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

Pei-Xhan Wu[1], Xiu-Fen Xiao[1], Min-Shin Ou[1], Yu-Chi Shu[1], and Shu-Ling Huang[1,2,3,*]

[1] International Master Program of Translation Medicine, College of Engineering and Science, National United University, Miaoli, Taiwan
[2] Department of Chemical Engineering, National United University, Miaoli, Taiwan
[3] PhD. Program in Materials and Chemical Engineering, National United University, Miaoli, Taiwan
Email: fifth.simone@nuu.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 (-NH2) of Mesalamine (Me). The structure of the HA-Me compound was identified using FTIR and H1-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 closed 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**

I. Introduction

With the advancement of medicine and technology, the medical quality has been greatly improved, so that the human's 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's a debilitating condition that can lead to life-threatening complications. IBD includes 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 [1]. For the UC, current clinical treatment requires the patients to maintain a fixed side-lying posture. After injecting a 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, muco-adhesive, and charge-targeted by the human body [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 thermos-sensitive property, which is liquid at low temperatures and gel state at high temperatures because of the hydrophobic association and junction [5].

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

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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 [6].

In this study, we developed a MC/HA-Me smart hydrogel with drug delivery for UC's treatment to improve the shortcomings of liquid suppositories and promote the medical comfort. HA-Me compound was synthesized using Hyaluronic Acid (HA) and anti-inflammatory Mesalamine (Me) drug by covalent organic polymers (amide bonds). Then, HA-Me compound mixed Methyl Cellulose (MC) by the hydrogen bonding forming MC/HA-Me hydrogels, which exhibited the thermosensitive properties. MC/HA-Me solutions were injected into the rectal from the anus at room temperature, as the rectal temperature of the human body increases [7], MC/HA-Me hydrogels cause the gelling because of the hydrogen bonds effect [8, 9]. 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 achieves the effect of directly releasing the drug at the affected area [10].

III. Experimental

A. Synthesis of HA-Me Compound

HA was dissolved in de-ionic water to form a 1% HA aqueous solution. HOBt (hydroxybenzotriazole) and Me were dissolved in DMSO (dimethyl sulfoxide) at concentration of 30 mg/mL and 5 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, HA-Me 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 H1-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 1 wt%~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 HA-Me concentration between 1 wt% to 5 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_2. Assess cell viability was measured with cell counting Kit-8. The cells were seeded in a 96-well plate (5×10^4 cells/mL) and cultured with six different concentrations of melamine for 24 hours. Optical Density (OD) values were determined using an all-wavelength photometer (ELISA) at 450 nm.

D. Nitric Oxide (NO) Assay

The evaluation of anti-inflammatory was carried out by Nitric Oxide (NO) assay. RAW264.7 cells (2×10^5 cells/mL) were seeded in 6-well plates and cultured for 24 hrs. Six different concentrations of melamine and LPS (lipopolysaccharide, 2 μg/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 100μL at intervals of 10, 20, 30 minutes…etc., measured the absorbance using an ELISA at 450 nm.

IV. Result and Discussion

A. Structural Identification by FTIR and H1-NMR Spectra

Fig. 1 shows schematics for the synthesis and appearance of HA-Me 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^{-1}~1610 cm^{-1}) 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^{-1}, 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 H^1-NMR spectrum of HA-Me compound. As shown in Fig. 3, new chemical shift peaks appear at δ_H7.0 ppm, δ_H7.6 ppm, and δ_H8.0 ppm, which are mainly the range where aromatic hydrocarbon groups, implying that Me can be grafted on HA forming a HA-Me compound.

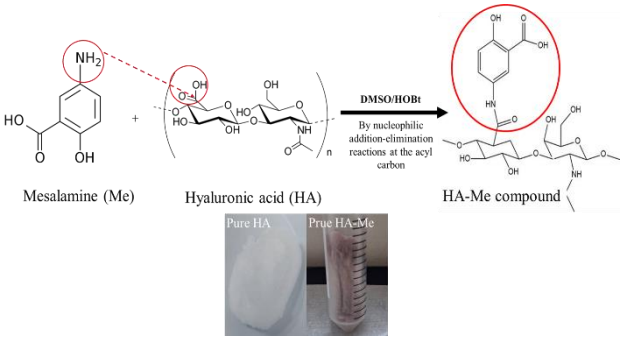

Fig. 1. Schematics of synthesis and appearance of HA-Me compound.

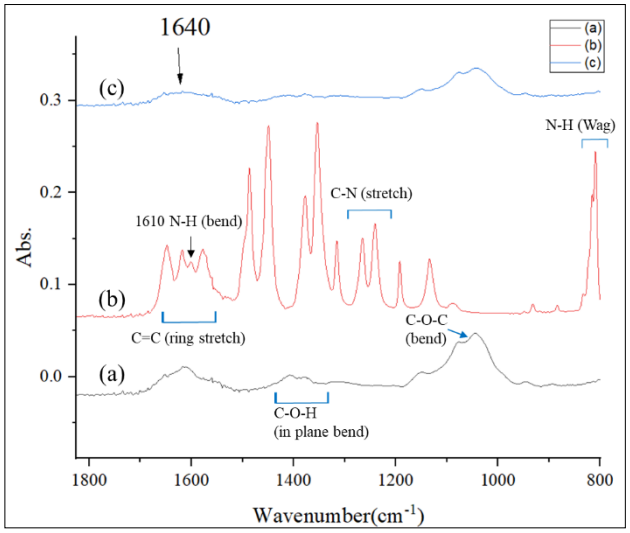

Fig. 2. Structural analysis of HA and HA-Me by FT/IR spectrum, (a) pure HA, (b) pure Me, (c) HA-Me.

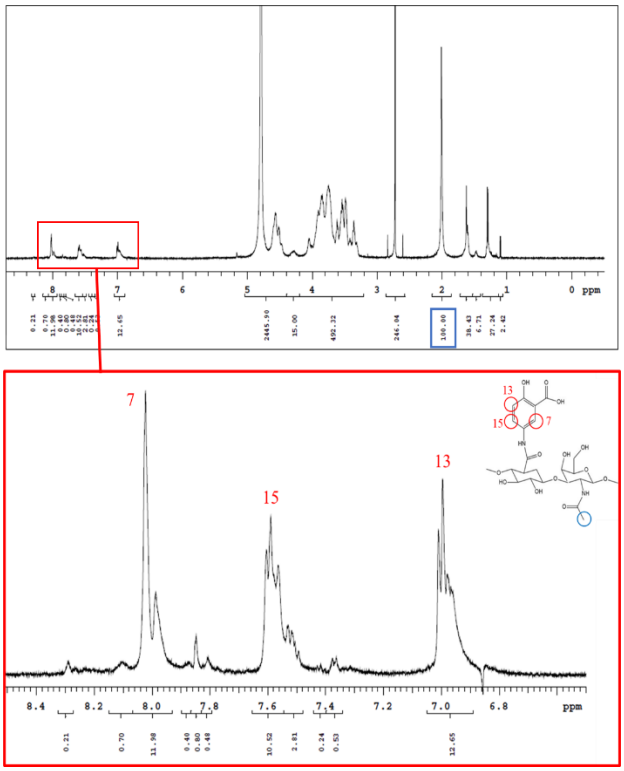

Fig. 3. Structural analysis of HA-Me by H1-NMR spectrum.

B. *Quantitative Analysis of Me Concentration in HA-Me Compounds*

(1) Preparation of the calibration curve

The OD values under various concentration for pure Me were listed in Table I. The Me drug concentration (x-axis) and the resulting OD value (y-axis) were made into a calibration curve, as shown in Fig. 4. The standard calibration linear curve was expressed as y = 8.9544 x +0.1053, the slope is 8.9544, the intercept is 0.1053 and the R2 value is equal to 0.9890.

TABLE I. THE OD VALUES OF DIFFERENT ME CONCENTRATION

Mesalamine concentration (mg/mL)	OD value
0.1250	1.185
0.0625	0.706
0.0313	0.428
0.0156	0.293
0.0078	0.206
0.0039	0.159
0.0020	0.134
0.0010	0.100
0.0005	0.083
0.0002	0.070
0.0000	0.063

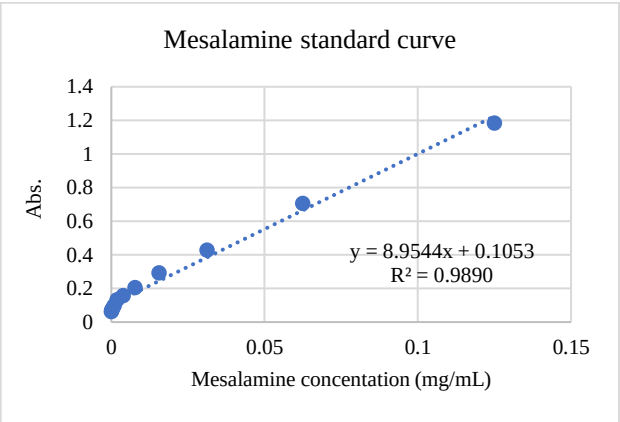

Fig. 4. The calibration curve of pure Me.

(2) Calculation of Me content in HA-Me compounds

The concentration of Me in HA-Me compound was obtained from the calibration curve, as shown in Fig. 4. Each micro-gram Me / HA-Me gram was calculated by the read OD value subtracted HA background value and intercept, as shown in Eqs. (1) and (2).

$$x = \frac{(\text{read OD value} - \text{background value}) - \text{intercept}}{\text{slope}} \quad (1)$$

$$x = \frac{(0.848 - 0.161) - 0.1053}{8.9544}$$

$$x = 6.496 \times 10^{-2}\ \text{mg/mL (after diluting 10 times)}$$

where, the read OD value was 0.848 after diluting 10 times and the background value of 1% HA was 0.161. The total grams of HA-Me compound was 0.0104 g. Therefore, the grams of Me contained in each gram of HA-Me compound (mg/g) can be expressed by Eq. (2).

$$\text{mg(Me)/g(HA-Me)} = \frac{6.496 \times 10^{-2}\ \text{mg/mL} \times 1\ \text{mL} \times \frac{10\ mL}{1\ mL}}{0.0104\ g} = 62.46\ \text{mg/g} \quad (2)$$

Therefore, each gram of HA-Me 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%HA-Me/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 cm~15 cm).

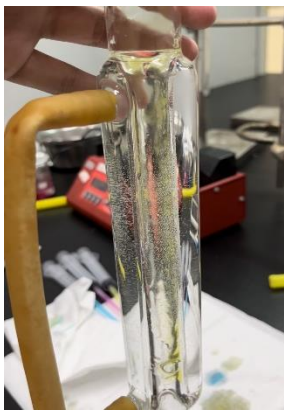

Fig. 5. The flowing distance and gelling time tests of MC/HA-Me hydrogels by a condenser tube simulated.

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%HA-Me/1.0 %NaCl	13.2	238
6.0%MC/4.0%HA-Me/1.0% NaCl	18.7	259
6.5%MC/6.0%HA-Me/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 thermos-sensitive 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.

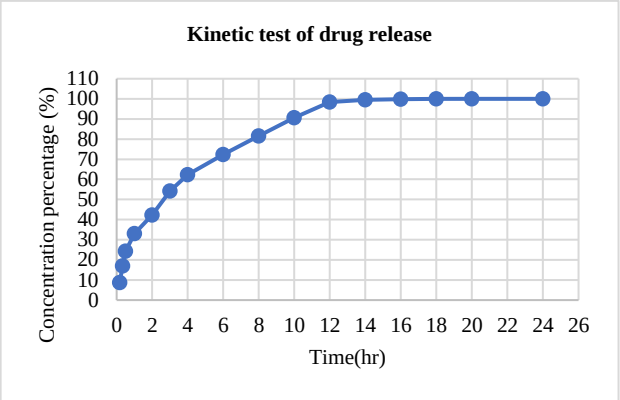

Fig. 6. Kinetic analysis of the drug release for MC/HA-Me hydrogels.

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 experiment were carried out below 50 mM. 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.

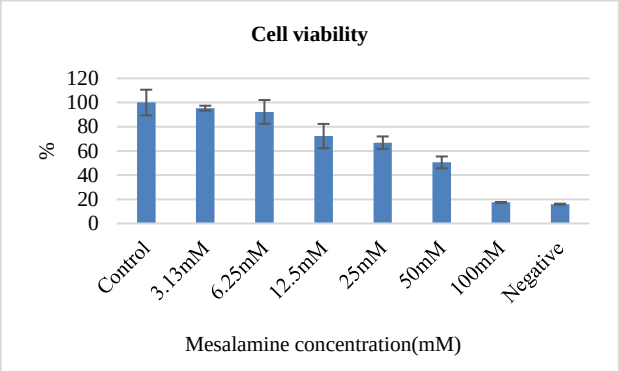

Fig. 7. Cell viability of the concentrations of mesalamine and control group.

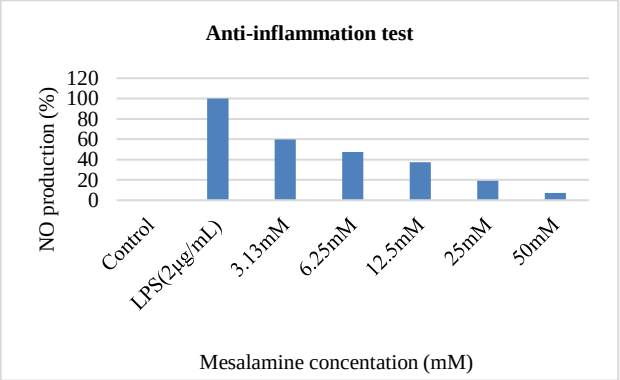

Fig. 8. Anti-inflammation test of various concentration for Me and control group.

(2) Anti-inflammatory effect of HA-Me 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. Fig. 9 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 co-culture experiments in the future.

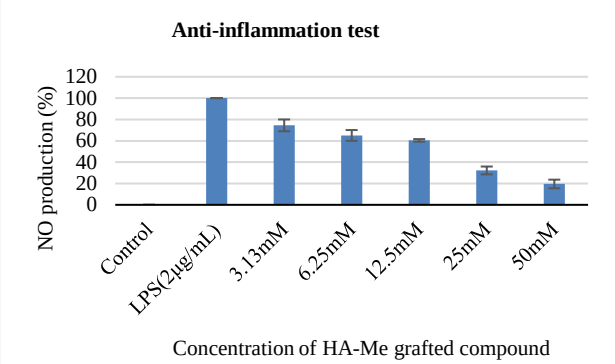

Fig. 9. Anti-inflammation test of various concentration for HA-Me compound and control group.

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^1-NMR spectra.
(2) HA-Me drugs were calculated with a concentration of 62.46 mg (Me)/g (HA-Me).
(3) Mixtures of Methylcellulose (MC) and HA-Me drugs were carried out by gelling in the process of simulating the intestine. The average flowing distance of the optimized formula hydrogels at about 4 minutes and 37℃ were 13.2 cm, which is closed to human rectal length.
(4) Sustained drug release time of MC/HA-Me smart hydrogels were achieved for up to 12 hours at 37℃.
(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's dosage, but also increase the release time.
(6) By a concentration of 12.5 mM HA-Me drug as a reference value for anti-inflammatory experiments will be carried out in vitro simulation-three-dimensional cell layers co-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 Yu-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.

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