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STUDY OF THE WOUND-HEALING ACTIVITY OF A HYDROGEL IMMOBILIZED WITH 6-AMINOPURINE

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Abstract: Rapid healing of traumatic and postoperative wounds in farm animals remains an important veterinary problem due to infection risk, dehydration of tissues and slow regeneration under field conditions. In this study a bioactive hydrogel containing immobilized 6-aminopurine was developed and its wound-healing activity was evaluated. The hydrogel matrix was obtained from biocompatible polysaccharides followed by ionic crosslinking and incorporation of the active compound to ensure prolonged release. Physicochemical properties of the material (swelling capacity, moisture retention and biodegradability) were characterized, and release kinetics of 6-aminopurine from the matrix were determined in physiological medium.

Biological activity was assessed on experimental skin wounds in farm animals by monitoring inflammation phase duration, granulation tissue formation, epithelialization rate and microbial contamination. Application of the hydrogel created a moist protective environment, reduced exudation and prevented secondary infection. Compared with untreated control wounds, the treated group demonstrated faster granulation, earlier epithelial layer formation and shorter healing period. The regenerating tissues showed reduced edema and more organized collagen fibers.

The results indicate that immobilization of 6-aminopurine in a hydrogel matrix provides controlled delivery of the bioactive compound and significantly enhances reparative processes. The developed preparation can be considered a promising antibiotic-free regenerative veterinary material for treatment of wounds and inflammatory skin lesions in livestock.

Keywords: 6-Aminopurine; hydrogel; wound healing; tissue regeneration; veterinary medicine; controlled release; epithelialization; livestock wounds.

Introduction

Skin injuries in livestock represent one of the most frequent veterinary problems encountered in animal husbandry. Mechanical trauma, surgical interventions, insect bites, infections and harsh environmental conditions often lead to open wounds accompanied by inflammation, microbial contamination and delayed tissue regeneration. In farm animals, the healing process is usually prolonged due to constant movement, contamination with soil and manure, dehydration of tissues and repeated mechanical irritation. Slow wound closure reduces productivity, increases treatment costs and raises the risk of secondary bacterial infection. Therefore, development of safe and effective regenerative preparations that accelerate tissue repair without excessive antibiotic use is an important task of modern veterinary pharmacology[1-24].

Traditional wound-healing agents used in veterinary practice mainly include antiseptics, antibiotics and fatty ointments. Although they suppress microbial growth, many of these treatments do not actively stimulate tissue regeneration and may cause resistance, tissue irritation or drying of the wound surface. Modern regenerative medicine focuses on creating materials that not only protect the damaged tissue but also regulate the biological phases of healing:

inflammation, proliferation and remodeling. In this context, hydrogels have attracted considerable attention as wound-dressing materials because of their high water content, permeability to oxygen and ability to maintain a moist environment favorable for cell migration and collagen synthesis.

Hydrogels based on biocompatible polymers can serve as carriers for biologically active compounds and ensure their controlled release directly at the injury site. Such systems reduce systemic exposure and prolong therapeutic activity. Immobilization of pharmacologically active molecules within a hydrogel matrix allows simultaneous protection of the wound surface, absorption of exudate and stimulation of cellular regeneration. The effectiveness of these systems strongly depends on the nature of the incorporated bioactive substance.

6-Aminopurine and its derivatives belong to a class of purine compounds known for their biological regulatory properties. Purine bases are structural components of nucleic acids and play an essential role in cellular metabolism, energy transfer and cell division. In biological systems, aminopurine derivatives can stimulate nucleic acid synthesis, enhance fibroblast proliferation and accelerate formation of granulation tissue. These properties suggest that local application of 6-aminopurine may promote reparative processes in damaged skin. However, direct administration of low-molecular compounds often leads to rapid diffusion and short-term activity, limiting therapeutic efficiency.

Immobilization of 6-aminopurine in a hydrogel matrix provides a promising solution by enabling sustained release and maintaining an effective concentration in the wound area. Such a delivery system may combine protective, antimicrobial-barrier and regenerative functions in a single preparation. Despite the known biological activity of purine derivatives, their application in veterinary regenerative biomaterials remains insufficiently investigated.

The aim of the present study was to develop a biocompatible hydrogel containing immobilized 6-aminopurine and to evaluate its wound-healing activity in livestock. Particular attention was paid to inflammation reduction, granulation tissue formation, epithelialization rate and overall healing time compared with untreated wounds.

Materials and Methods

All reagents used in the study were of analytical grade. 6-Aminopurine was selected as the biologically active compound. Sodium alginate and chitosan were used as biocompatible polymers for hydrogel formation, while calcium chloride served as an ionic crosslinking agent. Glycerol was added as a plasticizer to improve elasticity and water retention, and distilled water was used as the solvent.

The hydrogel was prepared by dissolving sodium alginate in distilled water at room temperature under continuous stirring until a homogeneous viscous solution was obtained. Chitosan was separately dissolved in dilute acetic acid solution and then mixed with the alginate solution to form a uniform polymer matrix. A calculated amount of 6-aminopurine was dissolved in warm distilled water and introduced into the polymer mixture under constant stirring to ensure even distribution within the matrix. Glycerol was added to improve mechanical stability and hydration properties. The resulting composition was then slowly introduced into calcium chloride solution, where ionic crosslinking occurred and a three-dimensional hydrogel network was formed. The formed hydrogel was washed with sterile distilled water to remove unreacted components and stored at low temperature in sterile conditions before biological testing.

Physicochemical properties of the obtained hydrogel were evaluated prior to biological experiments. Swelling behavior was studied in physiological saline at body temperature by recording weight changes over time. Moisture retention capacity was determined by measuring mass loss during incubation. Controlled release of 6-aminopurine was investigated in phosphate buffer medium at physiological pH, and samples were periodically analyzed spectrophotometrically to determine the amount of released substance. Biodegradability was assessed by monitoring gradual reduction of sample mass during incubation in aqueous medium.

The biological study was carried out on clinically healthy farm animals maintained under standard feeding and housing conditions. The experimental area on the dorsal skin was cleaned, shaved and disinfected. Standardized full-thickness skin wounds of equal size were created under local anesthesia using aseptic technique. Animals were divided into control and experimental groups. The experimental group received topical application of the prepared 6-aminopurine hydrogel once daily, while the control group was left untreated or received routine treatment(Fig-1).

Figure 1. General appearance and application scheme of the 6-aminopurine-immobilized hydrogel dressing on a skin wound surface showing formation of a moist protective layer and stimulation of granulation tissue development.

Healing progress was evaluated throughout the observation period. Changes in wound size were measured using planimetric methods and expressed as percentage reduction relative to the initial area. The duration of inflammation, formation of granulation tissue, onset of epithelialization and total healing time were recorded. Presence of edema, exudate and redness was also noted as indicators of inflammatory response.

Microbiological assessment was performed by taking swabs from the wound surface at different stages of healing and culturing them on nutrient media to determine microbial load. In addition, histological analysis was conducted on tissue samples collected from the wound margins. Samples were fixed, sectioned and stained, after which microscopic examination was carried out to evaluate fibroblast proliferation, collagen fiber organization and epithelial layer formation.

All experiments were performed repeatedly, and the obtained results were processed using standard statistical analysis. The data were expressed as mean values with standard deviation, and differences between groups were considered significant at a confidence level of $p < 0.05$.

Results and Discussion

The prepared hydrogel containing immobilized 6-aminopurine formed a uniform, elastic and transparent three-dimensional structure with high adhesion to moist tissue surfaces. The material maintained structural integrity during handling and application, which is important for veterinary use under field conditions. Swelling experiments showed rapid water uptake followed by stabilization, indicating formation of a hydrated network capable of maintaining a moist microenvironment at the wound surface. Moisture retention remained high over prolonged incubation, confirming that the hydrogel can prevent drying of damaged tissues and reduce scab formation.

Release studies demonstrated a prolonged liberation of 6-aminopurine from the polymer matrix. An initial moderate release phase was followed by a sustained diffusion stage, ensuring maintenance of a therapeutic concentration at the injury site. Such controlled release is essential because free low-molecular compounds are usually removed quickly by wound exudate. The immobilized form therefore provides longer biological activity and reduces frequency of treatment.

Macroscopic observation of wounds revealed clear differences between control and treated groups. In untreated wounds, inflammation persisted for several days and was characterized by edema, redness and serous exudate. In contrast, wounds treated with the hydrogel showed reduced swelling and earlier formation of a protective moist layer. Exudation decreased rapidly after application, suggesting barrier and protective effects of the hydrogel coating.

Reduction of wound area occurred faster in the treated animals. Granulation tissue appeared earlier and was more uniform in structure. The surface of the wound became covered with fine vascularized tissue, indicating activation of reparative processes. Early epithelialization was observed along the wound margins, followed by progressive closure of the defect. The healing period was shortened compared with the control group, demonstrating the regenerative potential of the developed preparation.

Microbiological examination showed lower microbial contamination in the hydrogel-treated wounds. Although the material did not contain classical antibiotics, the moist protective environment limited external contamination and supported natural immune defense mechanisms. Reduced infection risk is particularly important in livestock conditions where wounds are exposed to environmental microorganisms.

Histological analysis confirmed the macroscopic observations. Tissue sections from the treated group showed dense and well-organized collagen fibers, active fibroblast proliferation and formation of a continuous epithelial layer. Inflammatory cell infiltration was significantly lower than in untreated wounds, indicating faster transition from inflammatory to proliferative phase. Newly formed tissue demonstrated more ordered structure, suggesting improved remodeling.

The observed biological effect can be explained by the combined action of the hydrogel matrix and 6-aminopurine. The hydrogel provides hydration, oxygen permeability and mechanical protection, while 6-aminopurine acts as a metabolic regulator that stimulates nucleic acid synthesis and cell division. Sustained release of the compound maintains local stimulation of fibroblasts and epithelial cells, accelerating granulation and re-epithelialization.

Overall, the results indicate that immobilization of 6-aminopurine in a biocompatible hydrogel creates a multifunctional veterinary material capable of reducing inflammation, protecting the wound surface and promoting tissue regeneration. Such preparations may represent an effective alternative to traditional ointments and reduce the need for antibiotic therapy in routine livestock wound management.

Conclusion

The conducted study demonstrated that incorporation of 6-aminopurine into a biocompatible hydrogel matrix produces a multifunctional regenerative veterinary material with pronounced wound-healing activity. The developed hydrogel maintained a moist protective environment, reduced inflammatory manifestations and prevented excessive tissue dehydration, which are critical factors for effective repair of skin injuries in livestock.

Controlled release of 6-aminopurine ensured prolonged local action of the biologically active compound, leading to stimulation of fibroblast proliferation, accelerated granulation tissue formation and earlier epithelialization. Compared with untreated wounds, the treated lesions showed faster contraction, improved collagen organization and reduced healing time. Lower microbial contamination also confirmed the protective barrier function of the hydrogel dressing.

Thus, immobilization of 6-aminopurine in a hydrogel carrier significantly enhances reparative processes without the need for intensive antibiotic therapy. The obtained results indicate that the proposed preparation is a promising regenerative veterinary agent for treatment of traumatic and inflammatory skin lesions in farm animals and may be recommended for further clinical implementation and optimization of formulation composition.

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