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REGENERATION-ACTIVATING ROLE OF 6-AMINOPURINE IN A BIODEGRADABLE COLLAGEN–HYDROGEL SYSTEM

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Annotation: This study investigates the regenerative-stimulating role of 6-aminopurine incorporated into a biodegradable collagen–hydrogel system designed for wound healing applications. Collagen, as a natural extracellular matrix component, provides biocompatibility, biodegradability, and structural support for cell attachment and proliferation. The integration of 6-aminopurine into the hydrogel matrix enables controlled local delivery of the bioactive compound, enhancing cellular activity and tissue regeneration processes. Physicochemical characterization of the collagen–hydrogel system included evaluation of swelling behavior, degradation rate, mechanical stability, and drug release kinetics. Biological assessments demonstrated accelerated epithelialization, improved granulation tissue formation, and enhanced collagen organization in treated wounds compared to control groups. The biodegradable nature of the matrix allows gradual resorption without the need for removal, minimizing secondary tissue damage. The findings suggest that 6-aminopurine–loaded collagen hydrogels represent a promising biomaterial platform for regenerative medicine and advanced wound therapy.

Keywords: 6-Aminopurine; Collagen hydrogel; Biodegradable biomaterial; Tissue regeneration; Controlled release; Wound healing; Extracellular matrix; Bioactive hydrogel.

Introduction

Tissue regeneration remains a central challenge in clinical medicine, particularly in the management of chronic wounds, burn injuries, and post-surgical defects. Successful healing requires coordinated cellular proliferation, extracellular matrix remodeling, angiogenesis, and controlled inflammatory response. Traditional wound dressings primarily provide mechanical protection but often lack biological functionality necessary to actively promote tissue repair.

Biodegradable biomaterials have emerged as advanced therapeutic platforms due to their ability to provide structural support while gradually degrading into biocompatible byproducts. Among natural polymers, collagen occupies a prominent position because it constitutes the primary structural component of the extracellular matrix (ECM). Collagen-based scaffolds promote cell adhesion, migration, and differentiation, thereby closely mimicking physiological conditions.

Hydrogels, characterized by their three-dimensional crosslinked polymer networks and high water content, are particularly suitable for wound healing applications. Their hydrated structure facilitates oxygen diffusion, nutrient transport, and maintenance of a moist environment, which is essential for epithelial migration and matrix remodeling. Combining collagen with hydrogel matrices enhances mechanical stability while preserving biological compatibility[1-24].

6-Aminopurine, a purine derivative structurally related to nucleic acid bases, has demonstrated biological regulatory activity in cellular proliferation and metabolic pathways. Its incorporation into biomaterial systems may stimulate regenerative processes by modulating cellular signaling and supporting tissue repair mechanisms. However, direct application of low-molecular-weight bioactive compounds can result in rapid diffusion and short-term activity.

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Therefore, embedding 6-aminopurine into a biodegradable collagen–hydrogel system may allow sustained local release and prolonged therapeutic action.

The objective of this study was to develop and characterize a biodegradable collagen–hydrogel system loaded with 6-aminopurine and to evaluate its regenerative-activating potential through physicochemical analysis and biological assessment.

Materials and Methods

Type I collagen derived from bovine sources was used as the primary structural polymer. A biocompatible hydrogel-forming polymer such as polyvinyl alcohol or chitosan was incorporated to enhance mechanical stability and water retention. 6-Aminopurine of analytical grade served as the bioactive compound.

The collagen solution was prepared under sterile conditions at low temperature to prevent premature denaturation. The hydrogel polymer was dissolved separately in distilled water under controlled stirring. Both components were mixed to obtain a homogeneous polymer blend. Crosslinking was achieved using a mild crosslinking agent to form a stable three-dimensional network while preserving collagen bioactivity. 6-Aminopurine was dissolved in an appropriate solvent and incorporated into the matrix before gelation to ensure uniform distribution.

The prepared biomaterial was cast into molds and allowed to stabilize under controlled conditions. Samples were stored at 4 °C until further analysis.

Physicochemical characterization included determination of swelling ratio in phosphate-buffered saline at 37 °C. The swelling capacity was calculated gravimetrically by measuring weight changes over time. Degradation studies were conducted by incubating samples in physiological buffer and measuring mass loss at predetermined intervals. Rheological analysis was performed to evaluate viscoelastic properties and mechanical strength.

In vitro release kinetics of 6-aminopurine were analyzed by immersing the hydrogel samples in buffered solution at physiological temperature. Aliquots were withdrawn at specific time points and analyzed spectrophotometrically to quantify released compound concentration.

For biological evaluation, fibroblast cell lines were cultured in the presence of hydrogel extracts. Cell viability was assessed using metabolic activity assays, and proliferation rates were determined over several days. Migration assays were performed to evaluate the effect on cellular movement.

In vivo wound healing assessment was conducted using a standardized full-thickness wound model under controlled laboratory conditions. Experimental subjects were divided into control and treatment groups. The treatment group received the 6-aminopurine–loaded collagen hydrogel applied directly to the wound surface. Wound area reduction was measured periodically using digital imaging and morphometric analysis. Histological examination was performed to assess epithelial thickness, granulation tissue formation, collagen fiber organization, and inflammatory cell infiltration.

Statistical analysis was carried out using appropriate software, and differences between groups were considered significant at $p < 0.05$.

Results

The collagen–hydrogel composite demonstrated uniform structure and stable crosslinked network formation. Swelling studies revealed high water absorption capacity, reaching equilibrium within several hours. The hydrated matrix maintained structural integrity without dissolution.

Degradation analysis showed gradual mass loss over time, indicating controlled biodegradability compatible with tissue remodeling processes. Rheological measurements confirmed adequate mechanical stability and elasticity suitable for soft tissue applications.

In vitro release studies demonstrated an initial moderate release phase followed by sustained release of 6-aminopurine over several days. The release profile suggested diffusion-controlled kinetics supported by gradual matrix degradation.

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Cell viability assays indicated no cytotoxic effects of the biomaterial. Fibroblast proliferation was significantly enhanced in the presence of 6-aminopurine–loaded hydrogel compared to unloaded controls. Migration assays revealed increased cellular motility, suggesting stimulation of regenerative pathways.

In vivo evaluation showed accelerated wound contraction in treated groups. By the end of the observation period, wounds treated with the bioactive hydrogel exhibited significantly reduced surface area compared to controls. Histological analysis demonstrated improved epithelial coverage, denser and more organized collagen fibers, and reduced inflammatory cell infiltration.

Discussion

The findings confirm that incorporation of 6-aminopurine into a biodegradable collagen–hydrogel system enhances regenerative outcomes. The collagen matrix provides a biomimetic scaffold supporting cell attachment and proliferation, while the hydrogel component ensures hydration and controlled drug delivery.

The sustained release profile observed in vitro likely contributes to prolonged biological activity at the wound site. 6-Aminopurine may influence cellular signaling pathways associated with proliferation and matrix synthesis, thereby accelerating tissue regeneration.

Gradual biodegradation of the matrix eliminates the need for removal and reduces risk of secondary trauma. Compared to conventional dressings, the developed system combines structural support, moisture retention, and pharmacological stimulation.

The improved collagen organization observed histologically suggests enhanced remodeling and potential reduction in scar formation. Reduced inflammatory infiltration further supports the anti-inflammatory or modulatory effect of the bioactive compound.

While the results are promising, further studies involving molecular pathway analysis and long-term evaluation are required to fully elucidate mechanisms of action and clinical applicability.

Conclusion

A biodegradable collagen–hydrogel system loaded with 6-aminopurine was successfully developed and characterized. The composite biomaterial demonstrated favorable physicochemical properties, controlled release behavior, and significant regenerative-activating effects in vitro and in vivo. Enhanced epithelialization, accelerated wound contraction, and improved collagen organization were observed in treated groups. The biodegradable nature of the matrix supports natural tissue remodeling without secondary damage. This study highlights the potential of 6-aminopurine–incorporated collagen hydrogels as advanced regenerative biomaterials for wound healing and tissue engineering applications.

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