

# Development of a Novel Enema Smart Hydrogel for Ulcerative Colitis (UC) Treatment

Pei-Xhan Wu<sup>1</sup>, Xiu-Fen Xia<sup>2</sup>, Min-Shin Ou<sup>1</sup>, Yu-Chi Shu<sup>1</sup>, and Shu-Ling Huang<sup>1,2,3\*</sup>

<sup>1</sup> International Master Program of Translation Medicine, College of Engineering and Science, National United University, Miaoli, Taiwan

<sup>2</sup> Department of Chemical Engineering, National United University, Miaoli, Taiwan

<sup>3</sup> Ph.D. Program in Materials and Chemical Engineering, National United University, Miaoli, Taiwan  
Email: fifth.simone@nu.edu.tw (S.-L.H.)

\*Corresponding author

**Abstract** Inflammatory Bowel Diseases (IBD), including Ulcerative Colitis (UC) and Crohn's Disease (CD), are chronic autoimmune diseases with recurrent patterns that are not fully understood. In this study, a novel MC/HA-Me enema smart hydrogel was developed for treating UC. The HA-Me compound was synthesized through nucleophilic addition-elimination reactions at the acyl carbon (C=O) of Hyaluronic Acid (HA) with the amine groups (-NH<sub>2</sub>) of Mesalamine (Me). The structure of the HA-Me compound was identified using FTIR and <sup>1</sup>H-NMR spectroscopic techniques. The control of adhesion time to the intestinal wall was achieved by adjusting the ratio of MC and HA-Me compounds. An anti-inflammatory evaluation was conducted using Nitric Oxide (NO) assays with RAW 264.7 cells. The results indicated that the optimized formulation, consisting of 1 wt% NaCl, 6.5 wt% MC, and 4.5 wt% HA-Me, resulted in a gelling time of 238 seconds and a flow distance of 13.2 cm. This distance was close to the length of the human rectum (about 12 cm~15 cm). The hydrogel containing Me drug can slowly release for up to 12 hours. Considering the results of cell survival rate, the optimal concentration of MC/HA-Me was 12.5 mM. In the future, we plan to increase the synthesis amount of HA-Me and conduct in vitro organoid simulation and animal experiments to evaluate the feasibility of enema smart hydrogels for human applications.

**Keywords** hyaluronic acid, mesalamine, methylcellulose, enema smart hydrogel, Inflammatory Bowel Diseases (IBD), Ulcerative Colitis (UC), anti-inflammatory

Ulcerative Colitis (UC) and Crohn's Disease (CD), which are the chronic inflammatory intestinal diseases whose causes and pathogenesis are still not fully understood. UC is mainly complicated by the large intestine, especially the rectum, and the inflamed areas are continuously distributed. CD occurs in discontinuous distribution throughout the digestive tract, and the characteristics of the lesions make local treatment difficult. For the UC, current clinical treatment requires the patients to maintain a fixed side-lying posture. After injecting Mesalamine (Me) enema in the form of an intestinal tract, the drug is often excreted due to slight movement, and the drug rate is low.

Polymeric drug delivery systems are widely used in biomedical field. The main purpose is to control the release of drugs in local inflamed areas (such as mouth, stomach and intestines) and target specific areas for treatment. Both Hyaluronic Acid (HA) and Methylcellulose (MC) are biopolymers. HA shows many characteristics, such as biocompatible, absorbable, degradable, adhesive and charge-targeted by the human body [1, 2, 3]. The chemical structures rich in carboxyl groups (-COOH) and hydroxyl groups (-OH) can be utilized for chemical modification or covalent bonding of drugs [4]. MC mainly has thermosensitive property, which is liquid at low temperatures and gel state at high temperatures because of the hydrophobic association and junction [5].

## I. INTRODUCTION

With the advancement of medicine and technology, the medical quality has been greatly improved, so that the human lifespan can be extended. However, there are still many common diseases for which effective treatments have not yet been developed. This also brings unlimited challenges to the pharmaceutical industry, such as the Inflammatory Bowel Disease (IBD) from autoimmune disease. For some people, IBD is only a mild illness; however, for others, it is a debilitating condition that can lead to life-threatening complications. IBD includes

## II. LITERATURE REVIEW

In recent years, some literatures have investigated Covalent Organic Polymers (COPs) for drug delivery systems. Darieo et al. published an article on [pH dependent biocompatible room temperature covalent organic polymers for selective chemotherapeutic drug delivery], in 2024. This research included the synthesis of furan-based Covalent Organic Polymers (FRT-COP) and loaded anticancer drug Doxorubicin (DOX) and stabilized by Hyaluronic Acid (HA). FRT-COPDOX-HA were promised for selective chemotherapeutic drug delivery and shown the pH dependence. Utilizing the pH-sensitive and porous structure characteristics of FRT-COPDOX-HA by subcutaneous injection for each mouse cause the

hydrolytic breakdown of hydrazine bond at acidic pH. The drug can be accurately released in tumour micro environment. The results demonstrated that the polymer drug delivery system exhibits excellent biocompatibility and enhances therapeutic efficacy.

In this study, we developed a MC/HA-Me smart hydrogel with drug delivery for UC treatment to improve the shortcomings of liquid suppositories and promote the medical comfort. HAmE compound was synthesized using Hyaluronic Acid (HA) and antiinflammatory Mesalamine (Me) drug by covalent organic polymers (amide bonds). Then, HAmE compound mixed Methyl Cellulose (MC) by the hydrogen bonding forming MC/HA-Me hydrogels, which exhibited the thermosensitive properties. MC/HAmE solutions were injected into the rectal from the anus at room temperature as the rectal temperature of the human body increases. MC/HA-Me hydrogels cause the gelling because of the hydrogen bonds effect. Then, MC/HA-Me drug carrying hydrogels with the negative charges can be attracted to the positive charges of damaged site of intestinal mucosal that achieve the effect of directly releasing the drug at the affected area.

### III. EXPERIMENTAL

#### A. Synthesis of HAmE Compound

HA was dissolved in deionized water to form a 1% HA aqueous solution. HOBt (hydroxybenzotriazole) and Me were dissolved in DMSO (dimethyl sulfoxide) at a concentration of 30 mg/mL and 15 mg/mL, respectively. HA-Me product was carried out by mixing HA aqueous solution and Me/DMSO solution through overnight reaction at room temperature. Then, HAmE precursors were purified by dialysed for over 3 days, that followed by filtration and freeze-drying. The structural characteristics of HA-Me compound were analyzed using FT-IR/ATR and <sup>1</sup>H-NMR spectra.

#### B. Preparation of Hydrogel Formula

Due to the osmotic pressure of the human body, the concentration range of NaCl in the hydrogel formula must be between 1wt%~2 wt%. The fluidity of the hydrogel determines whether the drug can smoothly enter the intestine and cover the entire rectum. The concentration of HA-Me precursor affects the drug loading capacity of hydrogel. In addition, the gelling time of hydrogel is also crucial. Therefore, by adjusting the ratio ranges of MC concentration between 1 wt% to 4 wt% and 4 wt% to 7 wt% of MC can be obtained smart thermosensitive MC/HA-Me hydrogel, which can be found the most suitable gelling time and flowing distance.

#### C. Cell Viability Assay

The mouse macrophage cell line (RAW264.7) was cultured in DMEM containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin (PS) in an incubator at 37°C and 5% CO<sub>2</sub>. Assess cell viability was measured with cell

#### D. Nitric Oxide (NO) Assay

The evaluation of anti-inflammatory was carried out by Nitric Oxide (NO) assay. RAW264.7 cells (2 × 10<sup>5</sup> cells/mL) were seeded in 96-well plates and cultured for 24 hrs. Six different concentrations of melamine and LPS (lipopolysaccharide 2 g/mL) were added and cultured for 24 hrs. The cell supernatant (100 μL) was taken into a 96 well plate for NO measurement. OD values were determined an ELISA at 540 nm.

#### E. Kinetic Test of Drug Release

Using the optimized formula of 1wt% NaCl/6.5wt% MC/4.5wt% HA-Me drying hydrogel placed into a micro centrifuge tube, adding 1 mL of PBS and shaking the thermostat simulated the intestinal peristalsis. Extracting the absorbance using an ELISA at 450 nm.

### IV. RESULT AND DISCUSSION

#### A. Structural Identification by FTIR and <sup>1</sup>H-NMR Spectra

Fig. 1 shows schematics for the synthesis and appearance of HAmE product. The FT/IR spectrum of HA-Me compound because of lower content (Me 20%) was similar to pure HA, however, which can be seen the peak range (1630 cm<sup>-1</sup>~1610 cm<sup>-1</sup>) of -NH groups of benzyl ring become very weaker than that of pure Me. The peak (C=O) of an amide functional groups (CONH) for HA-Me compound was more obvious at 1640 cm<sup>-1</sup> as shown in Fig 2. It may be that because of carboxylic acid of HA with amine groups of Me reaction form a strength amide functional groups through nucleophilic addition elimination mechanism, which can also be verified in <sup>1</sup>H-NMR spectrum of HAmE compound. As shown in Fig 3, new chemical shift peaks appeared at δ<sub>H</sub>7.0 ppm, δ<sub>H</sub>7.6 ppm, and δ<sub>H</sub>8.0 ppm, which are mainly the range where aromatic hydrocarbon groups, implying that Me can be grafted on HA forming a HAmE compound.

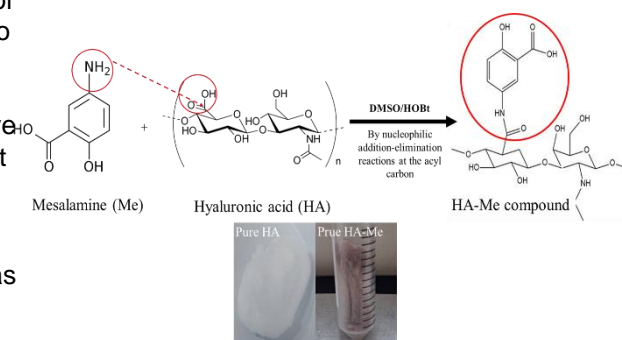


Fig. 1. Schematics of synthesis and appearance of HAmE compound.



Therefore, each gram of HMe grafted compound contained 62.46 mg of Me drug.

### C. Flowing Distance and Gelling Time

Using a condenser tube simulated the hydrogel injection of the rectal segment. Placing the hydrogel with mixed 1mL of PBS into a condenser tube by shaking the thermostat simulates the intestinal peristalsis to measure the flowing distance and gelling time as shown in Fig 5. The flowing distance and gelling time of various formula hydrogels were summarized in Table II. The MC/HA-Me with 6.5wt%MC/4.5wt%HAMe/1wt%NaCl was an optimal formula, which of the flowing distance and the gelling time were 13.2 cm and 238 second at 37°C respectively. This flowing distance was closed to the length of the human rectum (about 12~15cm).



Fig. 5. The flowing distance and gelling time test of MC/HA-Me hydrogels by a condenser tube simulated.

TABLE II. THE AVERAGE FLOWING DISTANCE AND GELLING TIME OF VARIOUS FORMULA HYDROGELS

Components	Flowing distance (cm)	Average gelling time (s) N = 3
6.5%MC/4.5%HAMe/1.0%NaCl	13.2	238
6.0%MC/4.0%HAMe/1.0%NaCl	18.7	259
6.5%MC/6.0%HAMe/1.0%NaCl	9.1	258

### D. Drug Release Analysis

The optimized hydrogel formula was shaken in a constant bath at 37°C to measure the kinetics of the drug release. Fig 6 found that the drug of MC/HA-Me hydrogels was released slowly and stably for up to 12 hours. Since the smart hydrogels with the thermosensitive occurred the gelling state at 37°C, so that the drug release time is slower than that of the liquid intestinal dosage forms. This gelling mechanism not only overcomes the shortcomings of liquid dosage forms that required maintaining a specific posture, but also has well the drug utilization rate and the patient compliance.

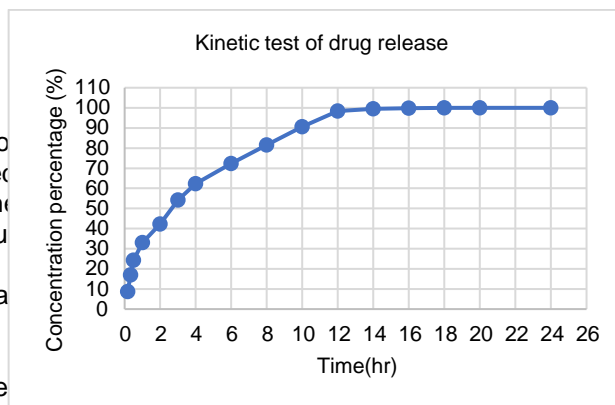


Fig. 6. Kinetic analysis of the drug release for MC/HA-Me hydrogels.

### E. Cell Viability and Anti-inflammation Tests

#### (1) Cell viability and Anti-inflammatory of pure Me

The cell viability of six different Me concentrations were shown in Fig 7, which can be seen that the IC50 of Me is 50mM. Thus, the concentration ranges for the anti-inflammatory experiments were carried out below 50mM. In anti-inflammatory experiments using pure Me drugs, the IC50 of Nitric Oxide (NO) was measured to be 6.25 mM, which means that 50% of NO can be inhibited at this concentration, as shown in Fig 8.

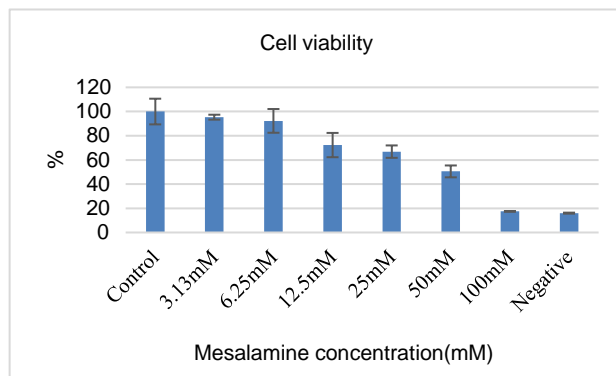


Fig. 7. Cell viability of the concentrations of mesalamine and control group.

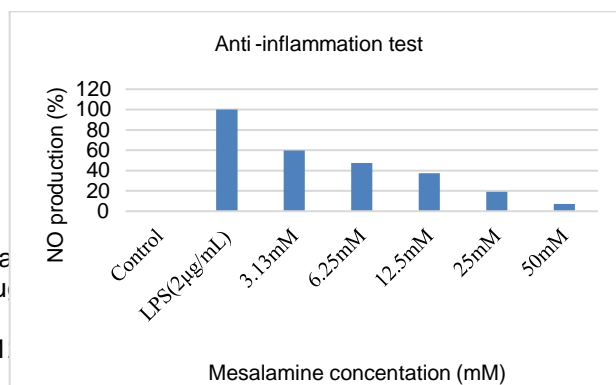


Fig. 8. Anti-inflammation test of various concentration for Me and control group.

#### (2) Anti-inflammatory effect of HAME compound

After understanding the anti-inflammatory mechanism of the pure Me drug, which can further explore the use of HA-Me compounds to conduct anti-inflammatory

experiments when Me is grafted onto HA. It found that the anti-inflammatory effect was reduced to compare with pure Me, which was speculated to be due to the effect of HA in the compound. Although the anti-inflammatory effect increased with increasing drug concentration, considering the results of cell survival rate, 12.5 mM was a suitable concentration in vitro simulation three dimensional cell layers culture experiments in the future.

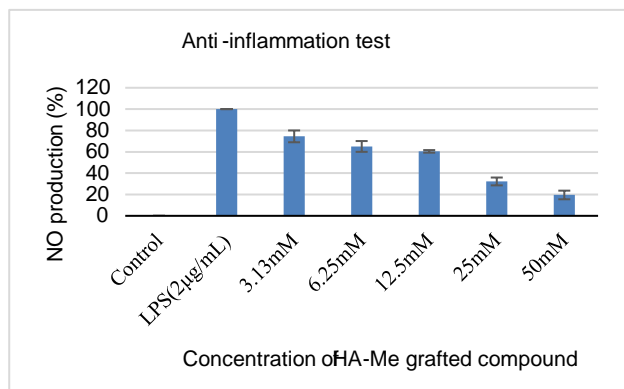


Fig. 9. Anti-inflammation test of various concentration for HA-Me compound and control group.

## V. CONCLUSION

Based on the above results and discussion, the following statements were summarized:

- (1) The HA-Me drugs were synthesized with Hyaluronic Acid (HA) and Mesalamine (Me), which of functional groups were verified by the FT/IR and H-NMR spectra.
- (2) HA-Me drugs were calculated with a concentration of 62.46 mg (Me)/g (HMe).
- (3) Mixtures of Methylcellulose (MC) and HMe drugs were carried out by gelling in the process of simulating the intestine. The average flowing distance of the optimized formula hydrogels at DERXW PLQXWHV DQG • ZHPU closed to human rectum length.
- (4) Sustained drug release time of MC/HMe smart K\GURJHOV ZHUH DFKLHYHG IRUKSWE NCUKRYUVDW.
- (5) Compared with the commercial liquid enema, MC/HA-Me smart hydrogels with thermo sensitivity and intestinal mucosal adhesion can not only reduce the drug dosage, but also increase the release time.
- (6) By a concentration of 12.5 mM HMe drug as a reference value for anti-inflammatory experiments will be carried out in vitro simulation three dimensional cell layers culture experiments in the future.

## CONFLICT OF INTEREST

The authors declare no conflict of interest

## AUTHOR CONTRIBUTIONS

Pei-Xhan Wu, Xiu-Fen Xiao, Min-Shin Ou, and Yi-Chi Shu conducted the research. Pei-Xhan Wu analyzed the data. Pei-Xhan Wu and Shu-Ling Huang wrote the paper. All authors had approved the final version.

## FUNDING

The authors are grateful for the financial support by National Science and Technology Council, Taiwan, (NSTC 1112221-E-239-001).

## REFERENCES

- [1] S. S. Seyedian, F. Nokhostin, and M. D. Malimir, "A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease," *Journal of Medicine and Life*, vol. 12, no. 2, pp. 113-122, Apr.-Jun 2019.
- [2] X. Li, et al., "Site-specific targeted drug delivery systems for the treatment of inflammatory bowel disease," *Biomedicine & Pharmacotherapy*, vol. 129, pp. 110486, September 2020.
- [3] S. M. Ruppert, et al., "Tissue integrity signals communicated by high-molecular weight hyaluronan and the resolution of inflammation," *Immunologic Research*, vol. 58, pp. 186-192, March 2014.
- [4] Y.-J. Jin, T. Ubonvan, and D. Kim, "Hyaluronic acid in drug delivery systems," *Journal of Pharmaceutical Investigation*, vol. 40, pp. 33-43, December 2010.
- [5] M. B. H. Othman, et al., "Thermal properties and kinetic investigation of chitosan/PMAA based dual-responsive hydrogels," *Industrial Crops and Products*, vol. 66, pp. 178-187, April 2015.
- [6] D. Thankachan, et al., "pH dependent biocompatible room temperature covalent organic polymers for selective chemotherapeutic drug delivery," *Microporous and Mesoporous Materials*, vol. 365, pp. 112903, February 2024.
- [7] L. Mayol, et al., "Effect of hyaluronic acid on the thermogelation and biocompatibility of its blends with methyl cellulose," *Carbohydrate Polymers*, vol. 112, pp. 480-485, November 2014.
- [8] F. Lee, J. E. Chung, and M. Kurisawa, "An injectable enzymatically crosslinked hyaluronic acid/tyramine hydrogel system with independent tuning of mechanical strength and gelation," *Soft Matter*, vol. 4, no. 4, pp. 880-887, February 2008.
- [9] R. Hoare and D. K. Khoshdel, "Hydrogels in drug delivery: Progress and challenges," *Polymer*, vol. 49, no. 8, pp. 1993-2007, April 2008.
- [10] Y. Luan and W. Xu, "The structure and main functions of laminin," *Journal of Cellular Biochemistry*, vol. 14, no. 6, pp. 639-647, March 2007.

Copyright © 2024 by the authors. This is an open access article distributed under the Creative Commons Attribution License [CC BY-NC-ND 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits use, distribution and reproduction in any medium, provided that the article is properly cited, the use is non-commercial and no modifications or adaptations are made.