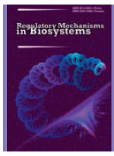

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The role of solid bone marrow aspirate concentrate in enhancing gingival wound repair in rabbits

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Healing of wounds involves factors such as inflammation and the formation of new tissue in the body's healing process. In this research project on the healing process of gingival wounds in rabbits using solid bone marrow aspirate concentrate (sBMAC), twelve male New Zealand White rabbits were split into two groups, one receiving saline treatment and the other treated with sBMAC. Histological and immunohistochemical examinations were conducted at two time points; three days and seven days after the surgery to evaluate the presence of inflammation and granulation tissue formation well as to observe angiogenesis and epithelialization processes. The findings indicate that sBMAC notably improved the stages of healing processes. On the 3rd day of observation wounds treated with sBMAC showed advancements in the formation of granulation tissue, angiogenesis and reepithelialization compared to the control group. By the 7th day both groups demonstrated progress in wound healing. However the sBMAC treated group exhibited structured granulation tissue, superior tissue maturity and heightened epithelial regeneration. The inflammatory response was also considerably reduced in the sBMAC group by Day 7, which suggests management of inflammation. The examination using immunohistochemistry showed levels of pan cytokeratin and TGF-β in the group treated with sBMAC, which indicates its effectiveness in stimulating the growth of cells and tissues.This discovery implies that sBMAC aids in speeding up the stages of wound healing and plays a part in improving tissue regeneration methods, for better soft tissue repair outcomes.

Keywords: solid bone marrow aspirate; gingival wound; inflammation; TGF-β; pan cytokeratin.

Introduction

The process of wound healing is quite intricate, in biology as it entails the movement of cells and their growth along with restructuring of the matrix and the formation of blood vessels (angiogenesis) (Morbidelli et al., 2021). The gum tissue healing process is similar to that of tissues in the body as it goes through a well-coordinated sequence comprising inflammation followed by cell growth and tissue reorganization phases (Dieterle et al., 2021). However some factors both internal to the body and external can impede the course of wound healing, leading to prolonged inflammation and delayed regeneration of tissues (Mamun et al., 2024).

Lately there has been a lot of interest, in medical methods like stem cell therapy and interventions using biomaterials due to their ability to improve tissue healing and speed up the recovery of wounds (Kolimi et al., 2022). Regenerative treatments have extensively made use of elements from bone marrow to aid in healing processes over time. Specifically, solid bone marrow aspirate (SBMA) is recognized for its abundance in mesenchymal stem cells (MSCs) hematopoietic stem cells, growth factors and cytokines. These components play a role in boosting cell growth, blood vessel formation and the creation of extracellular matrix. Studies in the past have shown that products derived from bone marrow can be effective in repairing bones and soft tissues like cartilage, which hints at their use in healing wounds in the mouth and gums (Oryan et al., 2014; Mohammadinejad et al., 2020).

In the field of dentistry it is essential to ensure thorough healing of gum wounds to support health and avoid any further infections or issues. Conventional treatment methods involve the use of antiseptics, antibiotics, and stitching techniques, for supportive care but do not directly encourage tissue regeneration (Jari Litany & Praseetha, 2022). Biological therapies that leverage the body's natural healing processes, such as SBMA application, could offer a successful strategy for speeding up the recovery process from gum injuries (Yan et al., 2023).

This research focuses on examining the impact of solid bone marrow aspirate (SBMA) on healing wounds in the gums of rabbits through studying tissue samples taken at time intervals to observe immunohistochemical alterations over time periods. The examination involves studying the presence of markers for healing, such as growth and regulation of epithelialization and growth factors, to assess the effectiveness of SBMA as a therapeutic intervention. The results of this research may help advance dentistry and establish a basis for upcoming clinical uses of treatments derived from bone marrow in repairing oral tissues.

Materials and methods

The study aimed to assess how solid bone marrow aspirate (SBMA) impacts the healing of gum wounds in rabbits, within a research setting approved by the Animal Ethics Committee at the College of Dentistry in Al Farabi University situated in Baghdad and conducted following the protocols for animal welfare.

Twelve male New Zealand White rabbits that were 28 weeks old and weighed between 3.5 and 4.0 kg were included in the research project. The rabbits were kept in a lab setting with temperature and humidity levels provided along with a nutritious diet and unrestricted access to fresh water. They were separated into two sets of six rabbits each. They were euthanized at two time intervals (after 3 and 7 days post-surgery P.O.) to gather samples.

Bone marrow was collected from the bones using a bone marrow aspiration needle, in sterile conditions following general anesthesia.

After collecting the bone marrow samples, they were promptly placed into tubes. Then the tubes were spun in a centrifuge according to these specified parameters: centrifuge at a speed of 750 times the force of gravity; the sample was spun for 12 minutes; the room temperature should ideally be between 22 to 25 °C; type of centrifuge being used is horizontal.

Following the centrifugation process, the clot components from the layer were gathered along with the buff coat. Then they were carefully administered to the gingival wound areas in the experimental group. The rabbits were split into two categories. In the control group of six participants; a 0 surgical cut was created in the gums. Then they were treated with normal saline at the site of the wound. SBMA group (consisting of six individuals). The surgical cut was carried out in the standard manner; however SBMA was directly applied to the wound site following the surgery. Each group was split into two depending on when they were sacrificed ; on the 3rd day after the surgery (with three individuals) and on the 7^{th} day after the surgery (with three individuals).

Before the surgery the medical team administered anesthesia by injecting a combination of drugs into the animals' muscles: butorphanol tartrate, medetomidine hydrochloride.

An incision measuring 0.05 cm was created in the gum using a scalpel following strict aseptic protocols to reduce the chances of infection. Following the administration of the treatments, close monitoring of the animals was conducted after surgery to guarantee their health and prevent any issues from arising.

At the scheduled times (days 3 and 7, after the surgery) euthanasia was carried out using anesthesia. Tissue samples were then removed from the wound areas and promptly preserved in 10% neutral buffered formalin for further examination, through histological and immunohistochemical methods.

The samples were gradually dehydrated with a series of alcohol solutions of increasing strength to achieve the desired result. The specimens were sliced into 5 μM sections, with a microtone and embedded in paraffin wax. Hematoxylin and eosin staining is commonly used to assess alterations within tissue structures. The growth of fibroblasts was examined at a level to observe the formation of tissue and the production of collagen.

Immunohistochemical staining was carried out to identify the presence of proteins linked to the process of wound healing. Cytokeratin acts as a sign of the renewal process, in tissues. Transforming growth factor beta (known as TGF beta) plays a role in the regulation of tissue restructuring and the healing process of wounds. The tissue samples underwent procedures for immunohistochemistry (IHC), where suitable primary and secondary antibodies were utilized to analyze protein expression levels through examination.

Data analysis was conducted using the version of SPSS software. Variations among groups were examined using a method known as analysis of variance (ANOVA). Data were expressed as mean ± standard deviation (SD) (one-way ANOVA, Duncan's test) at $P < 0.05$.

Results

The histological assessment of rabbit gingiva at days 3 and 7 post-operation provides valuable insights into the wound healing process and the potential impact of sBMAC treatment.

On day 3 in the control group, the wound site displayed epithelial detachment, inflammatory cell infiltration, inflammatory exudates, and minimal granulation tissue, indicating a delayed or less efficient healing process. By contrast, the sBMAC-treated group exhibited narrow wound edges, mild inflammatory infiltration, well-formed granulation tissue, and increased blood vessel formation, suggesting an accelerated healing response.

On day 7 the control group showed evidence of continued inflammatory cell infiltration, granulation tissue formation, and re-epithelialization, indicating progressive but ongoing tissue repair; the sBMAC group displayed similar histopathological features but with narrower wound edges and better granulation tissue organization, suggesting that wound closure and tissue maturation occurred more efficiently in this group.

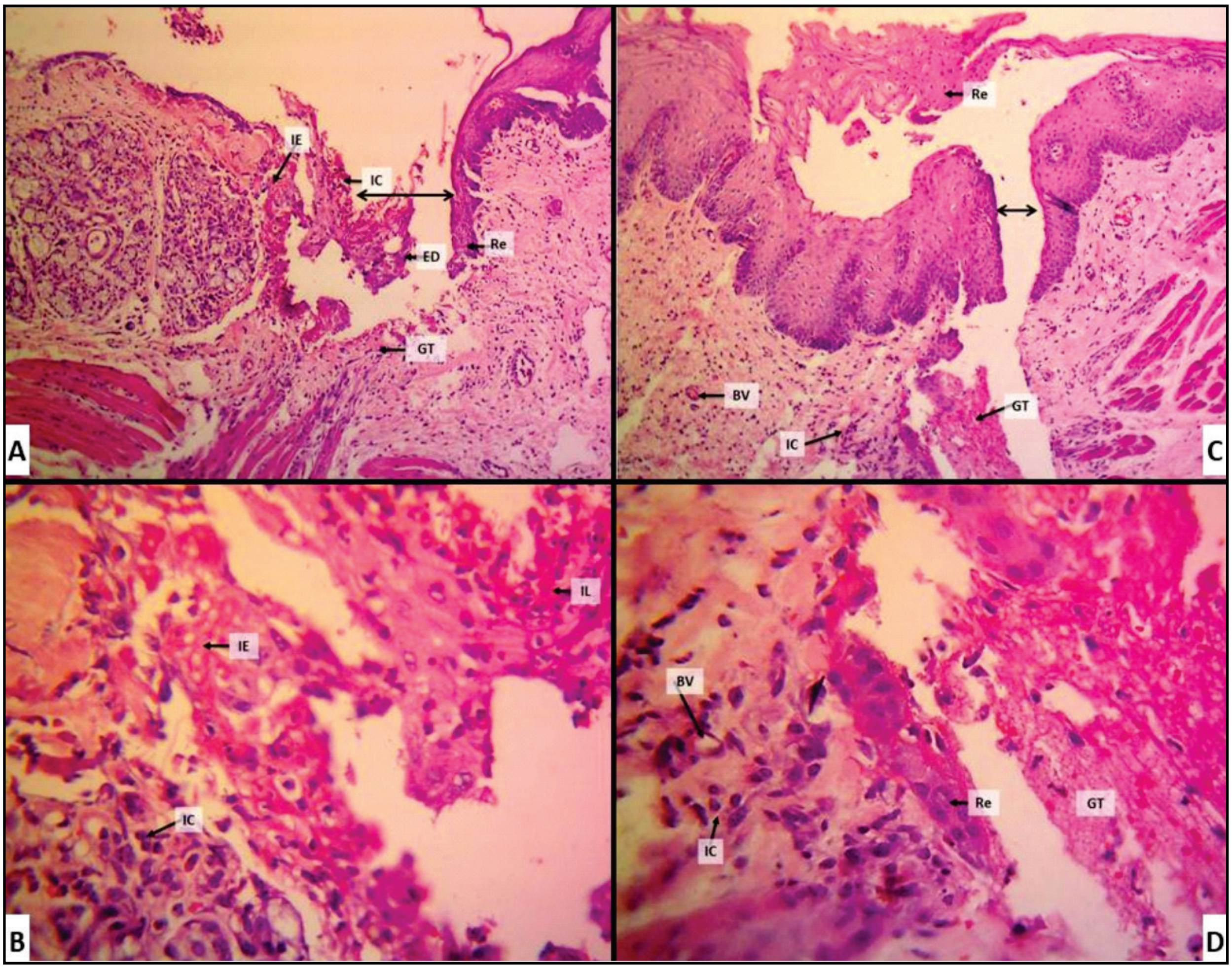

Fig. 1. Histological sections of rabbit's gingiva at day 3: *A* and *B* – control group (surgical wound only) reveals the site of wound with (↔) with detachment of epithelium (DE), inflammatory lesions (IL) with infiltration of inflammatory cells (score 3) (IC) and inflammatory exudate (IE) with sparse granulation tissue (score 1) (GT), and slight re-epithelialization (score 1) (Re); *C* and *D* – sBMAC group reveals site of wound in narrow edges (↔) with mild infiltration of inflammatory cells (score 1) (IC), healthy granulation tissue (score 2) (GT) with new blood vessels (BV) and healthy re-epithelialization (score 3) (Re); hematoxylin and eosin; *A* and *C* – 100X; *B* and *D* – 400X

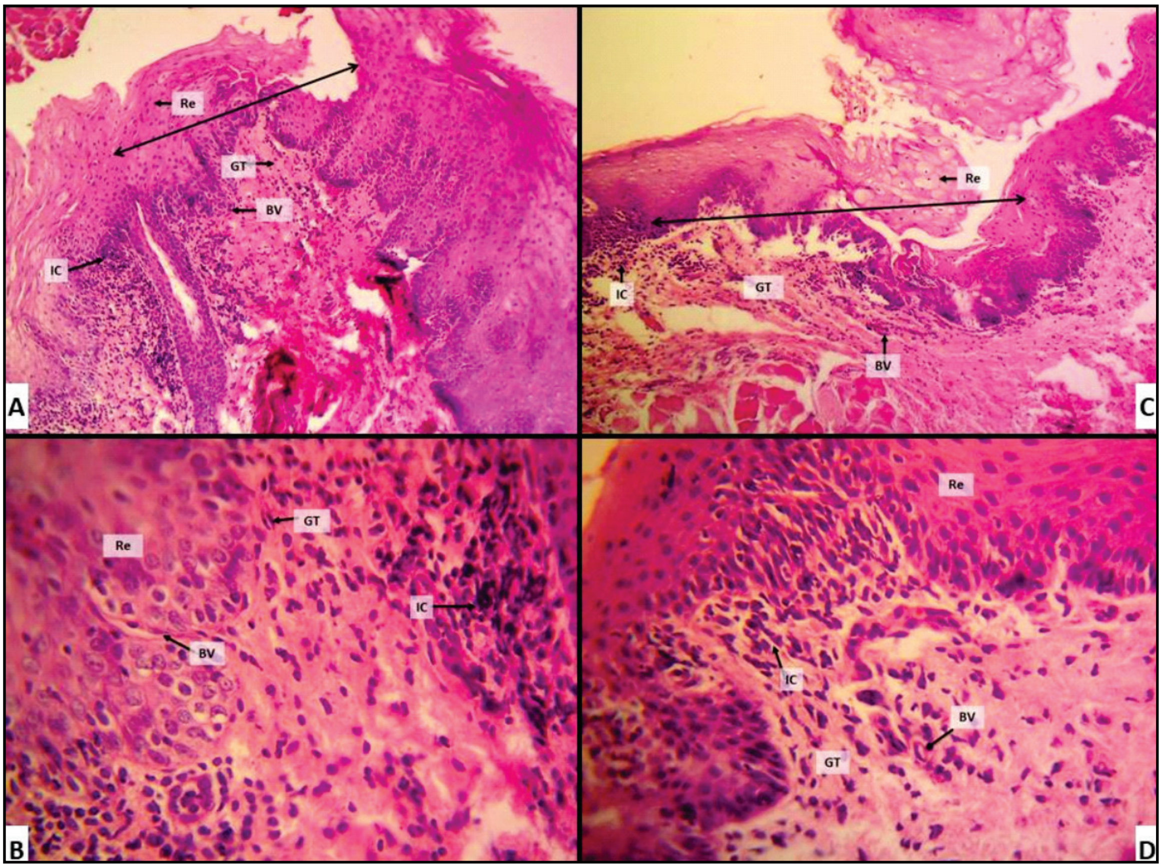

Fig. 2. Histological sections of rabbit's gingiva at day 7: *A* and *B* – control group (surgical wound only) reveals the site of wound with (↔) with infiltration of inflammatory cells (score 3) (IC), granulation tissue (score 2) (GT), with new blood vessels (BV) and healthy re-epithelialization (score 3) (Re); *C* and *D* – sBMAC group reveals site of wound in narrow edges (↔) with infiltration of inflammatory cells (score 2) (IC), healthy granulation tissue (score 2) (GT) with newlblood vessels (BV) and healthy re-epithelialization (score 4) (Re); hematoxylin and eosin; *A* and *C* – 100X; *B* and *D* – 400X

These findings align with the hypothesis that sBMAC promotes early and sustained wound healing by enhancing granulation tissue formation, reducing inflammation, and promoting better vascularization. The presence of narrower wound edges and increased angiogenesis in the sBMAC group supports its potential role in accelerating wound resolution.

The immunohistochemical analysis further supports the histological findings by revealing differential expression patterns of pan-cytokeratin and TGF-β, both of which are key markers in epithelial regeneration and wound healing.

Pan-cytokeratin, a marker for epithelial integrity, was moderately expressed in the control group at both time points, suggesting a slower epithelial regeneration process. In the sBMAC group, the intense expression of pan-cytokeratin at both days 3 and 7 indicates enhanced epithelial cell proliferation and differentiation, consistent with accelerated re-epithelialization observed histologically.

TGF-β plays a crucial role in wound healing by regulating inflammation, fibroblast activity, and extracellular matrix deposition. The control group exhibited moderate expression at day 3 and mild expression at day 7, suggesting a typical but slower progression of wound healing. The sBMAC group demonstrated intense TGF-β expression at both time points, implying a strong regenerative response and possible immunomodulatory effects that contribute to reduced inflammation and enhanced tissue repair.

Table 1 presents the histopathological scores of inflammation in rabbit gingival wounds, comparing the control group and the sBMAC group at different time intervals (days 3 and 7).

On day 3, the inflammation score in the control group was 2.66 ± 0.57, while in the sBMAC group, it was 2.0 ± 0.0. The difference between the groups was not statistically significant (P = 0.116). However, by day 7, the inflammation score in the control group remained relatively high (2.33 ± 0.57), whereas the sBMAC group showed a significant reduction (1.0 ± 0.0, P < 0.001).

Within-group comparisons over time indicate that in the control group, the inflammation scores did not change significantly between day 3 and 7 (P = 0.519). In contrast, the sBMAC group exhibited a significant decrease in inflammation from day 3 to 7 (P = 0.016), suggesting that the treatment effectively reduced inflammation over time. The presence of different letters (A and B) in the sBMAC group denotes a significant difference between time intervals at P < 0.05. Additionally, the asterisk (*) indicates a statistically significant difference between the sBMAC and control groups on day 7 (P < 0.05).

Table 1
Histopathological scores of the of the of inflammation in the rabbit gingival wound between control and sBMAC groups (x ± SD, n = 3)

Groups	Day 3	Day 7	*P*-value
Control group	2.66 ± 0.57	2.33 ± 0.57	0.519
sBMAC group	2.00 ± 0.00	1.00 ± 0.00	<0.001
P-value	0.116	0.016	–

Table 2 presents the histopathological scores of granulation tissue (GT) formation in rabbit gingival wounds, comparing the control and sBMAC groups at two time intervals (days 3 and 7).

On day 3, the control group had a significantly lower GT score (1.33 ± 0.57) compared to the sBMAC group (2.66 ± 0.57, P = 0.047), indicating that the sBMAC treatment enhanced granulation tissue formation in the early phase of healing. On day 7, the control group showed an increase in GT formation (2.66 ± 0.57), while the sBMAC group had a slightly lower score (2.0 ± 0.0). However, this difference was not statistically significant (P = 0.116).

In the control group, GT formation significantly increased from day 3 (1.33 ± 0.57) to day 7 (2.66 ± 0.57, P = 0.047), suggesting a natural progression of wound healing over time. In the sBMAC group, GT formation slightly decreased from day 3 (2.66 ± 0.57) to day 7 (2.0 ± 0.0), but this change was not statistically significant (P = 0.116).

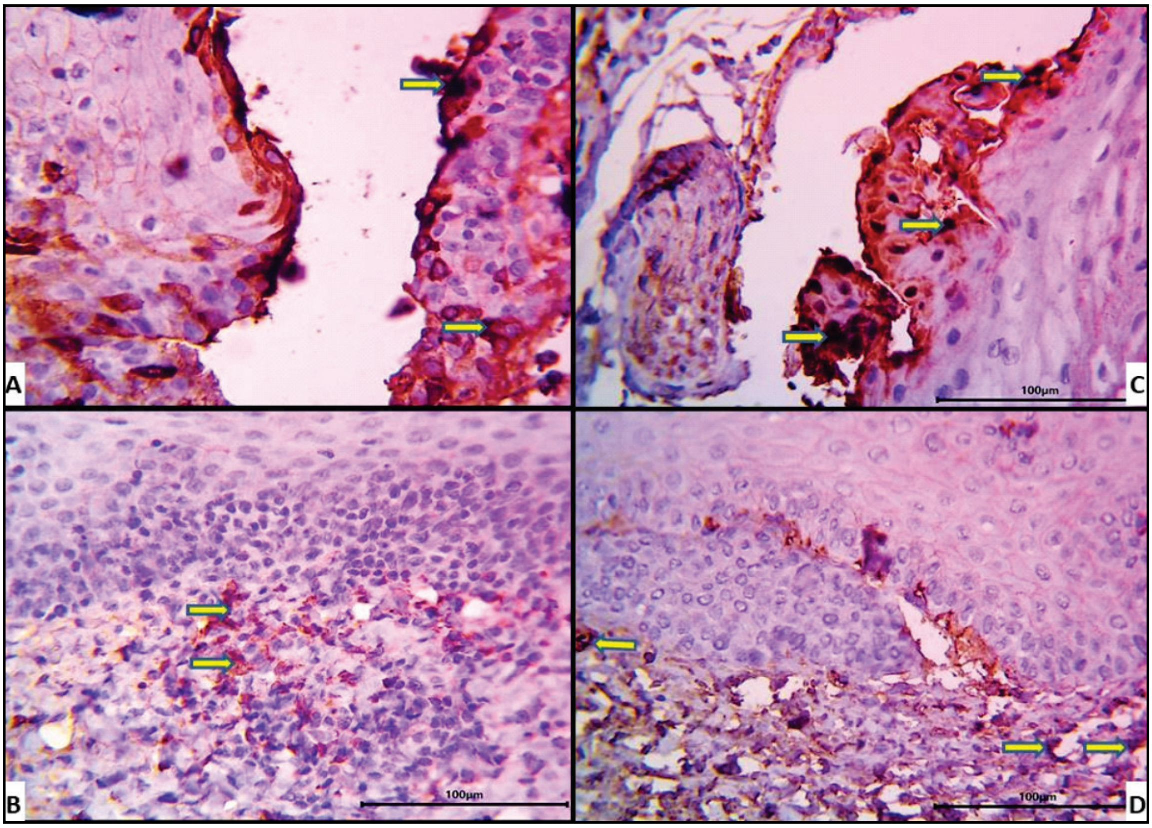

Fig. 3. Immunohistochemistry of pan cytokeratin (*A* and *C*) and TGF-B (*B* and *D*) of rabbit's gingiva at day 3: *A* – pan cytokeratin, control group (surgical wound only) reveals moderate expression in the epithelial cells of cytoplasmic patterns of mucosa (arrows); *B* – TGF-B, control group reveals moderate expression (arrows); *C* – pan cytokeratin, sBMAC group reveals intense expression in the epithelial cells' cytoplasmic patterns of mucosa (arrows); *D* – TGF-B, sBMAC group reveals intense expression (arrows); 400X

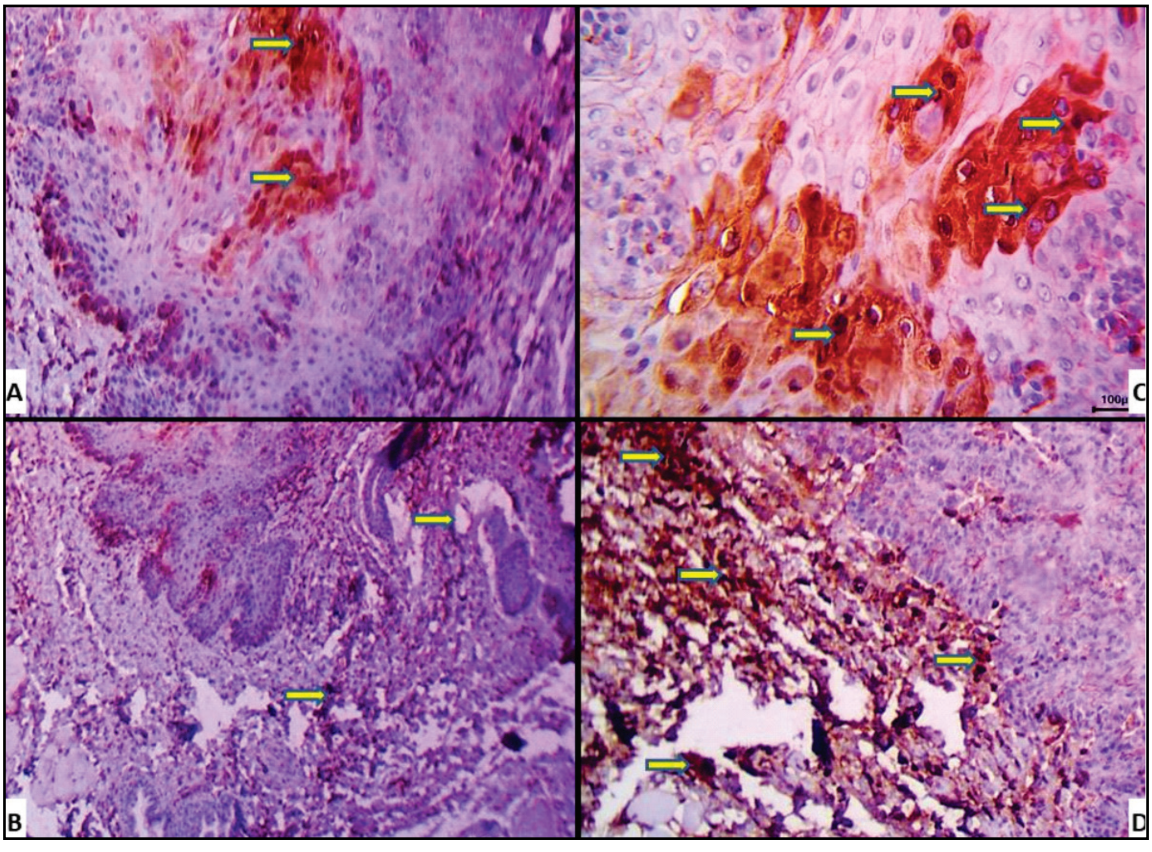

Fig. 4. Immunohistochemistry of pan cytokeratin (*A* and *C*) and TGF-B (*B* and *D*) of rabbit's gingiva at day 7: *A* – pan cytokeratin, control group (surgical wound only) reveals moderate expression in the epithelial cells of cytoplasmic patterns of mucosa (arrows); *B* – TGF-B, control group reveals mild expression (arrows); *C* – pan cytokeratin, sBMAC group reveals intense expression in the epithelial cells of cytoplasmic patterns of mucosa (arrows); *D* – TGF-B, sBMAC group reveals intense expression (arrows); 400X

Table 2
Histopathological scores of the granulation tissue GT in the rabbit gingival wound between control and sBMAC groups (x ± SD, n = 3)

Groups	Day 3	Day 7	*P*-value
Control group	1.33 ± 0.57	2.66 ± 0.57	0.047
sBMAC groups	2.66 ± 0.57	2.00 ± 0.00	0.116
P-value	0.047	0.116	–

Table 3 presents the histopathological scores of angiogenesis (new blood vessel formation) in rabbit gingival wounds, comparing the control and sBMAC groups at two time intervals (days 3 and 7).

On day 3, the sBMAC group exhibited a significantly higher angiogenesis score (1.33 ± 0.57) compared to the control group (1.0 ± 0.0, P = 0.016). This suggests that sBMAC treatment promoted early blood vessel formation in the healing process. On day 7, the sBMAC group had a higher angiogenesis score (2 ± 0) than the control group (1.33 ± 0.57), but the difference was not statistically significant (P = 0.374).

In the control group, angiogenesis slightly increased from day 3 (1.0 ± 0.0) to day 7 (1.33 ± 0.57), but this change was not statistically significant (P = 0.374). In the sBMAC group, angiogenesis increased from day 3 (1.33 ± 0.57) to day 7 (2.0 ± 0.0), but this difference also did not reach statistical significance (P = 0.116).

Table 3
Histopathological scores of the of angiogenesis (new blood vessels) in the rabbit gingival wound between control and sBMAC groups (x ± SD, n = 3)

Groups	Day 3	Day 7	*P*-value
Control group	1.00 ± 0.00	1.33 ± 0.57	0.374
sBMAC groups	1.33 ± 0.57*	2.00 ± 0.00	0.116
P-value	0.016	0.374	–

Table 4 presents the histopathological scores of re-epithelialization (Re) in rabbit gingival wounds, comparing the control and sBMAC groups at two time intervals (days 3 and 7).

On day 3, the sBMAC group exhibited a significantly higher re-epithelialization score (2.66 ± 0.57) compared to the control group (1.33 ± 0.57, P = 0.047), suggesting that sBMAC treatment enhanced early epithelial regeneration. On day 7, the sBMAC group continued to show a higher re-epithelialization score (3.66 ± 0.57) compared to the control group (3.0 ± 0.0), but the difference was not statistically significant (P = 0.116).

In the control group, re-epithelialization significantly increased from day 3 (1.33 ± 0.57) to day 7 (3.0 ± 0.0, p=0.007), indicating a natural progression of healing over time. In the sBMAC group, re-epithelialization also increased from day 3 (2.66 ± 0.57) to day 7 (3.66 ± 0.57), but this change was not statistically significant (P = 0.101).

Table 4
Histopathological scores of the re-epithelialization (Re) in the rabbit gingival wound between control and sBMAC groups (x ± SD, n = 3)

Groups	Day 3	Day 7	*P*-value
Control group	1.33 ± 0.57	3.00 ± 0.00	0.007
sBMAC groups	2.66 ± 0.57	3.66 ± 0.57	0.101
P-value	0.047	0.116	–

Discussion

The research was conducted to examine how solid bone marrow aspirate concentrate (known as sBMAC, for short) impacts the healing of gum wounds in rabbits. The results, from examining tissue samples and immune system markers on days 3 and 7 show that sBMAC notably speeds up the recovery process. By the 3rd day of treatment, with sBMAC (stem cell derived bone marrow aspirate concentrate) wounds displayed decreased inflammation and improved growth of granulation tissue well as early formation of blood vessels compared to the control group where standard inflammatory signs were seen and limited tissue regrowth occurred.

The significant rise in angiogenesis observed in the sBMAC group initially likely contributes to oxygen and nutrient supply enhancement, leading to enhanced tissue healing and recovery. By the end of the 7th day of observation, it was observed that while both groups displayed signs of healing advancement the group treated with sBMAC showed characteristics indicating structured granulation tissue formation, alongside narrower wound margins and enhanced epithelial regeneration maturation compared to the control group. The findings imply that sBMAC not only aids in tissue recovery but also contributes to long term tissue restructuring and regeneration processes.

Intense pan cytokeratin expression found in the sBMAC group through immunohistochemistry suggests regeneration. Additionally the robust TGF Beta expression indicates heightened activation, collagen synthesis and angiogenesis activities. Moreover the continuous presence of TGF Beta may also imply effects that could help alleviate long lasting inflammation and enhance the quality of healing processes (Koyanagi et al., 2022).

In general sBMAC shows promising capabilities, for speeding up and improving the healing of gum wounds by influencing inflammation levels and promoting blood vessel formation and skin cell regrowth as noted by Wadallah & Allawi (2025). The examination of tissue inflammation and the development of granulation tissue underscores the healing benefits of sBMAC in improving gum wound recovery.

By the third day of observation, inflammation levels were quite similar across all groups, which is expected for an acute reaction period. However by the seventh day those who received sBMAC treatment displayed notably lower inflammation scores compared to those in the control group. This indicates that sBMAC has an effective impact on managing and resolving inflammation over time. Further examination within each group confirms this trend as individuals in the sBMAC group experienced significant decreases in inflammation while there was no notable change in the control group, suggesting that inflammatory resolution was slower, for them.

In terms of GT formation, findings show that the group treated with sBMAC had scores by day 3 which suggests quicker granulation and initial tissue healing progressions. This rapid improvement is probably because sBMAC contains growth factors and precursor cells that stimulate function and angiogenesis, as mentioned by Cheng et al. (2021). By the seventh day the mark was reached when both sets of participants had high GT scores, which indicates that although sBMAC speeds up initial recovery process, the control group managed to catch up. sBMAC seems to help speed up the shift from the inflammatory to the proliferation phase and supports quicker development of granulation tissue. This faster healing process could have advantages in situations where swift tissue repair is essential (Karima & Kim, 2024).

The research emphasizes the impacts of solid bone marrow aspirate concentrate (known as sBMAC), showcasing its influence on the healing of gum wounds in rabbits by enhancing blood vessel formation and skin cell regeneration. On the third day of observation, the study group receiving sBMAC treatment showed improvements in blood vessel formation and skin regrowth compared to the control group This positive outcome is probably attributed to the influence of growth factors such as VEGFs and stem cells that play key roles in kickstarting the initial phases of tissue healing.

The rapid growth of blood vessels in the sBMAC group could potentially support the healing of wounds by reducing oxygen deficiency and enhancing the delivery of nutrients, to the injured tissue (Koyanagi et al., 2022). By the end of the week of the study period both sets of participants displayed progress; however the variations, in their outcomes were no longer deemed noteworthy. This implies that sBMAC may offer a time advantage by hastening the stages of recovery. Significant progress, in the control group's healing was observed over time during group analysis; on the other hand, the sBMAC treated wounds displayed high initial scores, with no notable improvement later on, suggesting that the majority of healing took place in the early stages.

Treatment with sBMAC encourages regeneration of blood vessels and skin cells, which could result in faster healing of wounds, reduced swelling and lower chances of infection, making it an encouraging method for improving the healing of soft tissues.

Conclusion

The latest results show that sBMAC significantly speeds up the healing process in rabbit gum wounds during the early stages of recovery on day 3, showing promising signs of faster skin regeneration with the help of stem cells and growth factors contained in the concentrate. Even though there was no difference between the groups by day 7; the improved response to early healing suggests that sBMAC could be beneficial in promoting rapid closure of wounds and reducing complications linked to delayed skin regeneration. The findings suggest that using sBMAC may be beneficial in clinical settings for improving the healing of tissues,

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