RESEARCH PAPER

In Vitro release and Anti- inflammatory potential of a Troxerutin-Loaded Chitosan–Xanthan Gum Scaffold for Cartilage Regeneration

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ABSTRACT

Cartilage regeneration remains a major challenge because articular cartilage has limited intrinsic healing capacity owing to its avascular nature. The present study aimed to develop and evaluate a troxerutin-loaded chitosan–xanthan gum (CDX) scaffold as a porous biomaterial for sustained drug delivery and potential cartilage tissue engineering applications. The scaffold was prepared using natural biopolymers and characterized through in vitro drug release, release kinetics, antioxidant, and anti-inflammatory studies. The optimized formulation exhibited an interconnected porous structure and sustained troxerutin release for up to 96 hours, with a cumulative drug release of 96 ± 3.56%. Release kinetics showed the best fit to the Higuchi model, while the Korsmeyer–Peppas indicated anomalous diffusion involving both drug diffusion and polymer relaxation. The scaffold retained significant antioxidant activity in the FRAP and nitric oxide scavenging assays and demonstrated measurable anti-inflammatory activity in the protein denaturation assay. These findings suggest that the troxerutin-loaded chitosan–xanthan gum scaffold is a promising biomaterial platform for localized drug delivery and warrants further in vitro cellular and in vivo investigation for cartilage regeneration.

Keywords: Troxerutin; Chitosan; Xanthan gum; Scaffold; Cartilage regeneration; Sustained drug release; Tissue engineering; Antioxidant; Anti-inflammatory

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1. INTRODUCTION

Articular cartilage possesses limited intrinsic healing capacity because it is avascular, alymphatic, and lacks neural tissue. Cartilage degeneration in osteoarthritis is associated with oxidative stress, inflammatory cytokines, and extracellular matrix degradation [1] . Biomaterial scaffolds based on natural polymers are increasingly investigated to provide structural support, controlled drug delivery, and a favorable microenvironment for chondrocyte growth [4–9].

Chitosan is a biodegradable polysaccharide with excellent biocompatibility, swelling ability, antimicrobial activity, and structural similarity to glycosaminoglycans [3-4,8]. Xanthan gum forms a polyelectrolyte complex with chitosan, improving hydration, mechanical integrity, and drug encapsulation [2,9,14]. encapsulation. Troxerutin is a flavonoid derivative with antioxidant and anti-inflammatory properties that may protect cartilage from reactive oxygen species and inflammatory mediators [11,17,20].

The present study investigates a troxerutin-loaded chitosan–xanthan gum scaffold evaluates its drug release behavior, antioxidant activity, and anti-inflammatory potential as a candidate biomaterial for cartilage regeneration.

Chitosan is a biocompatible, hydrophilic polysaccharide with film-forming and tissue-engineering properties, whereas xanthan gum is an anionic polysaccharide that can contribute to hydration, structural organization and matrix stability. Their combination can generate a polyelectrolyte complex through interactions between protonated amino groups of chitosan and carboxyl groups of xanthan gum. Such interactions can influence scaffold porosity, swelling, drug retention and structural integrity [5–7].

Troxerutin is a flavonoid glycoside with antioxidant and anti-inflammatory properties. These characteristics make it of interest for biomaterial systems intended for inflammatory and degenerative tissue conditions [11]. However, incorporation of a bioactive compound into a polymeric scaffold is important for maintaining local exposure and controlling its release. A chitosan–xanthan gum matrix may provide a hydrated, porous environment for prolonged drug delivery. The present study was undertaken to evaluate for in vitro drug release, release kinetics, antioxidant activity and anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1 In Vitro Drug Release and Kinetic Analysis

The in vitro release study was performed using the dialysis membrane diffusion method. A known amount of drug-loaded scaffold was placed inside a pre-soaked

dialysis membrane and immersed in release medium maintained at 37 ± 0.5°C under continuous stirring. Aliquots were withdrawn at predetermined intervals and replaced with fresh medium. Samples were analyzed at 355 nm and cumulative percentage release was calculated. Release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models, and the model with the highest R^2 was considered the best fit.

2.2 Antioxidant Activity

Antioxidant activity was evaluated by the FRAP assay using freshly prepared acetate buffer, TPTZ and ferric chloride reagent. Samples were incubated with FRAP reagent at 37°C for 30 min in the dark, and absorbance was measured at 593 nm. Ascorbic acid was used as the reference standard. Nitric oxide scavenging activity was additionally evaluated using sodium nitroprusside and Griess reagent, with absorbance measured at 546 nm.

2.3 Anti-Inflammatory Activity

Anti-inflammatory activity was assessed using the egg-albumin protein-denaturation method. Formulation samples and egg albumin in phosphate-buffered saline were incubated at 37°C and subsequently heated to induce protein denaturation. Absorbance was measured at 660 nm. Ibuprofen was used as the standard.

2.4 Statistical Analysis

The source scaffold document reports comparative formulation observations and fitted release models. No complete numerical ANOVA dataset or p-values were provided in the scaffold results; therefore, no additional statistical significance values have been introduced in this manuscript.

3. RESULTS AND DISCUSSION

3.1 In Vitro Drug Release

Troxerutin exhibited sustained release over 96 h, with cumulative release reaching 96 ± 3.56% at the end of the study. The early release phase was attributed primarily to diffusion of drug located near the scaffold surface, whereas the subsequent slower phase was associated with diffusion through the hydrated polymer matrix and gradual dissolution or erosion of the scaffold. The prolonged release profile indicates that the chitosan–xanthan gum matrix can modulate troxerutin release.

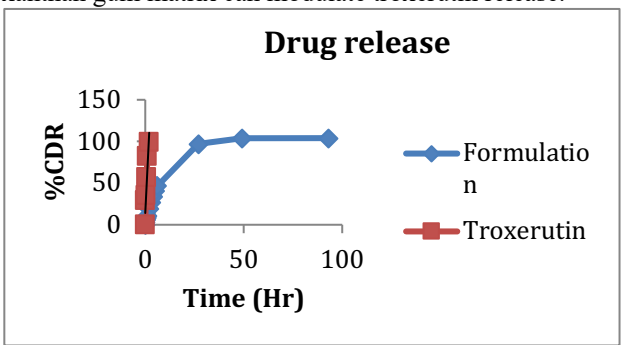


Figure 1. In vitro cumulative drug-release profile of troxerutin from the optimized CDX scaffold over 96 h.

3.2 Drug Release Kinetics

The release data were best fitted by the Higuchi model, indicating a predominant diffusion-controlled contribution to drug release [1,17]. The Korsmeyer–Peppas model gave an n value of 0.69, supporting anomalous transport rather than simple Fickian diffusion. The source results indicate that both penetration of the dissolution medium and polymer-chain relaxation/swelling contributed to release from the scaffold.

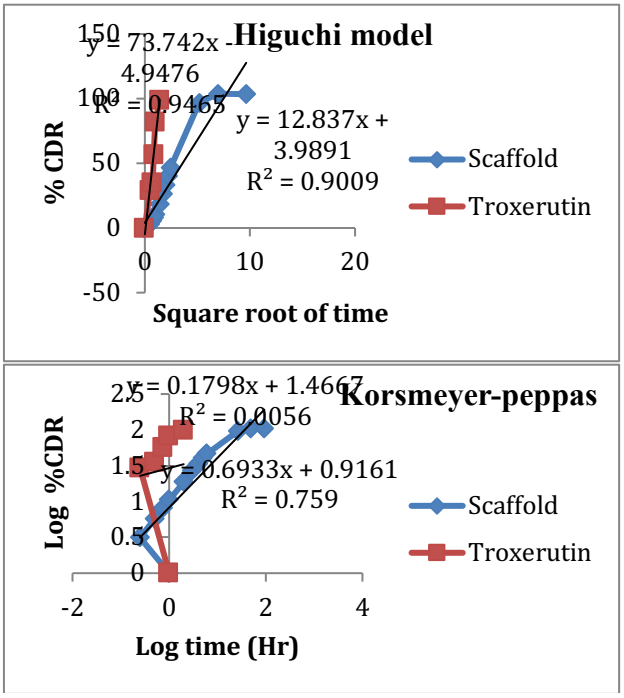


Figure 2. Drug-release kinetic analysis of the optimized CDX scaffold using the (A) Higuchi and (B) Korsmeyer–Peppas models.

3.3 Antioxidant Activity

The optimized scaffold retained antioxidant activity after fabrication. In the FRAP assay, its antioxidant capacity was reported as equivalent to 27.4 µg/mL ascorbic acid. The source report attributed this activity primarily to troxerutin, whose hydroxyl-rich flavonoid structure can contribute to electron donation and reactive oxygen species scavenging. The preservation of activity after scaffold fabrication indicates that the processing conditions did not eliminate the measured antioxidant property.

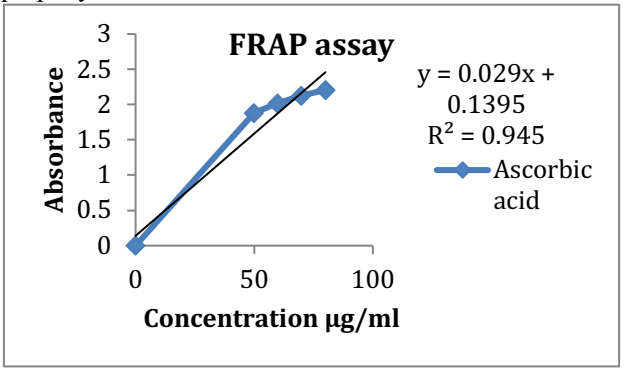
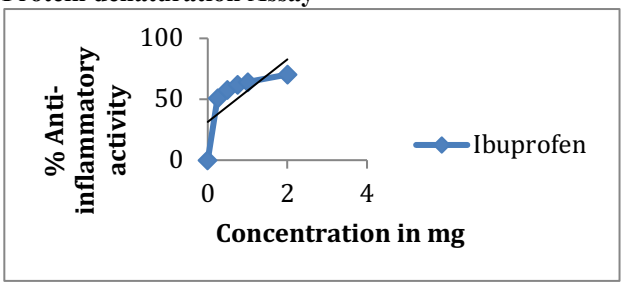


Figure 3. FRAP assay calibration plot using ascorbic acid as the reference standard.

3.4 Anti-Inflammatory Activity

Protein-denaturation testing showed an IC50 of 0.627 mg for ibuprofen and 2.7 mg for the optimized formulation. The formulation therefore exhibited measurable inhibition of protein denaturation, supporting retention of anti-inflammatory activity. The source report proposed that sustained availability of troxerutin from the matrix may contribute to the observed activity.

Protein denaturation Assay



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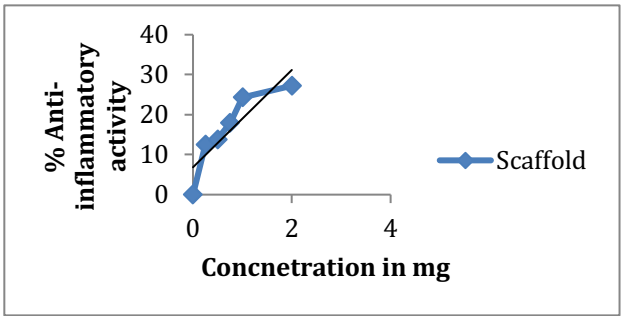

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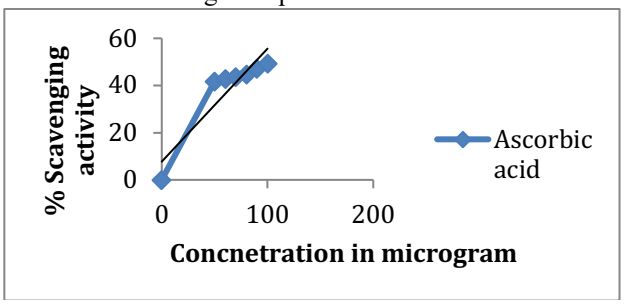
Figure 4 . Protein-denaturation assay showing anti-inflammatory activity of (A) ibuprofen and (B) the optimized CDX scaffold.

3.5 Nitric Oxide Scavenging Activity

The optimized formulation showed an IC50 of 14.74 mg in the nitric oxide scavenging assay, whereas ascorbic acid showed an IC50 of 100.36 µg. Although the formulation was less potent than the reference standard, measurable free-radical scavenging activity was retained. This result, together with the FRAP and protein-denaturation findings, supports the biological activity of troxerutin following incorporation into the scaffold.



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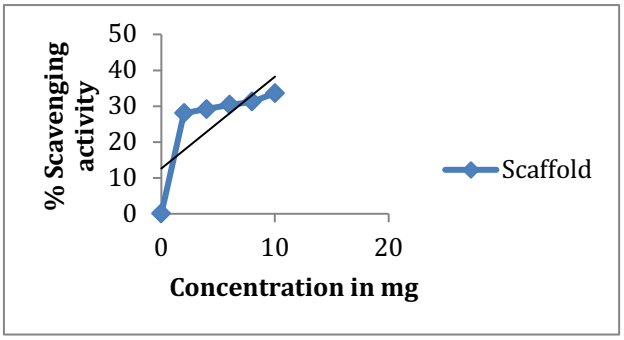

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Figure 5. Nitric oxide scavenging activity of (A) ascorbic acid and (B) the optimized CDX scaffold.

The sustained 96-hour release profile and Higuchi kinetic model indicate diffusion-controlled drug release, whereas the Korsmeyer–Peppas exponent suggests anomalous transport involving diffusion and polymer relaxation. Controlled release is beneficial for maintaining prolonged local exposure of troxerutin during the inflammatory phase of cartilage repair [10,16,17].

The retained antioxidant and anti-inflammatory activities demonstrate preservation of troxerutin bioactivity after scaffold fabrication. These biological properties are relevant because oxidative stress and inflammatory signaling contribute to cartilage degeneration. Although the present findings are limited to in vitro characterization, they justify further evaluation in chondrocyte culture, mesenchymal stem-cell models, mechanical testing, and animal studies [9,12 -15].

The high encapsulation efficiency and drug loading indicate effective incorporation of troxerutin within the chitosan–xanthan gum network . This may be favorable for maintaining the drug in a dispersed state within the polymeric matrix, although improved solubility or bioavailability should be confirmed experimentally rather than inferred solely from solid-state characterization.

The 96-h release profile and Higuchi model fit indicate that the scaffold can provide prolonged release rather than immediate drug discharge. The Korsmeyer–Peppas n value of 0.69 further indicates that diffusion occurs together with polymer relaxation and swelling. Such a release mechanism may be useful for maintaining local exposure to a bioactive compound during the early stages of tissue repair.

Troxerutin-related antioxidant and anti-inflammatory activities provide an additional biological rationale for the scaffold. Oxidative stress and inflammatory signaling are important contributors to cartilage degeneration, and the preservation of measurable antioxidant and anti-inflammatory activity after scaffold fabrication is therefore relevant to the proposed application [10-11,18,20]. However, the present data are in vitro physicochemical and biochemical findings and should not be interpreted as direct evidence of cartilage regeneration or therapeutic efficacy in vivo.

Taken together, the CDX scaffold showed a favorable combination of porous morphology, hydration capacity, drug incorporation, controlled release and retained biological activity. These characteristics justify further studies involving mechanical strength, cytocompatibility, chondrocyte or mesenchymal stem-cell responses, extracellular matrix deposition and appropriate animal models before any translational claims are made [19].

4. CONCLUSION

A troxerutin-loaded chitosan–xanthan gum scaffold was successfully developed and characterized in the previous publication . Troxerutin release was sustained for 96 h and was best described by the Higuchi model, with a Korsmeyer–Peppas exponent of 0.69 indicating anomalous transport. Antioxidant and anti-inflammatory activities were retained following fabrication. Overall, the optimized CDX scaffold represents a promising experimental biomaterial platform for troxerutin delivery and warrants further in vitro cellular and in vivo studies focused specifically on cartilage regeneration and safety.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

6. ACKNOWLEDGEMENT

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