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Mesenchymal Stem Cell-injected Omental Patch More Effective Promoting Wound Healing in Bowel Perforation Animal Model

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ABSTRACT

Introduction: Bowel perforation (BP) occurs as the complication of many gastrointestinal problems. Omental patch (OP) is one of the methods to place omentum flaps in the perforated area. Mesenchymal stem cells (MSCs) may increase regeneration process in all tissues. **Aim:** to demonstrate the role of MSC in accelerating of wound healing process by analyzing fibroblast and collagen appearance in perforated bowel conditions. **Methods:** Using a BP rabbit model, 18 rabbit were randomly assigned into three groups: combination of umbilical cord (UC)-MSCs injection and OP (T1), OP only (T2) and vehicle control (Veh). Hematoxylin-eosin staining and Masson's trichrome staining were performed to analyze the level of fibroblast and collagen. Wound length were measured using standardized caliper. **Results:** The study showed a significant ($P<0.05$) increase of fibroblast and collagen amount on T1 and T2, in which T1 was higher than T2. This result was also followed by the decrease of wound length. **Conclusion:** The combination of MSCs and OP-sutured in perforated bowel are better to accelerate wound healing than OP only in BP cases.

Keywords: Mesenchymal stem cells, fibroblast, collagen, wound length, Bowel perforation.

1. INTRODUCTION

Bowel perforation (BP) is the most disastrous complication of gastroduodenal ulcers, that potentially lead to peritonitis and eventually sepsis (1, 2). Although the incidence of BP is 7 to 10 in 100.000 population, the BP is a life-threatening catastrophe that requires immediate surgical repair (3). The one minimum invasive technique for BP treatment is using a laparoscopic closure (4). However, It takes more operative time and necessarily needs trained personnel that not available everywhere yet. This procedure is not the best choice to control the BP in the majority of hospitals. On the other hand, the simple closure of the BP using the omentum patch (OP) has been suggested method (5). Most of them were healed completely, nevertheless under a certain condition such as elderly patients, they have a high risk of death, owing to abnormal healing and gastrointestinal leakage (6). While majority studies highlight the benefits of mesenchymal stem cells (MSCs) in repairing tissue damage including in accelerating wound healing (7, 8). Therefore, combining the MSCs to OP in regard to control BP is a rational option to achieve the optimum healing.

MSCs as multipotent stromal progenitor cells express several surface markers such as CD105, CD90, and CD73, and less express CD11b, CD14, CD19 or CD79a, CD45, CD34, or Human Leucocyte Antigen (HLA) class II. Most studies reported that MSCs shown the capacity to differentiate into multiple tissue-forming cell lineages and promote tissue regeneration by releasing various growth factor (9). On the other hand, omentum tissues also contain mesothelial cells that have properties of MSCs known as adipose-derived MSCs (AD-MSCs). Specifically, AD-MSCs have the ability to promote tissue repair and differentiate into cartilage, bone, tendon, and fat under specific conditions (10). Previous studies reported that MSCs have been used to repair a variety of organ injuries including lung, peritoneum, kidney, and liver, with encouraging results (11-15).

Under certain condition, MSCs can migrate into the damaged tissues and show immunomodulatory capabilities to inhibit an inflammatory response

(9). Furthermore, the active MSCs may activate fibroblast cells to synthesize higher amounts of collagen including extracellular matrix to accelerate wounds healing (16). Nevertheless, the little bit amount of MSCs in the normal omentum tissues and mostly inactive state is a point crucial in healing acceleration. Therefore to activate and optimize the MSCs are needed. Thus, injecting MSCs to OP sutured in BP site could give a better outcome in wound healing process rather than MSCs or OP procedure only. However, there is no yet study reported the combination of MSCs and OP in BP. In this study, we investigated the effects of the combination of MSCs and OP-sutured in perforated bowel to accelerate wound healing of BP cases by analyzing the increase of fibroblast quantity as one of the healing process markers.

2. AIM

The aim of this study was to demonstrate the role of MSCs in accelerating of wound healing process by analyzing fibroblast and collagen appearance in perforated bowel conditions.

3. MATERIAL AND METHODS

Isolation and culture of MSCs

MSCs were isolated from female New Zealand white rabbits' umbilical cords. Phosphate Buffer Solution (PBS) (Gibco TM Invitrogen, NY, USA) with 5% Penstrep antibiotic were used as a transport medium. The Wharton's jelly was separated from umbilical cord and minced evenly then placed into the 75 cm2 flask containing Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, Louis St, MO) mixed with 10% Fetal Bovine Serum (FBS) (Gibco) TM Invitrogen, NY, USA), and 100 IU/mL penicillin/streptomycin (Sigma-Aldrich). The cultured Wharton's Jelly was incubated in 5% CO2 and 370c incubator. The medium was changed in three days intervals and the MSCs will emerge in seven to 10 days. After reaching 80% confluence, the MSCs were passaged by trypsin. The 4th passage was used for the experiment. This study was approved by the Institutional Review Board of the Ethics Committee of the Medical Department, Sultan Agung Islamic University, Semarang, Indonesia.

BP animal models

Eighteen New Zealand white male rabbits weighing between 2000-3000 grams were veterinary at 240C with 12h light-dark cycle and fed with water and food. Briefly, sutured gastric perforation in rabbits was performed under sterile conditions, as described previously the rabbits were anesthetized by intraperitoneal administration of ketamine (80 mg per kg body weight) after fasting for 12 h. A midline abdominal incision was made to expose The stomach was exposed along the midline abdominal incision. A 2-cm vertical perforation was made at the gastric body using a scalpel, allowing the gastric lumen to open into the abdominal cavity. Subsequently, the gastric perforation was disinfected with iodophor, and closed employing 5/0 non-absorbable suture (Ethicon, Johnson & Johnson, Somerville, New Jersey, USA) at 4-mm intervals.

Administration of MSCs

Eighteen New Zealand white male rabbits were selected and divided randomly into three groups (n = 6). Vehicle group (Veh) received no treatment and intervention, only sutured on the incision's mark. The treatment group has had an OP (T1) and MSCs-injected OP (T2) on the abraded ileum. Tissue was obtained from the rabbits after 14 days of observation and stored in phosphate-buffered saline (PBS, Sigma) with 1% (v/v) penicillin-streptomycin (Figure 1).

In Vitro Differentiation

The MSCs were grown in 10 % fetal bovine serum (FBS) in DMEM and osteogenic induction medium containing 10 mmol/L β-glycerophosphate, 10-7 mol/L/0.1 μM dexamethasone, 50μmol/L ascorbate-2-phosphate (Sigma-Aldrich, Louis St, MO), at 37°C and 5% CO2. The fixed cells were stained with 0.2% Alizarin Red solution (Sigma-Aldrich) to represent calcium deposition (cells used were from the 4th passage).

Histopathological evaluation

Wound tissues from Veh and treatment group were taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with Hematoxylin and eosin (H&E) then observed under a microscope for the histopathological changes such as fibroblast proliferation, collagen formation, and angiogenesis.

Wound length measurement

Wound length measured before the rabbit was terminated. Wound length measured using calipers and calculated every rabbit in each group. All measurement was recorded in millimeters.

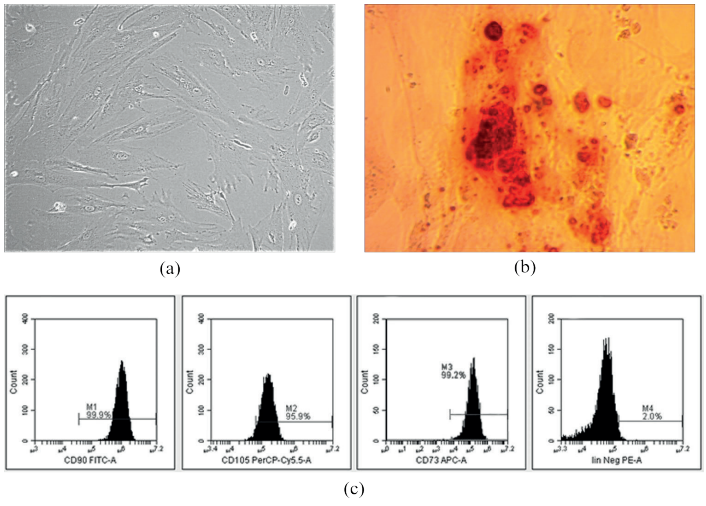

Figure 1. a) MSCs candidates from the in vitro culture showed fibroblast-like cell characteristics, b) Differentiation assays revealed that UC-MSCs could differentiate into osteocytes- via staining by Alizarin red which enabled osteocytes to appear red among the MSCs population, c) Flow cytometry analysis of UC-MSCs related immunophenotypes. Most UC-MSCs expressed positive markers (CD90, CD105, and CD73)

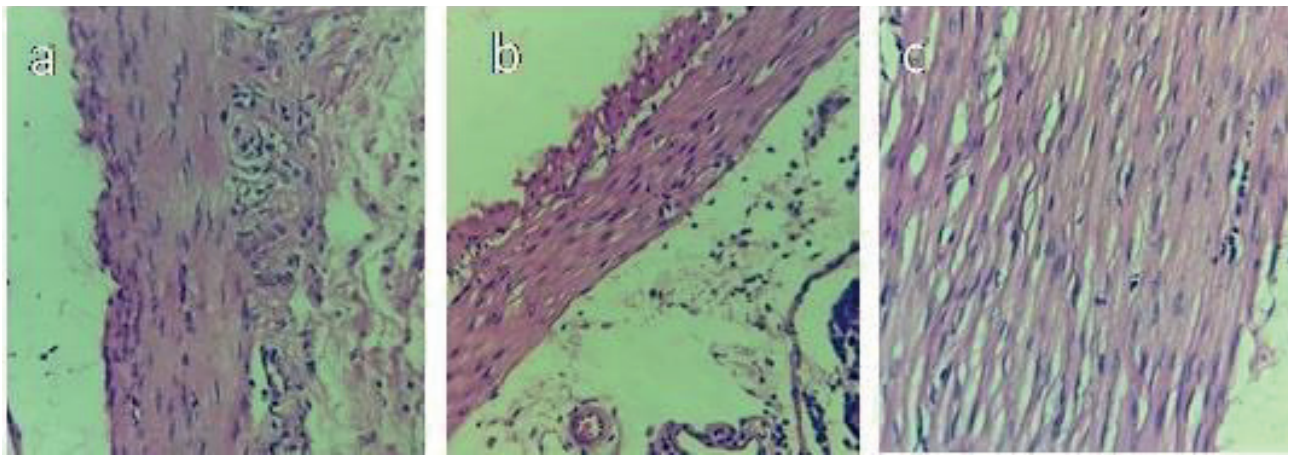

Figure 2. Histogy analysis after H&E staining of paraffin-embedded bowel section a) vehicle group which lowest level of fibroblast and collagen, b) treatment group has had an OP only and c) MSCs-injected OP group

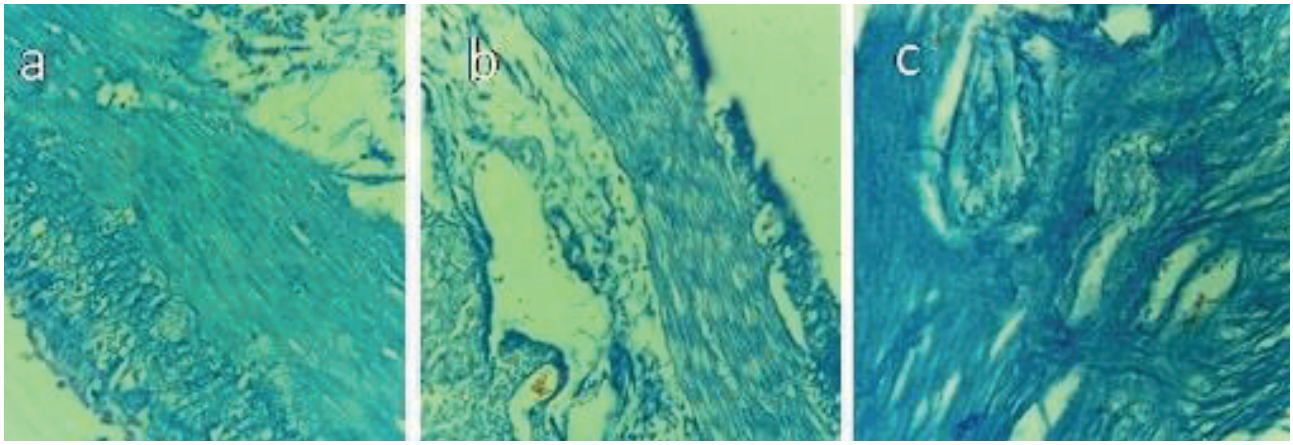

Figure 3. Histological apperance of the healing perforated wound a) vehicle group b)) treatment group has had an omental patch only and c) MSCs-injected omental patch group

Statistical analysis

Statistical comparisons were performed using the One-Way ANOVA and Kruskal Wallis test (SPSS 23.0), and results were expressed as mean + SDs. Statistical correlations were performed using Pearson and Spearman tests. P < 0.05 was considered statistically significant.

4. RESULTS

MSCs culture and characterization

Spindle-like shaped cells adhered to the culture flasks and differentiated into osteoblasts after being induced by the osteoblast differentiation medium. The characteristics of MSCs were expressing high levels of CD73 (99.2%), CD90 (96.7%), and CD105 (67.1%) in flow cytometric analysis.

Histology Evaluation

To evaluate the effects treatment of UC-MSCs to BP animal model after 14 days, the healed wounds of the animals were stained by H&E. The Veh showed 86.67 + 0.76 of mean fibroblast. In T1 (OP only) demonstrated a better fibroblast visualization by 106.17 + 0.477. The highest fibroblast visualized on T2 (OP and MSCs site injection) with 12.2±0.73. There was a significant difference among all groups ($p<0.05$). The result showed significant increase of collagen formation ($p<0.05$) of in Veh (11.33±0.211), T1 (12.83±0.167), and T2 (13.67±0.211) (Figure 2 and 3).

Wound length measurement

The wound healing was evaluated by wound length enclosure measurement after 14 days. The measurement showed significant difference (P<0,05) between the wound length in Veh, T1, and T2 (respectively 0,92 + 0,155; 0,65 + 0,108; 0,02 + 0,007 mm).

5. DISCUSSION

In BP cases, the OP method may accelerate wound healing by increasing some specific neovascularization and reducing complications afterward due to these omentum-contained MSCs (17-19). A little bit amount of these MSCs in the omentum tissue and mostly inactive state lead to inadequately repair in tissue damage including the BP cases. Injecting MSCs to OP-sutured in the BP site could accelerate wound healing process. We assumed this combination may help in sealing the perforation of the damaged area through neovascularization formation and cellular proliferation to accelerate the BP healing and potentially prevents recurrence. Therefore, to explore the effects of the combination of MSCs and OP-sutured in perforated bowel, we used the BP animal model as previous study and analyzed fibroblast quantity as one of the healing process markers.

This study showed a significant difference in fibroblast levels among treatment groups in which the highest level was in the combinations of OP and MSCs group. The fibroblast enhancement following MSCs administration had been considerably studied in some reports that associated with a collagen increase leading to the wound healing acceleration. This is in line with a previous study that reported MSCs become active in the injury area to promote wound closure whether using the release of paracrine molecules or transdifferentiation mechanisms (20-22). MSCs signaling has also been shown to positively regulate cell survival, proliferation, and migration, besides increasing fibroblast gene expression (23-25). This finding was supported by in vitro study that reported there were not fibroblast cells apoptosis when treated with MSCs. The rule of MSCs in wound healing mechanism was proposed by reepithelization improvement, angiogenesis formation, granulation tissue growth, extracellular matrix restoration, following the in-situ inflammation process is under control (26).

In this study, we supposed the controlled inflammation in BP areas triggered the proliferation and maturation of fibroblasts (27). There were double paracrine signaling loops between fibroblast cells and mesothelium containing endogenous MSCs, known as crosstalk or dynamic reciprocity to normalize tissue homeostasis after injury (28). Acute inflammatory cells at the wound sites initially promote fibroblast migration however the active exogenous MSCs concurrently with endogenous MSCs of omental release paracrine molecules to activate fibroblasts. The active fibroblasts known as myofibroblastic release platelet-derived grow factor (PDGF) and basic fibroblast growth factor (bFGF/FGF-2) to synthesize collagen and promote cross-linking for supporting wound closure (29, 30). This is in line with our founding in which there were a significant increase of collagen levels in the treatment group particularly in the combination between MSCs and OP groups and also positive correlation to fibroblast levels. The collagen accumulation dramatically affect wound healing.

This study has several limitations in which we did not analyze several growth factors secreted MSCs such as VEGF, FGF, and PDGF. Therefore, the understanding of the process underneath of the BP must be explored to get a comprehensive healing mechanism in BP cases.

6. CONCLUSION

In summary, this study demonstrates the combination of the MSCs and OP-sutured in perforated bowel are more active to accelerate wound healing in BP cases.

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- **Conflict of interest:** None declared.
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