Journal of Microbiology, Immunology and Infection 58 (2025) 486–489

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

journal homepage: www.e-jmii.com

Unanticipated cytotoxicity of fusidic acid in antibiotic-loaded hydrogel

Jing-Gu Jiang [a,1], Yu-Chun Chen [b,1], Ni-En Jiang [c], Chun-Hsing Liao [d,e,*], Chih-Hung Chang [e,f,**]

[a] Division of Hematology and Oncology, Department of Medicine, Far Eastern Memorial Hospital, New Taipei City, Taiwan
[b] Department of Chemical Engineering, National United University, Miaoli County, Taiwan
[c] Department of Orthopaedic Surgery, Far Eastern Memorial Hospital, New Taipei City, Taiwan
[d] Department of Medicine, Far Eastern Memorial Hospital, New Taipei City, Taiwan
[e] School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan
[f] Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Taoyuan City, Taiwan

ARTICLE INFO

Keywords:
Antibiotics
Fusidic acid
Cytotoxicity
Cell viability
Cell apoptosis

ABSTRACT

This study evaluated the cytotoxicity of fusidic acid-loaded HA hydrogels on different cell types. Results show significant cytotoxicity, especially at concentrations above 0.1 %, with MC3T3 cells being the most sensitive. These findings highlight the importance of considering cell-specific responses and cytotoxic effects when designing antibiotic-loaded hydrogels for biomedical applications.

1. Introduction

Aseptic techniques are used in surgeries, but 2–4% of patients still develop surgical site infections (SSI), a major cause of morbidity, mortality, and imposing an additional economic burden.[1] *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), is the primary pathogen, with vancomycin resistance posing challenges.[2] Fusidic acid is an alternative treatment with fewer complications.

In situ drug delivery systems with antibiotics, such as antibiotic-loaded hydrogels, offer promising antibacterial effects and enhance patient protection during surgery. Our previous study showed that an oxidized hyaluronic acid/adipic acid dihydrazide hydrogel loaded with vancomycin is easy to prepare, biocompatible, and effective for local antibiotic delivery.[3] With the emergence of MRSA strains exhibiting reduced responsiveness to common antibiotics, this study aimed to explore the feasibility of incorporating fusidic acid as an anti-MRSA agent into the hydrogel model.

2. Methods

2.1. Preparation of antibiotic-loaded hydrogel

We developed an antibiotic-laden hydrogel by combining adipic acid dihydrazide (ADH), oxidized hyaluronic acid (oxi-HA), and fusidic acid. Briefly, hyaluronic acid (HA) (1 % w/v) was dissolved in double-distilled water and oxidized with sodium periodate (2.67 % w/v) for 24 h in the dark. The reaction was terminated with ethylene glycol, and oxi-HA was purified by dialysis and freeze-dried to yield the final product.

To fabricate the antibiotic-loaded hydrogels, oxi-HA (6 %) and ADH (8 %) were separately dissolved in normal saline containing fusidic acid at concentrations of 0.01 %, 0.1 %, 0.5 %, and 1 %. These solutions were gently mixed to produce HA Hydrogel-F001, HA Hydrogel-F01, HA Hydrogel-F05, and HA Hydrogel-F1, respectively.

2.2. Cell viability evaluation by WST-8 assay

For cell viability assays, hydrogels (0.1 mL) were incubated with 1

* Corresponding author. Department of Medicine, Far Eastern Memorial Hospital, No. 21, Section 2, Nanya S. Road, Banqiao District, New Taipei City, 22060, Taiwan.
** Corresponding author. Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Department of Orthopedic Surgery, Far Eastern Memorial Hospital, No. 21, Section 2, Nanya S. Road, Banqiao District, New Taipei City, 22060, Taiwan.
E-mail addresses: liaochunhsing@gmail.com, liaochunhsing@nycu.edu.tw (C.-H. Liao), orthocch.home@gmail.com (C.-H. Chang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jmii.2025.02.004
Received 24 January 2024; Received in revised form 13 January 2025; Accepted 22 February 2025
Available online 28 February 2025

mL of standard medium at 37 °C for 72 h to prepare extraction media. Then, 200 μL of the extraction medium was added to MC3T3 osteoblasts, L929 fibroblasts, and porcine mesenchymal stem cells (MSCs) in 96-well plates and cultured for 1 and 3 days at 37 °C under 5 % CO_2.

Control groups included standard medium (positive control), 0.1 % Triton X (negative control), and hydrogel extraction medium without fusidic acid (HA hydrogel group). Cell viability was assessed using WST-8 assays, where mitochondrial activity reduced tetrazolium salt to formazan, measured at 450 nm.

2.3. Cell apoptosis analysis by flow cytometry

Apoptosis was assessed using flow cytometry with the Annexin V Apoptosis Kit-FITC (Novus Biologicals, Minneapolis, MN, USA). Cells (3×10^4 per well) were seeded in 24-well plates and treated with hydrogel extraction medium. After treatment, cells were washed with PBS, detached with trypsin, and incubated with Annexin V dye for 30 min at room temperature, followed by PI staining for another 30 min. Stained cells were analyzed using a CytoFlex flow cytometer (Beckman Coulter, Brea, CA, USA).

2.4. Statistical analysis

All experiments were conducted at least in triplicate, and the mean ± standard deviation was calculated. Analysis of variance (ANOVA) was performed to assess the impact of antibiotic-loaded hydrogels on cell viability. Flow cytometry data were collected and analyzed using CytExpert software (Beckman Coulter, Brea, CA, USA).

3. Results

3.1. Cell toxicity of fusidic acid - loaded hydrogels

The cell compatibility of the fusidic acid-loaded hydrogel was evaluated by assessing the viability of MSCs, MC3T3, and L929 cells using the WST-8 assay. Interestingly, a slight increase (15 % for MSCs) or decrease (~10 % for L929) in cell viability was observed compared to the positive control group. These results suggest that the slight variations in cell viability may result from differences in cellular responses to the HA Hydrogel. However, given the relatively small percentage changes, the overall impact on cell viability appears minimal, further supporting the HA Hydrogel's non-cytotoxic nature, as illustrated in Fig. 1(A).

In addition, the cell compatibility of the antibiotic-loaded hydrogel containing varying concentrations of fusidic acid was assessed. A significant decline in absorbance values was observed for all three cell types on days 1 and 3. Notably, MC3T3 cells were the most sensitive to fusidic acid, with cell viability decreasing to approximately 70 % when the concentration exceeded 0.01 % (HA Hydrogel-F001 group). For L929 cells, growth inhibition was observed at a fusidic acid concentration of 0.1 % (HA Hydrogel-F01 group), with cell viability dropping to 52 % after 24 h of incubation. In contrast, MSCs exhibited higher

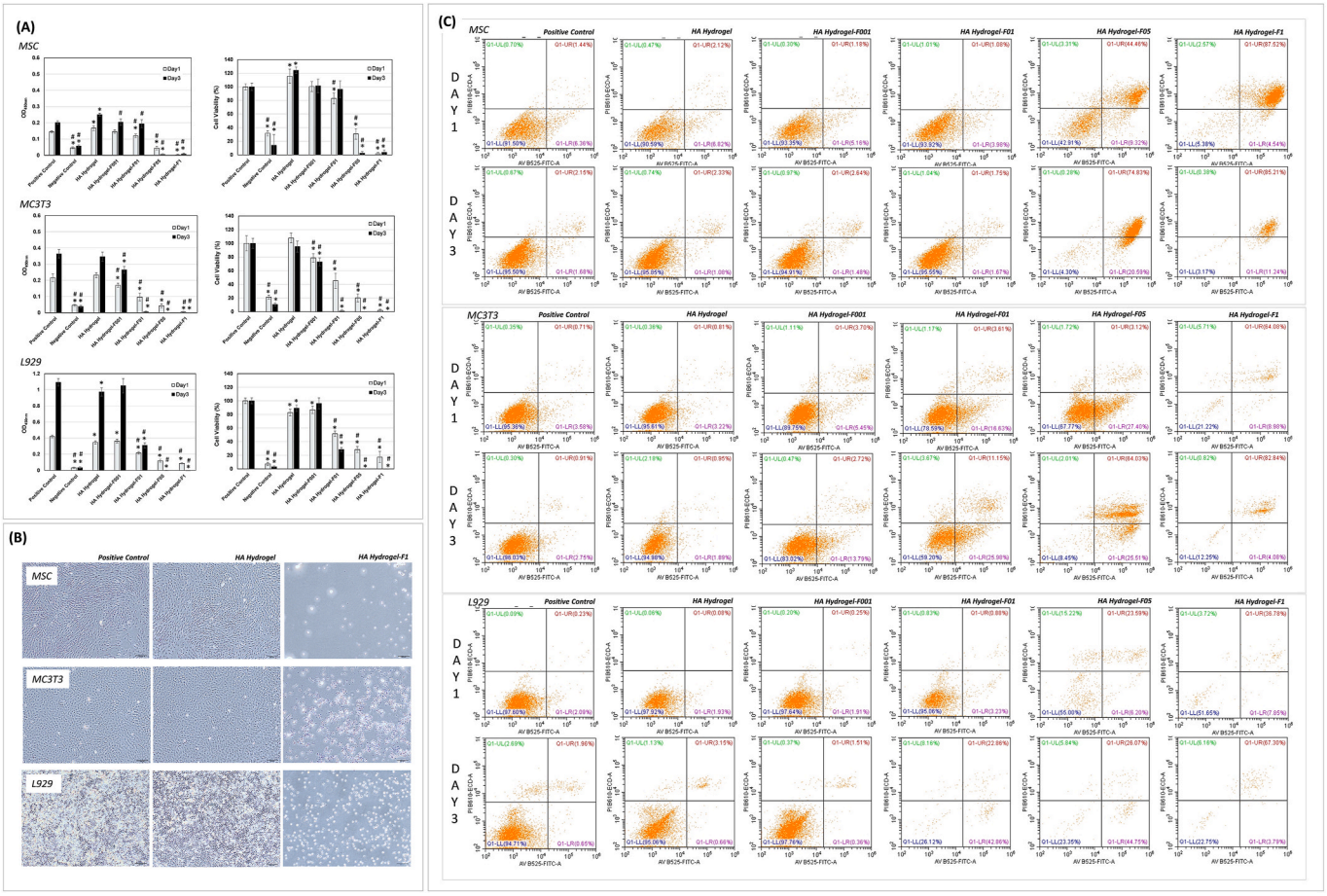

Fig. 1. (A) Cell viability of porcine bone marrow mesenchymal stem cells (MSC), mouse osteoblasts (MC3T3), and mouse fibroblasts (L929) cultured with HA hydrogels containing varying concentrations of fusidic acid on day 1 and 3. (B) Morphology of MSC, MC3T3, and L929 cells cultured in standard medium, HA hydrogel, and HA Hydrogel-F1 extraction medium on day 3, highlighting changes in cell attachment and shape. (C) Annexin V/Propidium iodide flow cytometry analysis showing apoptosis and necrosis in MSC, MC3T3, and L929 cells after exposure to HA hydrogels with different concentrations of fusidic acid on days 1 and 3. * indicates $p < 0.05$ compared with the positive control; # indicates $p < 0.05$ compared with the HA hydrogel group.

tolerance to fusidic acid, maintaining approximately 80 % viability at a concentration of 0.1 % (HA Hydrogel-F01 group). However, at 0.5 % fusidic acid (HA Hydrogel-F05 group), MSC viability significantly decreased to 30 % after 24 h. These findings suggest that the cytotoxic effects of fusidic acid are dose-dependent and vary significantly among cell types, with MC3T3 and L929 cells being more susceptible compared to MSCs.

3.2. Possible mechanism of fusidic acid cytotoxicity

The cell viability tests revealed that fusidic acid exhibited local toxicity even at ultra-low concentrations (0.01 %). MSCs showed a slight decrease in cell viability at this concentration, while MC3T3 displayed noticeable growth inhibition. Significant cell death was observed at 0.5 % fusidic acid (HA Hydrogel-F05 group) on days 1 and 3. For both MC3T3 and L929 cells, viability sharply declined in (HA Hydrogel-F01 group (0.1 % fusidic acid), and no viable cells were observed in the HA Hydrogel-F1 group (1 % fusidic acid), as shown in Fig. 1(B).

Flow cytometry was used to analyze cell apoptosis and necrosis using Annexin V and PI staining. Annexin V binds to phosphatidylserine exposed during early apoptosis, while PI detects late apoptosis or necrosis. Results revealed increased Annexin V and PI signals in all three cell types on days 1 and 3, indicating elevated apoptotic and necrotic populations (Fig. 1(C)). Apoptotic cells were prevalent within the first 24 h, while necrotic cells became dominant after 72 h.

In MSCs, exposure to HA Hydrogel-F05 and Hydrogel-F1 extracts resulted in 9.3 % apoptotic and 44.5 % necrotic cells after 24 h, increasing to 20.6 % and 74.8 %, respectively, by day 3. For MC3T3 cells, the Hydrogel-F01 extract induced 16.6 % apoptosis at 24 h, rising to 26.0 % by day 3, while the Hydrogel-F05 extract induced apoptosis rates of 27.4 % at 24 h and 25.5 % by day 3. Necrosis was most pronounced with the F1 extract, reaching 82.8 % by day 3.

L929 fibroblasts showed 42.9 % and 44.8 % apoptotic cells with the F01 and F05 extracts, respectively, after 3 days. Necrosis was significant with the F1 extract, showing 64.1 % at 24 h and increasing to 67.3 % by day 3.

These results demonstrate that HA antibiotic-loaded hydrogel extracts exert cell type- and concentration-dependent cytotoxic effects. MC3T3 and L929 exhibited higher apoptosis with the HA Hydrogel-F01 and HA Hydrogel-F05 extracts. These findings underscore the need to account for cell-specific responses when designing hydrogels for biomedical applications, ensuring both efficacy and safety.

4. Discussion

This is the first study to demonstrate the cytotoxicity of fusidic acid in HA hydrogels, showing significant toxicity even at low concentrations like 0.1 %. Antibiotics are often used before surgery to prevent infections, and *in situ*-forming antibiotic-loaded hydrogels offer a novel way to reduce bacterial adherence and biofilm formation on implants. Fusidic acid, introduced in the 1960s, is a potent antibacterial agent against gram-positive bacteria, available in topical and systemic forms.[4] Studies have shown it effectively inhibits bacterial growth when incorporated into PMMA and chitosan-based constructs for wound treatment.[5,6] Systemic use is generally well-tolerated with minimal adverse effects. Fusidic acid works by disrupting bacterial translation through its binding to elongation factor G (EF-G). In our study, fusidic acid-loaded HA hydrogels caused significant reductions in cell viability, particularly at concentrations of 0.5 % and 1 %.

A previous study reported that oral administration of 500 mg fusidic acid twice daily resulted in a serum concentration of about 100 mg/L within 2–3 h, corresponding to 0.01 % fusidic acid.[7] This concentration matches that used in the HA Hydrogel-F001 group, suggesting relevance between systemic and local concentrations. The observed slight toxicity at 0.01 % in the hydrogel supports that low concentrations can affect cellular viability. Notable adverse reactions have been reported at systemic concentrations exceeding 1500 mg, but the difference between systemic and local delivery concentrations remains unclear.[8]

Fusidic acid-induced cytotoxicity may result from mutations in EF-G, disrupting cellular growth and size regulation.[9] Its antitumor activity is linked to inhibiting EF-G, affecting protein synthesis in bacteria and mitochondria.[10] Flow cytometry indicated that the toxicity is due to apoptosis, not necrosis, highlighting the need to consider broader effects when using fusidic acid locally around implants.

Several factors influence antibiotic release in local drug delivery systems. In our study, fusidic acid consistently showed cytotoxicity across three cell types. Clinically, its use in surgical sites to prevent SSIs should be carefully considered due to significant cytotoxicity, with further research needed for specific settings like open fractures.

Our study has limitations, such as relying solely on *in vitro* results, so *in vivo* studies and evaluations of the hydrogel's long-term stability are necessary for a more comprehensive understanding.

In conclusion, this study revealed dose-dependent cytotoxicity of fusidic acid-loaded HA hydrogels across various cell types, with MC3T3 cells being the most sensitive. Fusidic acid at 0.1 % concentration showed slight toxicity, and higher concentrations (0.5 % and 1 %) led to significant cell death. These results suggest that local application of fusidic acid-loaded hydrogels in clinical settings requires careful consideration of potential cytotoxic effects.

CRediT authorship contribution statement

Jing-Gu Jiang: Data curation, Writing – original draft. **Yu-Chun Chen:** Conceptualization, Data curation, Project administration, Visualization. **Ni-En Jiang:** Data curation, Formal analysis. **Chun-Hsing Liao:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Chih-Hung Chang:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision.

Funding

We would like to thank the National Science and Technology Council, Taiwan (NSCT -105-2221-E−418-001, NSCT -106-2221-E−418-001, NSCT-113-2221-E−239-003, NSCT-112-2221-E−239 -016), Far Eastern Memorial Hospital, Taiwan (FEMH-2016-C-005, 105FN14), Taichung Veterans General Hospital/National United University Joint Research Program (TCVGH-NUU1118901, TCVGH-NUU1128903) and AOTRAUMA (AOTAP16-08) for financial support. These funding sources had no role in the study design or conduct, or in the decision to submit it for publication.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Acknowledgments

We thank for Far Eastern Memorial Hospital Core Laboratories I & II for providing facilities and instruments.

References

1. de Lissovoy G, Fraeman K, Hutchins V, et al. Surgical site infection: incidence and impact on hospital utilization and treatment costs. *Am J Infect Control*. 2009;37:387–397.
2. Brown NM, Goodman AL, Horner C, et al. Treatment of methicillin-resistant Staphylococcus aureus (MRSA): updated guidelines from the UK. *JAC Antimicrob Resist*. 2021;3, dlaa114.
3. Liao CH, Chen CS, Chen YC, et al. Vancomycin-loaded oxidized hyaluronic acid and adipic acid dihydrazide hydrogel: bio-compatibility, drug release, antimicrobial activity, and biofilm model. *J Microbiol Immunol Infect*. 2020;53:525–531.
4. Acid Fusidic. In: *Drugs and Lactation Database (LactMed®)*. Bethesda (MD): National Institute of Child Health and Human Development; 2006.

5. Buranapanitkit B, Srinilta V, Ingviga N, et al. The efficacy of a hydroxyapatite composite as a biodegradable antibiotic delivery system. *Clin Orthop Relat Res*. 2004: 244–252.
6. Thakur K, Sharma G, Singh B, et al. Chitosan-tailored lipidic nanoconstructs of Fusidic acid as promising vehicle for wound infections: an explorative study. *Int J Biol Macromol*. 2018;115:1012–1025.
7. Vaillant L, Machet L, Taburet AM, et al. Levels of fusidic acid in skin blister fluid and serum after repeated administration of two dosages (250 and 500 mg). *Br J Dermatol*. 1992;126:591–595.
8. Christiansen K. Fusidic acid adverse drug reactions. *Int J Antimicrob Agents*. 1999;12 (Suppl 2):S3–S9.
9. Macvanin M, Johanson U, Ehrenberg M, et al. Fusidic acid-resistant EF-G perturbs the accumulation of ppGpp. *Mol Microbiol*. 2000;37:98–107.
10. Nyfeler B, Hoepfner D, Palestrant D, et al. Identification of elongation factor G as the conserved cellular target of argyrin B. *PLoS One*. 2012;7, e42657.