EXPERIMENTAL WORKS

UDC 615.471 https://doi.org/10.15407/biotech18.02.014

COLLAGEN MATRIX WITH INCREASED HEMOSTATIC PROPERTIES: COMPARATIVE ANALYSIS OF HEMOSTATIC EFFICACY

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Received 2025/02/21
Revised 2025/03/06
Accepted 2025/04/30

Aim. To develop collagen matrices with increased hemostatic properties and to evaluate their effectiveness in comparison with commercial products.

Methods. Collagen was obtained from calfskin by acid hydrolysis, identified by SDS-PAGE, and sterilized in an autoclave. The enzymatic clotting activator was purified from *Echis multisquamatis* venom by ion exchange chromatography. The activity of the activator was evaluated with the chromogenic substrate S2302. Ready-made modified matrices were obtained by lyophilization. Hemostatic efficacy was tested in the Wistar Han rat hepatic hemorrhage model, compared with commercial materials.

Results. Optimal concentrations of collagen (300 μg/cm^2) and enzyme activator (10 μg/cm^2) were selected for the manufacture of collagen matrices with increased hemostatic properties. Studies on the rat parenchymal bleeding model showed that these matrices provide faster bleeding control compared to commercial hemostatic materials. After the removal of collagen matrices, bleeding did not resume.

Conclusions. The developed collagen matrices with increased hemostatic properties demonstrate high efficiency, stable clot formation, and minimal risk of rebleeding, which confirms their prospects in surgery.

Keywords: collagen matrix, enzyme activator, haemostatic materials, comparison.

Uncontrolled bleeding remains one of the leading causes of trauma-related deaths in both military and civilian settings. A significant proportion of fatalities occur before patients arrive at the hospital or within the first hour of injury. This underscores the critical importance of effective hemostatic materials that can quickly stop bleeding and reduce the risk of complications [1]. Modern hemostatic agents such as coagulants, chemical agents, and physical barriers all have their advantages, but none of them is a one-size-fits-all solution.

Collagen matrices are a perspective material for stopping bleeding due to their biocompatibility and ability to stimulate platelet adhesion and promote blood clotting. However, standard collagen

Citation: Baidakova, K. B., Kucheryavyi, Y. P., Korolova, D. S. (2025). Collagen matrix with increased hemostatic properties: comparative analysis of hemostatic efficacy. *Biotechnologia Acta*, 18(2), 14–16. https://doi.org/10.15407/biotech18.02.014

matrices have certain limitations which necessitate their modification. One of the promising approaches is to add specific clotting activators to the matrices, which can accelerate clot formation and improve the material's effectiveness [2]. In this study, we used a prothrombin activator that acts exclusively on the final stage of blood clotting. This not only enhances the hemostatic effect of the material but also makes it more controlled and safer.

This study aimed to develop and comparatively analyze the effectiveness of collagen matrix with increased hemostatic properties with commercial hemostatic materials.

Methods. Collagen was isolated from calfskin by acid hydrolysis, characterized by SDS-PAGE, and sterilized by autoclaving (134 °C, 210 kPa, 35 minutes). The absence of gram-negative and gram-positive microorganisms and yeast assessed sterility. The enzymatic blood coagulation activator (EA) was purified from *Echis multisquamatis* venom by ion-exchange chromatography. The activity of the enzyme activator in combination with collagen matrices and the quality of immobilization was evaluated using the chromogenic substrate S2302 (HD-Pro-Phe-Arg-pNA×2HCl). Modified collagen matrices were prepared by lyophilization using the LyoQuest Telstar system. The hemostatic efficacy was assessed in a rat model of liver bleeding by comparing it with commercial hemostatic materials, including the hemostatic gelatin sponge 'CUTANPLAST Dental' (Mascia Brunelli, Italy) [3], corrugated hemostatic bandage 'HEMOSTATIC' (Senta Pharm, Ukraine) and hydrogel bandage 'HYDROBYNT №1' (V-CUBE, Ukraine) [4]. The comparison was performed by the time of stopping bleeding, the amount of blood loss, and the resumption of bleeding after the removal of the hemostatic agent. Local tolerability was assessed in Wistar Han rats by subcutaneous implantation in accordance with ISO 10993-6:2011.

Results. The study involved the development of collagen matrix with increased hemostatic properties, optimizing their composition to achieve maximum effectiveness. Several variants of collagen content were tested (100, 200, 300, 400 and 500 μg/cm^2). Analysis of the physicochemical properties showed that a concentration of 300 μg/cm^2 provides good mechanical strength, flexibility, and biodegradability. At lower concentrations, the structure of the matrices was too delicate, while higher values resulted in excessive density, which made it difficult to adapt them to the wound surface.

We also examined the effect of different concentrations of the enzyme activator (4, 8, 10 and 16 μg/cm^2). The optimization of the concentration of the enzyme activator allowed us to establish that the most effective value was 10 μg/cm^2. Lower concentrations did not provide sufficient activation of prothrombin, which slowed down the process of blood clot formation. At the same time, no significant improvement in efficacy was observed when the concentration exceeded 10 μg/unit. Still, there was a risk of excessive release of the activator into the general bloodstream.

The study of the stability of the activator immobilization showed that in the buffer solution, the release of the enzyme from the matrices was less than 10%. In the blood plasma, its diffusion from the clot was less than 5%. This indicates the effective immobilization of the activator in the collagen structure and its controlled release at the site of injury.

Collagen matrices 3×3 cm in size and weighing 3 mg were prepared and modified with the enzyme activator. Microbiological testing confirmed their sterility, and subcutaneous implantation testing showed good local tolerance.

We compared the hemostatic effectiveness of collagen matrix with increased hemostatic properties with the hemostatic gelatin sponge 'CUTANPLAST Dental', corrugated hemostatic bandage 'HEMOSTATIC,' and hydrogel bandage 'HYDROBYNT №1' in a rat parenchymal liver bleeding model (Fig.). The study included a comparison of the bleeding time after the usage of different hemostatic agents, measurement of blood loss, and whether bleeding resumed after the removal of the hemostatic material from the wound surface.

Experimental results have shown that collagen matrices modified with an enzyme activator provide bleeding control in an average of 20 seconds. This is faster than when using 'CUTANPLAST Dental,' 'HYDROBYNT No 1', and 'HEMOSTATIC,' for which the stopping time was longer. In all cases of use of modified collagen matrices, there was no rebleeding after their removal.

Discussion. The developed collagen matrix with increased hemostatic properties has a significant potential for use in clinical practice. The combination of biocompatibility, biodegradability, and active influence on the blood coagulation system makes them a promising solution for local hemostasis. Their use can be particularly useful in surgery, traumatology, and emergency medicine, where fast and reliable bleeding control is critical.

Unlike many traditional hemostatic materials, collagen matrices modified with an enzyme activator not only create a physical barrier to blood but also directly affect the clotting process,

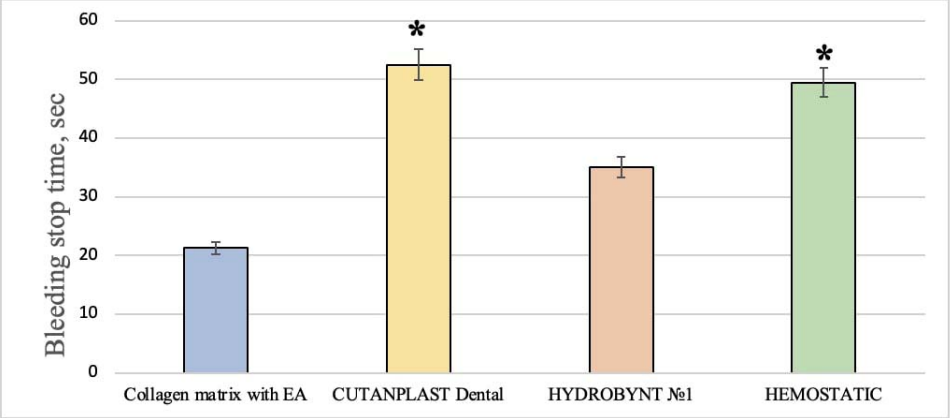

Figure. **Comparison of the bleeding stop time of the collagen matrix with increased hemostatic properties with the 'CUTANPLAST Dental' (Mascia Brunelli, Italy), 'HYDROBYNT №1' (V-CUBE, Ukraine), and 'HEMOSTATIC' (Senta Pharm, Ukraine)**
* $P \leq 0.05$ compared to collagen matrix modified with enzyme activator

promoting the rapid formation of a stable clot. This can provide better results in patients with a disrupted coagulation system, where passive hemostatic methods may not be effective enough.

Conclusions. We have developed collagen matrices with increased hemostatic properties that have demonstrated high efficiency compared to commercial hemostatic agents. They provide rapid and stable clot formation, significantly reducing the time to stop bleeding. The results confirm the prospects of their use as an effective hemostatic material with a minimal risk of bleeding reopening after removal.

Authors' contribution

KB was involved in the manufacture of collagen matrices with increased hemostatic properties; YP performed the in vivo experiments in a rat parenchymal liver bleeding model; DK supported and organized the research.

Funding source

The research was financed by the project No. 0124U000251, "Research and search for ways to regulate molecular mechanisms of intravascular and extravascular thrombosis" (2024–2028).

Acknowledgment

The authors gratefully thank the Ukrainian Armed Forces for such an opportunity to live and work in a peaceful Kyiv.

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