingly, score 0=no evidence, score 1=occasional collagen fibers, score 2=light scattering, score 3=abundant collagen fibers, and score 4=dense collagen bundles under 100× magnification (24). All sections were coded and examined blindly blindly by two observers and the results were presented as the mean score.

Statistical analysis

The semi-quantitative scores were analyzed using Kruskal Wallis followed by Mann-Whitney test and the results were shown as the mean and interquartile range (25 and 75% quartile). The quantitative results were analyzed using one-way ANOVA and Tukey post hoc test for multiple comparisons. The experimental data were presented as mean ± standard deviation (SD). All statistical analyses were done in Minitab (version 16.0, Minitab Inc., Boston, USA), and $P<0.05$ were considered as statistical significance.

Results

Culture properties

Morphologically, the cultured cells had a spindle-shaped cell body with flat elongated oval nuclei and long lamellipodia. In the scratch assay, the cells were loosely connected during migration which was the characteristic of fibroblasts (Fig.3). The migration rate of fibroblast in the culture plate was 26.5 μm/hours. *In vitro* hydroxyproline content after 48 hours culture was 1.20 ± 0.12 mg/ml of cell homogenate. According to the trypan blue exclusion assay, cell viability was above 95% before transplantations.
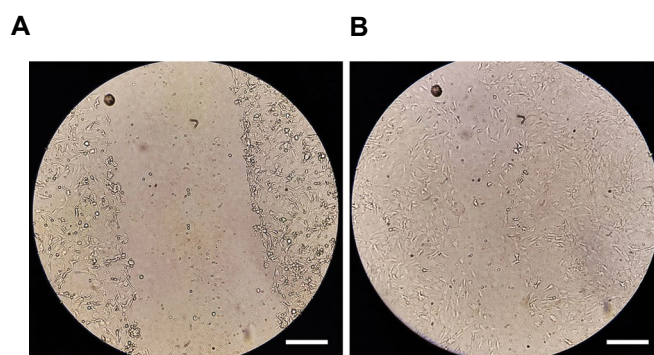

Fig.3: The *in vitro* wound-healing assay. **A.** Scratch assay on monolayer fibroblast culture (0 hour). **B.** Fibroblasts migrated after 24 hours to close the distance between two edges of the wound (scale bars=300 μm).

Bodyweight

After 7 days, an increase in the body weight was observed in the sham (19.66 ± 3.24 g) and treatment (6.50 ± 3.24 g) groups versus their preoperative values. However, a decrease in body weights of the control rats was observed (4.83 ± 1.38 g) when compared to the baseline values. Significant differences were found among the three groups in terms of post-operative body weight change (P<0.001).

Macroscopic necropsy findings

No adhesion was observed in the sham group (score 0). Adhesion formation was detected in the control group (mean score=2, range 1-3) in which the adhesions

were formed mostly to small intestines and omentum. In the fibroblast transplanted group, mild adhesions to the omentum were observed (mean score=0.5, range 0-1, Fig.4). The semi-qualitative statistical comparison showed that adhesions were significantly lower in the fibroblast transplanted group in comparison with the control samples (P=0.03). In control rats, mild to moderate anastomotic leakage into the abdomen were observed (mean score=1, range 0-2). No leakage was found in sham and treatment groups (score 0). Statistical analysis showed a significant increase in the extent of leakage between the control group (P=0.00) compared to sham and treatment groups. Peritonitis was not observed in the samples (score 0). Statistically, no significant changes were observed among the three groups for peritonitis (P=0.10).
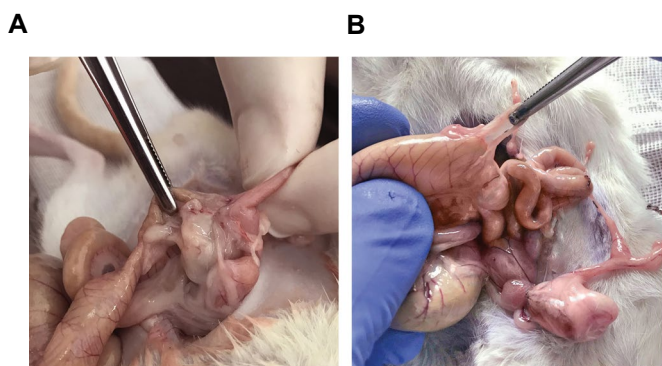
A   **B**

Fig.4: After euthanasia, the rats underwent necropsy to evaluate the adhesion score. **A.** Moderate adhesion was observed in the control group. The colon anastomotic site was covered by small intestines and greater omentum and precise sharp dissection were required for sampling. **B.** In the treatment group, mild omental adhesions were found around the colon anastomosis. Using blunt dissection and mild traction the omentum was easily detached from the colon.

Mechanical properties

Statistical analysis of tensile test showed a significant increase in the mechanical properties of repairs including maximum load, yield load and energy absorption in the fibroblast received group when compared to the control group (P=0.01). Figure 5 represents the results of mechanical properties in the experimental groups.

Bursting pressure

In the sham group, the bursting pressure (228.5 ± 24.90 mm Hg) was significantly higher in comparison with the other experimental groups (P=0.01). According to the statistical analysis, no significant difference was observed between the control (142.67 ± 34.51 mm Hg) and treatment (150.00 ± 15.65 mm Hg) groups (P=0.15).

Histopathology findings

A significant reduction of infiltrated inflammatory

cells was observed in the treatment group compared to the control group (P=0.03, Fig.6). The mean score for infiltration of inflammatory cells was 3 (range=1-4) in the control group versus 1.5 (range=1-2) in the treatment group. In terms of angiogenesis, a significantly lower score was obtained in the control group (mean=2.5, range: 1-3) as compared to the treatment group (mean=3.5, range: 3-4, P=0.03). According to the MTC staining, fibroblast transplantation resulted in a significant increase in collagen deposition (P=0.001) with a parallel orientation of collagen bundles within the granulation tissue. In contrast, the haphazard orientation of collagen bundles was observed in control samples (Fig.6). The mean score for collagen deposition were 1.5 (range=1-3) in control group versus 3.5 (range=3-4) for treatment group. The difference between these two groups was statistically significant (P=0.02).
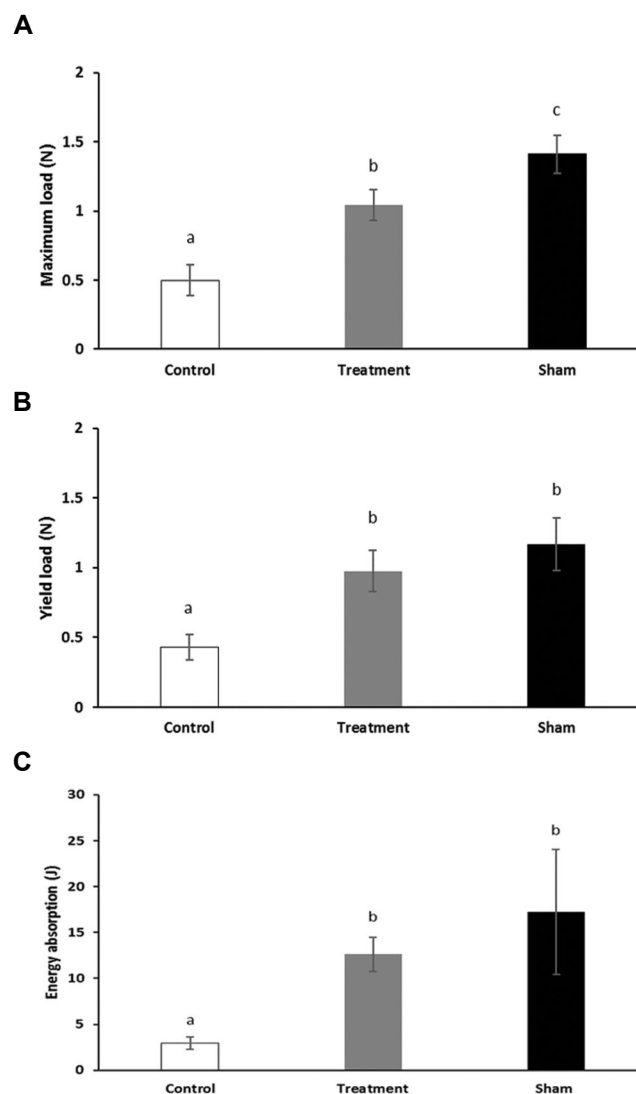
A

B

C

Fig.5: Mechanical properties of colon anastomosis of different groups derived from the load-displacement curves. **A.** Maximum load, **B.** Yield load, and **C.** Energy absorption. Data are presented as mean ± standard deviation. abc; Different letters indicate significant differences among the groups at P<0.05.
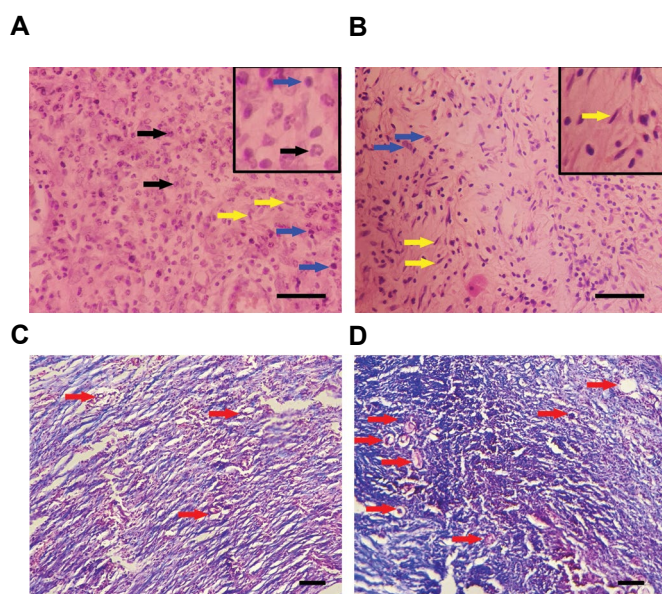
Fig.6: Photomicrograph of granulation tissue in the colon anastomotic site. A significant increase in the infiltration of inflammatory cells was observed in **A.** The control group compared to **B.** The fibroblast transplanted group (H&E, 400×, Insets 1000×). Neutrophils and lymphocytes are shown with black and blue arrows, respectively. Yellow arrows show mature fibroblasts within the granulation tissue. Masson's trichrome staining of colon anastomotic site in **C.** The control group and **D.** Treatment groups. Collagen bundles were sparse and randomly oriented within the granulation tissue of the control group. However, in the fibroblast transplanted group, a dense network of collagen bundles was observed. Blue areas indicate collagen deposition (MTC, 40×). Red arrows show newly formed vessels within the granulation tissue. The number of new vessels was significantly higher in the treatment group (MTC, 40×, scale bars=100 μm).

Discussion

Anastomotic dehiscence and leakage are known as the most serious complications of colorectal colon anastomosis (25) which often occur during the first-week post-operation (4). Following anastomosis, the tensile strength of the anastomotic site is reduced due to inflammatory responses. To prevent dehiscence collagen synthesis is crucial to provide compensatory strength (6). Collagen is synthesized by fibroblasts, which are the main cell type within the stroma. By providing structural scaffolding and modulating the secretion of growth factors, the fibroblasts have a critical role in wound remodeling and homeostasis (26). Here, we examined the effects of intramural transplantation of allogeneic fibroblasts on the healing of colonic anastomosis in a rat model.

In the present study, the isolated and cultured cells were verified as fibroblasts based on their spindle-shaped morphology as the defining characteristics of fibroblasts (27), plastic-adherence properties (28), cell migration pattern, (29), and *in vitro* hydroxyproline synthesis (30). *In vitro* scratch assay is a well-developed method to examine cell migration because it is easy to perform on adherent cell lines including fibroblasts, epithelial and endothelial cell lines (31). According to Suarez-Arnedo et al. (19), the most important advantages of the scratch assay are the low requirements of specialized equipment and costly reagents. Reportedly, the above-mentioned cell lines can be determined based on their pattern of migration in which

a loosely connected population indicates the fibroblasts. Whereas the epithelial and endothelial cells are embedded in sheets of cells during migration (29).

The present study revealed that fibroblast transplantation resulted in a lower adhesion and leakage score. Reduced inflammation, improved angiogenesis, and organized collagen deposition were detected in the fibroblast treated group. The transplantation also improved the mechanical properties of the repairs after 7 days.

Previously, promising results were obtained following systemic injection of stromal cells in experimental colonic anastomosis (7). The systemic transplantation of cells can significantly reduce the rate of cellular engraftment (32), in contrast, local transplantation of stem cells would improve the effectiveness of cell therapy. In the literature, there are few studies addressing the effects of intramural injection of cells on the healing of intestinal anastomosis. According to Shen et al. (33), submucosal injection of bone marrow stromal cells could prevent degenerative changes of the small intestine in rats (33). Adas et al. (7) reported that injection of bone marrow stromal cells could accelerate the healing of ischemic model colonic anastomosis in rats through improving the histopathological parameters and elevating the bursting pressure. Yoo et al. (34) transplanted ASCs in a rat colonic anastomosis model and reported higher bursting pressure, increased collagen deposition, improved angiogenesis, and minimal bodyweight loss in comparison with the control group.

Although MSCs are used as the ideal source for cell-therapy, there are major shortcomings in practice. To reach a therapeutic number of cells, multiple *in vitro* expansions are required which increase the possibility of mutagenesis and dysfunction of the cells. The *in vitro* expansions also would increase the duration and cost of treatment. Fibroblasts could be easily isolated in large quantities compared to the mesenchymal stromal cells. A shorter doubling time is required for fibroblast expansion which could effectively reduce the cost of treatment (13).

Fibroblasts are believed to be an important source of anti-inflammatory mediators (35). Inflammation is a critical phase during the healing process; however, prolonged inflammation may lead to massive collagen degradation in the repair site. A Short inflammatory phase provides the early commencement of the proliferation phase and early collagen synthesis. Collagen deposition is the key player in the prevention of anastomotic leakage (36). Mechanical stress from the strong colonic wall motility increases the potential for dehiscence and leakage after anastomosis.

The anastomotic site should also possess sufficient strength to resist the mechanical stress during the fecal passage. In this regard, the bursting pressure and tensile strength tests are used to evaluate the anastomotic strength. The bursting pressure reflects the resistance of the anastomotic site against the increased intraluminal pressure, and the tensile strength represents the anastomotic resistance to longitudinal forces resulted

from contractions of the intestinal muscular layer (37).

It has been reported that the evaluation of bursting pressure is reliable only during the early phase of anastomotic repair (i.e., the first three days) (38). In this study, no statistical difference was found between the control and treatment groups. Thus, we assumed that measurement on day 7, was too late to observe any changes.

According to Iwanaga et al., the tensile strength test is the standard method of assessing the biological aspects of anastomotic repair (39). In this study, the maximum load and yield load were significantly higher in response to fibroblast transplantation suggesting a higher tolerance against colonic motility and therefore greater resistance to the dehiscence and leakage. Low energy-absorption may lead to an increased risk of tissue overload (e.g. dehiscence) under mechanical stress. The tensile test in this study revealed higher energy-absorbing capacity in fibroblast transplanted samples when compared to the controls. Sufficient energy-absorbing capacity is required to store and release the mechanical forces without damage to the anastomotic site (40).

In the present study, following anastomosis the mean bodyweights were decreased in both experimental groups compared to the sham rats, however, the change was lower in the fibroblast treated group. It has been reported that bodyweight loss is directly linked to decreased anastomotic strength and lower deposition of collagen. Thus, it could be stated that fibroblast transplantation could prevent the adverse outcomes of an inferior anastomosis leading to the catabolic state after the surgery.

Conclusion

The present study provided strong pieces of evidence on the ameliorative effects of fibroblast transplantation on the healing of colonic anastomosis in rats. Our results showed that serious complications of colonic anastomosis could be avoided by intramural fibroblast transplantation.

Acknowledgments

Authors' Contributions

M.B., H.M.; Contributed to conception and design. N.S.; Contributed to the experimental work and drafted the manuscript. A.-A.T., H.M.; Data acquisition. M.B.; Contributed to data analysis revision and final approval of the manuscript. All authors read and approved the final manuscript.

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