

# Evaluation of the regenerative effect of chitosan scaffold and hyaluronic acid with and without mesenchymal stem cells on wound healing in rats

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Received: 05.01.2024

Accepted: 19.08.2024

## Abstract

This study aimed to evaluate the restorative effect of chitosan hyaluronic acid scaffold (CHAS) with and without mesenchymal stem cells (MSCs) on the wound healing process in rats. The different wound treatment groups were as follows: no treatment or control (C), wound treatment with CHAS, wound covering with CHAS with MSCs. The wound healing effect was measured by measuring the wound area in each mouse on days 3, 5, 9, and 14. Then, for histopathological evaluation in the above days, each wound and 5 mm of normal skin tissue around each wound were separated and fixed. The results demonstrated that on the third and fifth days after the wound formation, the area of the remaining wound in the CHAS group was significantly smaller than the CHAS with MSCs but no significant difference was observed in the group C. Also, the area of the remaining wound on the ninth and fourteenth days in the studied groups did not show a significant difference. However, on day 14, the mean wound area in the CHAS group with MSCs was smaller than the other two groups. Histological examinations of the wound site were studied in terms of collagen arrangement, inflammation, vascular formation, granulation tissue, and epithelial regeneration. Studies in terms of collagen arrangement, granulation tissue formation, and vascular formation showed that on the third day. There was a significant difference between the groups, while no statistically significant difference was found between the groups in terms of inflammation and epithelial regeneration on the studied days. All these results demonstrate that there is no significant difference between the CHAS group and the CHAS group with MSCs as well as in group C.

**Key words:** Chitosan, Hyaluronic acid, Mesenchymal stem cell, Wound healing

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## References

- Ahmed, S., Ali, A., & Sheikh, J. (2018). A review on chitosan centered scaffolds and their applications in tissue engineering. *International journal of biological macromolecules*, *116*, 849-862.
- Amorim, S., Reis, C. A., Reis, R. L., & Pires, R. A. (2021). Extracellular matrix mimics using hyaluronan-based biomaterials. *Trends in biotechnology*, *39*(1), 90-104.
- Bakshi, P. S., Selvakumar, D., Kadirvelu, K., & Kumar, N. (2020). Chitosan as an environment friendly biomaterial—a review on recent modifications and applications. *International journal of biological macromolecules*, *150*, 1072-1083.
- Berce, C., Muresan, M.S., Soritau, O., Petrushev, B., Tefas, L., Rigo, I., Ungureanu, G., Catoi, C., Irimie, A., & Tomuleasa, C. (2018). Cutaneous wound healing using polymeric surgical dressings based on chitosan, sodium hyaluronate and resveratrol. A preclinical experimental study. *Colloids and Surfaces B: Biointerfaces*, *163*, 155-166.
- Bianchera, A., Catanzano, O., Boateng, J., & Elviri, L. (2020). The place of biomaterials in wound healing. *Therapeutic dressings and wound healing applications*, 337-366.
- Boateng, J., & Catanzano, O. (2015). Advanced therapeutic dressings for effective wound healing—a review. *Journal of pharmaceutical sciences*, *104*(11), 3653-3680.
- Borda, L. J., Macquhae, F. E., & Kirsner, R. S. (2016). Wound dressings: a comprehensive review. *Current Dermatology Reports*, *5*, 287-297.
- Chakravorty, N., & Shukla, P. C. (2023). Regenerative Medicine: Emerging Techniques to Translation Approaches. Praphulla Chandra: Amazon.co.uk: Books, 2, 121-125
- Chen, J., Yang, J., Wang, L., Zhang, X., Heng, B. C., Wang, D.-A., & Ge, Z. (2021). Modified hyaluronic acid hydrogels with chemical groups that facilitate adhesion to host tissues enhance cartilage regeneration. *Bioactive Materials*, *6*(6), 1689-1698.
- Deng, C., Zhang, P., Vulesevic, B., Kuraitis, D., Li, F., Yang, A. F., Griffith, M., Ruel, M., & Suuronen, E. J. (2010). A collagen–chitosan hydrogel for endothelial differentiation and angiogenesis. *Tissue Engineering Part A*, *16*(10), 3099-3109.
- Eivazzadeh-Keihan, R., Noruzi, E. B., Mehrban, S. F., Aliabadi, H. A. M., Karimi, M., Mohammadi, A., Maleki, A., Mahdavi, M., Larijani, B., & Shalan, A. E. (2022). The latest advances in biomedical applications of chitosan hydrogel as a powerful natural structure with eye-catching biological properties. *Journal of Materials Science*, 1-37.
- El Sadik, A. O., El Ghamrawy, T. A., & Abd El-Galil, T. I. (2015). The effect of mesenchymal stem cells and chitosan gel on full thickness skin wound healing in albino rats: histological, immunohistochemical and fluorescent study. *PloS one*, *10*(9), e0137544.
- Farahani, M., & Shafiee, A. (2021). Wound healing: From passive to smart dressings. *Advanced Healthcare Materials*, *10*(16), 2100477.
- Frenkel, J. S. (2014). The role of hyaluronan in wound healing. *International wound journal*, *11*(2), 159-163.
- Garg, T., Chanana, A., & Joshi, R. (2012). Preparation of chitosan scaffolds for tissue engineering using freeze drying technology. *IOSR J. Pharm*, *2*(1), 72-73.
- Graça, M. F., Miguel, S. P., Cabral, C. S., & Correia, I. J. (2020). Hyaluronic acid—Based wound dressings: A review. *Carbohydrate polymers*, *241*, 116364.
- Guo, Y., Wang, M., Liu, Q., Liu, G., Wang, S., & Li, J. (2023). Recent advances in the medical applications of hemostatic materials. *Theranostics*, *13*(1), 161.
- Gupta, A., & Kumar, P. (2015). Assessment of the histological state of the healing wound. *Plastic and Aesthetic Research*, *2*, 239-242.
- Ha, D. H., Kim, H.-k., Lee, J., Kwon, H. H., Park, G.-H., Yang, S. H., Jung, J. Y., Choi, H., Lee, J. H., & Sung, S. (2020). Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration. *Cells*, *9*(5), 1157.

- Harrell, C. R., Jankovic, M. G., Fellabaum, C., Volarevic, A., Djonov, V., Arsenijevic, A., & Volarevic, V. (2019). Molecular mechanisms responsible for anti-inflammatory and immunosuppressive effects of mesenchymal stem cell-derived factors. Tissue engineering and regenerative medicine. *Journal of Dermatological Treatment*, 33(1), 2-22.
- Hussain, Z., Thu, H. E., Katas, H., & Bukhari, S. N. A. (2017). Hyaluronic acid-based biomaterials: a versatile and smart approach to tissue regeneration and treating traumatic, surgical, and chronic wounds. *Polymer Reviews*, 57(4), 594-630.
- Jiang, W., & Xu, J. (2020). Immune modulation by mesenchymal stem cells. *Cell proliferation*, 53(1), e12712.
- Johnson, V., Chow, L., Harrison, J., Soontarak, S., & Dow, S. (2022). Activated mesenchymal stromal cell therapy for treatment of multi-drug resistant bacterial infections in dogs. *Frontiers in Veterinary Science*, 9, 925701.
- Kawano, Y., Patrulea, V., Sublet, E., Borchard, G., Iyoda, T., Kageyama, R., Morita, A., Seino, S., Yoshida, H., & Jordan, O. (2021). Wound healing promotion by hyaluronic acid: Effect of molecular weight on gene expression and in vivo wound closure. *Pharmaceuticals*, 14(4), 301.
- Kirwan, H., & Pignataro, R. (2015). The skin and wound healing. *Pathology and Intervention in Musculoskeletal Rehabilitation*, 25(8), 125-129.
- Kolimi, P., Narala, S., Nyavanandi, D., Youssef, A. A. A., & Dudhipala, N. (2022). Innovative treatment strategies to accelerate wound healing: trajectory and recent advancements. *Cells*, 11(15), 2439.
- Krasnodembskaya, A., Song, Y., Fang, X., Gupta, N., Serikov, V., Lee, J.-W., & Matthay, M. A. (2010). Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem cells*, 28(12), 2229-2238.
- Li, C., Cao, Z., Li, W., Liu, R., Chen, Y., Song, Y., Liu, G., Song, Z., Liu, Z., & Lu, C. (2020). A review on the wide range applications of hyaluronic acid as a promising rejuvenating biomacromolecule in the treatments of bone related diseases. *International journal of biological macromolecules*, 165, 1264-1275.
- Nour, S., Imani, R., Chaudhry, G. R., & Sharifi, A. M. (2021). Skin wound healing assisted by angiogenic targeted tissue engineering: A comprehensive review of bioengineered approaches. *Journal of Biomedical Materials Research Part A*, 109(4), 453-478.
- Okur, M. E., Karantas, I. D., Şenyiğit, Z., Okur, N. Ü., & Sifaka, P. I. (2020). Recent trends on wound management: New therapeutic choices based on polymeric carriers. *Asian Journal of Pharmaceutical Sciences*, 15(6), 661-684.
- Oliveira, A., Simoes, S., Ascenso, A., & Reis, C. P. (2022). Therapeutic advances in wound healing.
- Pountos, I., Panteli, M., Georgouli, T., & Giannoudis, P. V. (2014). Do mesenchymal stem cells have a role to play in cutaneous wound healing? *Cell & Tissue Transplantation & Therapy*, 6, 11.
- Prajapati, V. D., & Maheriya, P. M. (2019). Hyaluronic acid as potential carrier in biomedical and drug delivery applications. In *Functional Polysaccharides for Biomedical Applications* (pp. 213-265). Elsevier.
- Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S., & Saparov, A. (2021). Immunology of acute and chronic wound healing. *Biomolecules*, 11(5), 700.
- Ribeiro, J. C. V., Forte, T. C. M., Tavares, S. J. S., Andrade, F. K., Vieira, R. S., & Lima, V. (2021). The effects of the molecular weight of chitosan on the tissue inflammatory response. *Journal of Biomedical Materials Research Part A*, 109(12), 2556-2569.
- Sharifi S., Hoseini, S.A., Karimi, I., Bigham- Sadegh, A., & Shirian, S. (2022). Therapeutic Effects of Ozone Therapy on Experimental Fracture Healing in the Rabbit Model. *Iranian Veterinary Journal*, 18 (3) 31-40.
- Scharnweber, D., & Saalbach, A. (2019). Hyaluronan/collagen hydrogels containing sulfated hyaluronan improve wound healing by sustained release of heparin-binding EGF-like growth factor. *Acta biomaterialia*, 86, 135-147.
- Shafiq, M., Ali, O., Han, S.-B., & Kim, D.-H. (2021). Mechanobiological strategies to enhance stem cell functionality for regenerative medicine and tissue engineering. *Frontiers in Cell and Developmental Biology*, 9, 747398.
- Shariatnia, Z. (2019). Pharmaceutical applications of chitosan. *Advances in colloid and interface science*, 263, 131-194.

- Shojaei, F., Rahmati, S., & Banitalebi Dehkordi, M. (2019). A review on different methods to increase the efficiency of mesenchymal stem cell-based wound therapy. *Wound Repair and Regeneration*, 27(6), 661-671.
- Son, Y. J., John, W. T., Zhou, Y., Mao, W., Yim, E. K., & Yoo, H. S. (2019). Biomaterials and controlled release strategy for epithelial wound healing. *Biomaterials science*, 7(11), 4444-4471.
- Tabassum, N., Ahmed, S., & Ali, M. A. (2021). Chitooligosaccharides and their structural-functional effect on hydrogels: A review. *Carbohydrate polymers*, 261, 117882.
- Thönes, S., Rother, S., Wippold, T., Blaszkiewicz, J., Balamurugan, K., Moeller, S., ... & Anderegg, U. (2019). Hyaluronan/collagen hydrogels containing sulfated hyaluronan improve wound healing by sustained release of heparin-binding EGF-like growth factor. *Acta biomaterialia*, 86, 135-147.
- Van Buul, G., Villafuertes, E., Bos, P., Waarsing, J., Kops, N., Narcisi, R., Weinans, H., Verhaar, J., Bernsen, M., & Van Osch, G. (2012). Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture. *Osteoarthritis and Cartilage*, 20(10), 1186-1196.
- Yang, F., Bai, X., Dai, X., & Li, Y. (2021). The biological processes during wound healing. *Regenerative medicine*, 16(04), 373-390.
- Yang, L., Zhang, L., Hu, J., Wang, W., & Liu, X. (2021). Promote anti-inflammatory and angiogenesis using a hyaluronic acid-based hydrogel with miRNA-laden nanoparticles for chronic diabetic wound treatment. *International journal of biological macromolecules*, 166, 166-178.
- Yazarlu, O., Iranshahi, M., Kashani, H. R. K., Reshadat, S., Habtemariam, S., Iranshahi, M., & Hasanpour, M. (2021). Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. *Pharmacological research*, 174, 105841.