Drug-Loaded Mucoadhesive Patch with Active Delivery and Controlled Releasing Ability

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Herein, a mucoadhesive patch for gastrointestinal tracts with active delivery, hyperthermia, and controlled drug release function using a magnetically actuated capsule is proposed to overcome the drug delivery and efficiency challenges of wireless structures. The proposed patch has excellent adhesion to the gastrointestinal tract and contains anticancer drug doxorubicin and magnetic nanoparticles. This enables hyperthermia and the controlled release of the loaded drug under an alternating magnetic field (AMF). In addition, it can be delivered to multiple intestinal target lesions using a magnetically actuated capsule. Characteristic analyses of the proposed patch are performed, such as morphology, adhesion force measurement with intestine, temperature change under an AMF, and drug release. The feasibility of the patch delivery into the gastrointestinal tract is verified through locomotion performance tests and ex vivo patch delivery experiments using a magnetically actuated capsule. Finally, through an in vitro therapeutic effect test, the death of tumor cells using the proposed patch is confirmed. As a result, the possibility that the multiple mucoadhesive patches can be delivered to target lesions in a digestive tract through a magnetically actuated capsule and can treat the lesions through hyperthermia and active drug release using an AMF stimulation is verified.

1. Introduction

Gastrointestinal cancer has a high incidence worldwide[1,2] and invasive surgical resection is the main form of treatment.[3–5] Surgical resection can be a large burden on a patient due to the sequelae of an affected area and the relatively long recovery period due to the invasiveness.[6–8] Various studies on the treatment of the gastrointestinal tract using wireless structures have been conducted as an alternative to gastrointestinal treatment. Generally, wireless structures are noninvasive compared to conventional resection and have advantages such as patient convenience and ease of operation.[9–11] For example, a capsule delivering insulin-loaded microneedles to the small intestine was reported, where the capsule could be moved in the digestive tract using peristalsis. However, the drug loaded on the microneedles equipped in the capsule could only be exposed and delivered in a specific pH range.[12] Compared with subcutaneous injection, this minimally invasive method using a capsule delivers the drug directly to the small intestine, and thus demonstrates better drug absorption efficiency. However, drug-loaded microneedles cannot be rapidly delivered to a lesion because the capsule was moved by peristalsis, and it could be exposed, and only delivered to a lesion site in a specific pH range. We recently presented a multilayer drug-loaded microneedle patch delivery method using a magnetically actuated capsule.[13] Drugs could be delivered quickly and accurately to multiple lesion sites because multilayer microneedle patches are equipped in the magnetically actuated capsule, and the capsule is actively operated using an external magnetic field. However, the microneedle patch may be easily separated from the lesion site due to weak adhesion between the microneedle patch and the intestine. In addition, the treatment efficiency is low because the drug loaded in the patch is passively released.

The mussel-inspired adhesive hydrogel is a verified tissue adhesion material in a wet environment and has been widely studied and utilized in various fields.[14–16] In general, mussel adhesion is caused by catechol, a functional group of 3,4-dihydroxyphenylalanine.[17] A related study reported using a fabricated drug-loaded patch with catechol to deliver a drug.[18] In addition, a hydrogel with a therapeutic drug and magnetic nanoparticles (MNPs) was fabricated and delivered to a lesion, successfully achieving the hyperthermia treatment and controlled release of the drug from the hydrogel using an alternating magnetic field (AMF).[19] However, these methods are limited to drug delivery to the skin. Until now, research on hyperthermia treatment and controlled drug release using patches delivered in the gastrointestinal tract has not been reported.

To overcome the limitations of previous studies, this study proposes a polyvinyl alcohol (PVA) and chitosan–catechol
(mucoadhesive) patch with excellent adhesion to the gastrointestinal tract, active delivery using magnetically actuated capsules, hyperthermia, and controlled drug release using an AMF (Figure 1). The magnetically actuated capsule consists of a capsule body, cover, patch delivery assembly, and multiple mucoadhesive patches, as shown in Figure 1a. In addition, the capsule can be opened or closed by rotating the cover. Therefore, it can be moved while protecting the patches in the closed state, and the patches can be delivered to the lesion sites in the open state. The operation process of the open–close mechanism of the magnetically actuated capsule was described in Video S1, Supporting Information. The proposed mucoadhesive patch consisted of PVA and chitosan–catechol layers. Catechol-conjugated chitosan is known to have excellent biodegradability, biocompatibility, and mucoadhesive properties.\textsuperscript{20–22} In particular, catechol is a functional group of the mussel adhesive protein and is known to have excellent adhesion in wet environments.\textsuperscript{23} In addition, doxorubicin (DOX), a representative anticancer drug, and MNPs for hyperthermia and active drug release are loaded onto the chitosan–catechol layer, as shown in Figure 1b.\textsuperscript{24–27} The PVA layer prevents the flow of fluids, such as intestinal mucus, between the patches. Three proposed patches are equipped in the proposed capsule, and each patch is delivered to multiple target lesions in the gastrointestinal tract using the patch delivery assembly in the capsule. The proposed patches have a ring shape with an inner diameter $D_{\text{inner, patch}}$ and are inserted and assembled into a center bar with a bottom diameter $D_{\text{center, bar}}$, which is smaller than $D_{\text{inner, patch}}$, as shown in Figure 1b. In other words, the proposed patches are not separated from the center bar of the patch delivery assembly without

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\caption{a) Magnetically actuated capsule with multiple mucoadhesive patches. b) Patch delivery assembly consisting of a mucoadhesive patch, center bar, and neodymium magnet. c) Delivery process of multiple mucoadhesive patches to target lesions using a magnetically actuated capsule.}
\end{figure}
an external force. The adhesion of the patch to the gastrointestinal tract surface is generated when the patch is exposed from the capsule and in contact with the gastrointestinal tract. As the capsule is aligned in different directions, the patch is separated from the center bar of the patch delivery assembly and delivered to the lesion site. The treatment process in the gastrointestinal tract using the proposed patch is shown in Figure 1c. In detail, the proposed capsule moves to the tumor lesion site using an external magnetic field, and then performs the treatment process by repeating the process of inserting and delivering the patch, as shown in Figure 1c(i)–(iii). After the proposed patch is delivered to the lesion site, an AMF is applied, as shown in Figure 1c(iv). The temperature is increased by the MNPs loaded in the patch under the AMF. Subsequently, hyperthermia occurs and the DOX release is achieved, leading to the death of the tumor cells, as shown in Figure 1c(v).

The proposed mucoadhesive patch has the following features. 1) It can be actively delivered to multiple target lesion sites using a magnetically actuated capsule. 2) It is fabricated using a mussel-inspired hydrogel that demonstrates biocompatibility, biodegradability, and improved adhesion to wet gastrointestinal tract surfaces. 3) It can perform a hyperthermia treatment and a controlled drug release at the desired time through a temperature rise using an AMF as an external stimulus.

Various experiments were performed to validate the delivery and therapeutic feasibility of the proposed mucoadhesive patch. First, adhesion force measurements were taken to confirm the sequential delivery of the proposed patches to the gastrointestinal tract. In addition, the temperature change and active drug release of the proposed patch were tested under AMF stimulation. In addition, basic tests of movement and alignment of the magnetically actuated capsule and patch delivery were performed. The possibility of patch delivery to various lesion sites in the gastrointestinal tract was verified through an ex vivo test. Finally, an in vitro therapeutic effect test was performed using HT-29 tumor cells to confirm the therapeutic performance of the proposed patch.

2. Results and Discussion

2.1. Fabrication and Characterization of Proposed Mucoadhesive Patch

The proposed mucoadhesive patch was fabricated by sequentially casting the patch material PVA solution, drug, and MNP-loaded chitosan–catechol solution into the polydimethylsiloxane (PDMS) mold, as shown in Figure 2a. First, the PVA solution was poured into a PDMS mold and dried overnight at room temperature. Then, the drug (DOX) and MNP-loaded chitosan–catechol solution were poured onto the hardened PVA layer in the PDMS mold and frozen in a refrigerator (−4 °C) and deep freezer (−80 °C). The final mucoadhesive patch was obtained after a freeze-drying process.

Catechol used in the proposed mucoadhesive patch is a functional group of mussel-inspired protein and has been widely used as an ideal drug delivery material because it has high adhesion in a wet environment, high strength, high stiffness, and water resistance. The catechol has biodegradability, and it can be dissolved in the gastrointestinal tract after the loaded drug is released. According to the study, through in vitro tests, it was shown that chitosan–catechol has excellent binding to mucus, which exists in the surface of the digestive organs and maintains mucosal adhesion for up to 10 h. That is, chitosan–catechol can be considered an appropriate material in terms of mucosal adhesion, strength, rigidity, and water resistance as a drug carrier in the gastrointestinal tract. In addition, as chitosan–catechol has biodegradability, it can be dissolved in the gastrointestinal tract after the loaded drug is released.

The overall shape and cross-sectional images of the fabricated mucoadhesive patch were observed using a CAMscope (SOMETECH, Korea), as shown in Figure 2b. The mucoadhesive patch had a diameter and inner diameter (Dpatch Inner) of ≈7.8 and 1.6 mm, respectively. The PVA layer had a thickness of ≈250 μm, where the biocompatible and biodegradable PVA exhibited low solubility. Subsequently, the PVA layer prevented the flow of fluids, such as intestinal mucus, between the patches. The chitosan–catechol layer had a thickness of ≈1.4 mm. Hyperthermia and accelerated drug release are performed using AMF stimulation to treat lesions in the gastrointestinal tract because the chitosan–catechol layer contained the therapeutic drug DOX and MNPs. Scanning electron microscopy (SEM) (UHR FE-SEM, SU8230, Hitachi) images of the chitosan–catechol layers in the mucoadhesive patches prepared by the freeze-drying process after being frozen in a refrigerator (−4 °C) and deep freezer (−80 °C) are shown in Figure 2c,d, respectively. It is generally known that the lower the freezing temperature before the freeze-drying of the hydrogel, the smaller the pore size. It is clear that the chitosan–catechol layer fabricated after freezing at −80 °C had a smaller pore size compared to that fabricated after freezing at −4 °C.

The adhesion force of the proposed mucoadhesive patch on the porcine small intestine surface was measured using a load cell, as shown in Figure 2e. The adhesion forces of the two types of patches frozen at different temperatures (−4 and −80 °C) before freeze-drying were measured after contact with the porcine small intestine surface for 60 and 120 s, where each experiment was performed six times. The mucoadhesive patch with a frozen temperature of −4 °C in the freezing stage demonstrated the adhesion forces of ≈0.28 and 0.38 N after contact for 60 and 120 s, respectively. However, the mucoadhesive patch with a frozen temperature of −80 °C in the freezing stage demonstrated the adhesion forces of ≈0.48 and 1.22 N after contact for 60 and 120 s, respectively. That is, the lower the freezing stage temperature, the smaller the pore size of the mucoadhesive patch, which achieved a higher adhesion force to the small intestine surface. Therefore, among the two types of patches, the one frozen at a lower temperature was more suitable in terms of delivery to the gastrointestinal tract and was used in the subsequent experiments.

The temperature change of the proposed mucoadhesive patch was measured when an AMF was applied, as shown in Figure 2f. The MNPs loaded in the patch generated heat from Neel and Brownian losses under an AMF stimulation. The proposed mucoadhesive patch was exposed to an AMF for 15 min, and its temperature changes were measured at two initial temperatures:
room temperature (22.9 °C) and body temperature (37 °C). As a result, the temperatures of the patches increased to 43.8 and 46 °C after 15 min, respectively. In general, 40–43 °C can induce apoptosis of the tumor cells by hyperthermia, thus it is expected that the proposed mucoadhesive patch can treat lesions through hyperthermia under an AMF stimulation.[35,36]

Finally, as shown in Figure 2g, the drug release from the proposed mucoadhesive patch in phosphate-buffered saline (PBS) was measured in the cases with and without AMF stimulation, where two PBS solutions (pH 7.4 and 2.0) were used. When the AMF was applied and was not applied, approximately 89.59% and 53.59% of the DOX were released from the patch in the pH 7.4 PBS after 60 min, respectively. Similarly, when the AMF was and was not applied, ≈80.63% and 50.38% of the DOX were released from the patch in the pH 2.0 PBS after 60 min, respectively. That is, it was confirmed that the applied AMF stimulation promotes the release of the loaded DOX from the proposed mucoadhesive patch. In addition, we could expect that the proposed patch will show a similar drug release profile in various organs with different pH in the gastrointestinal tract (small intestine: pH 6.6–7.5,[37] large intestine: pH 6.5,[38] and stomach: pH 1–2.5[39]).

2.2. Multiple Delivery of Proposed Mucoadhesive Patch Using Magnetically Actuated Capsule

The proposed mucoadhesive patches were delivered to multiple target lesions in the intestine using a magnetically actuated capsule. The capsule had a length of 27 mm and a diameter of 13 mm, as shown in Figure 3a,b. It is generally known that the small and large intestines have inner diameters of less than

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Figure 2. a) Fabrication process of a mucoadhesive patch. b) Fabricated mucoadhesive patch. c,d) SEM images of the chitosan–catechol layer produced after freeze-drying for −4 °C and −80 °C, respectively. e) Adhesion force between a porcine small intestine and mucoadhesive patch (n = 6). f) Temperature change of mucoadhesive patch under an AMF application from the initial room temperature and 37 °C, respectively. g) Drug release from DOX-loaded mucoadhesive patch.
25 and 48 mm, respectively. Therefore, it is expected that the magnetically actuated capsule can move sufficiently in the small and large intestines. The alignment and locomotion performances of the capsule were evaluated using an electromagnetic actuation (EMA) system, as shown in Figure 3e. The capsule was aligned and moved to the desired direction using the uniform and gradient magnetic fields generated by the EMA system, as shown in Figure 3f,g. The delivery process of the proposed mucoadhesive patches to the porcine small intestine using a magnetically actuated capsule is shown in Figure 3h–j. First, the capsule moved to the target lesion with the cover closed (Figure 3h). Then, the capsule was rotated on the target lesion, vertically erected, the cover was opened, and the patch protruded and contacted the lesion site (Figure 3i). Subsequently, the capsule was rotated, and the patch was separated from the center bar in the capsule and delivered to the target lesion (Figure 3j). Here, the rotational torque and propulsion force acting on the capsule were ≈0.00642 and 0.0205 N, respectively. And, the magnetic force acting on the capsule when the patch is inserted into the intestinal surface was about 0.0207 N. The diameter of the
center bar ($D_{\text{center, bar}}$) was $\approx 2$ mm, which was larger than the inner diameter of the patch ($D_{\text{inner, patch}} = 1.6$ mm). Therefore, the patch could not be separated from the capsule without an external force. The patch was separated and delivered from the capsule when the adhesive force was generated by the contact of the patch with the intestinal wall. The mucoadhesive patch delivery process using a porcine small intestine is described in Video S2, Supporting Information.

2.3. Ex Vivo Patch Delivery Test on Porcine Intestinal Surfaces

Ex vivo tests were conducted on a porcine small intestine and stomach to verify the delivery feasibility of the proposed mucoadhesive patches to various target lesions in the gastrointestinal tract, as shown in Figure 4. Here, a magnetically actuated capsule equipped with three proposed patches was used in the experiments. The results of the ex vivo test on the porcine small intestine are shown in Figure 4a. After attaching the porcine small intestine to the 3D printed phantom, three target lesions were marked using dye, and the magnetically actuated capsule was operated with the EMA system. The capsule with the closed cover was moved to each target lesion and aligned in the vertical direction (z-axis), and then the cover of the capsule was opened. Subsequently, a z-axis gradient magnetic field was applied to the patch for 2 min to contact the intestinal surface, a uniform magnetic field was applied to rotate the capsule, and the patch was delivered to the lesion site. Another gradient magnetic field was then generated, the cover was closed, and the capsule moved to the next lesion site in the same way. Similarly, an ex vivo test was performed on the porcine stomach, as shown in Figure 4b. After attaching a porcine stomach to a commercial stomach phantom, the proposed patches were delivered to the surface of the porcine stomach using a magnetically actuated capsule with a contact time of 2 min each. Through ex vivo tests on the porcine small intestine and stomach, it was confirmed that the proposed mucoadhesive patches could be sequentially delivered in the gastrointestinal tract using a magnetically actuated capsule. Ex vivo patch delivery test on porcine small intestine and stomach is shown in detail in Videos S3 and S4, Supporting Information, respectively.

However, to improve the clinical usefulness of the magnetically actuated capsule, the locomotion performance of the capsule needs to be improved. In addition, as narrow and curved sections exist in real digestive organs, and substances such as

![Figure 4](image-url)
food in the digestive process exist, they may affect the locomotion of the magnetically actuated capsule. Therefore, for the stable locomotion of the proposed capsule to a lesion site in the gastrointestinal tract by the magnetic actuation, the locomotion performance of the capsule should be improved. For this, the structure of a magnetically actuated capsule, an external magnetic field system, and a robust control algorithm can be considered.

In addition, to deliver the proposed patch to the desired lesion, the locations of the lesion and the capsule should be known. First, an imaging camera can be mounted on the wireless capsule to determine the location of the lesion. In addition, researches such as a method of providing 3D information in real time through a capsule camera and improving diagnostic performance through machine learning were conducted. Therefore, the lesion localization and diagnosis technology through the integration of an imaging camera can be applied to the proposed capsule. Also, a Hall effect sensor or an inertia measurement unit can be used to determine the position of the capsule. In addition, the localization of a wireless-capsule endoscope using radio-frequency identification (RFID) is also widely used. Therefore, it is expected that a sensor, electromagnetic wave device, or RFID can be integrated into the capsule to recognize the location of the proposed capsule.

Figure 5. In vitro therapeutic effects of the mucoadhesive patch using HT-29 cells. a–f) Viability of HT-29 cells after 24 h for control, MP, MP + MNPs without and with an AMF, and MP + DOX + MNPs without and with an AMF, respectively. g) CCK-8 analysis graph of the HT-29 cells viability to verify the therapeutic effect of the mucoadhesive patch \((n = 4), * p < 0.0005, ** p < 0.005, \text{ and } \text{ns} \ p > 0.05.\)
2.4. In Vitro Therapeutic Effect Test of Proposed Mucoadhesive Patch Using HT-29 Cells

To verify the therapeutic performance of the proposed mucoadhesive patch, an in vitro therapeutic effect test was conducted using HT-29 human colorectal cancer cells. After HT-29 cells were cultured in a 35 mm cell culture dish, the proposed patch was placed on the cultured cells, as shown in Figure 5. The viability of HT-29 cells was evaluated for each group after treatment with the proposed patch and a 24 h incubation. The six groups were control, mucoadhesive patch (MP), MP including MNPs (MP + MNPs) with and without an AMF, and MP including DOX and MNPs (MP + DOX + MNPs) with and without an AMF. After treatment with a patch for each group, HT-29 cells were observed under a microscope and fluorescence microscope through 4′,6-diamidino-2-phenylindole (DAPI) staining, as shown in Figure 5a-f. Compared with the control group, the MP group demonstrated that the mucoadhesive patch had little effect on the viability of HT-29 cells. In the MP + MNPs without an AMF group, a little release of chitosan-coated MNPs induced a little cell death. Moreover, the MP + MNPs with an AMF group showed more apoptosis of the HT-29 cells than the MP + MNPs without an AMF group, indicating that hyperthermia was effective for cell death. In addition, it was verified that the drug released from the patch caused cell death in HT-29 cells for the MP + DOX + MNPs without an AMF group. Finally, the MP + DOX + MNPs with an AMF group demonstrated the lowest viability of HT-29 cells among the six groups, indicating that the hyperthermia and released DOX achieved the highest therapeutic effect.

In addition, the cell viability of each group was also analyzed after 24 h of sample preparation for the CCK-8 assay (Figure 5g). Compared with the control group, the MP group achieved approximately 95.0% cell viability, which means that the mucoadhesive patch did not have cell toxicity. Moreover, the MP + MNPs groups without an AMF achieved cell viabilities of approximately 90.5%, indicating that a little released chitosan-coated MNPs had a little adverse effect on cell growth. The MP + MNPs groups with an AMF achieved cell viabilities of approximately 36.8%. This signifies that the mucoadhesive patch could induce apoptosis of the tumor cells through hyperthermia using an AMF. Finally, the MP + DOX + MNPs groups without an AMF achieved cell viabilities of approximately 56.0%. This means that the mucoadhesive patch still does not induce sufficient tumor cell death for treatment because it does not release sufficient DOX without AMF. The MP + DOX + MNPs groups with an AMF achieved cell viabilities of approximately 25.2%. Therefore, it was determined that the mucoadhesive patch could be used as a cancer treatment through hyperthermia and drug release.

3. Conclusion

This study proposed a mucoadhesive patch with an active delivery using a magnetically actuated capsule, hyperthermia, and controlled drug release using an AMF stimulation. The proposed mucoadhesive patch was fabricated based on chitosan–catechol, which demonstrates biocompatibility, biodegradability, and excellent adhesion in wet environments. In addition, the hyperthermia and active drug release using an AMF stimulation were possible and could be used for cancer treatment because MNPs and DOX were loaded in the patch. Three proposed mucoadhesive patches were equipped in the magnetically actuated capsule and sequentially delivered to multiple lesions in the gastrointestinal tract to perform medical treatment. The delivery feasibility of the proposed patch to the gastrointestinal tract was verified through an adhesion test between the patch and the surface of the small intestine. It was confirmed that hyperthermia and active drug release were possible through the temperature change and drug release tests of the patches under an AMF stimulation. In addition, an ex vivo test confirmed that the proposed patches could be delivered to various lesions in the gastrointestinal tract using a magnetically actuated capsule. Finally, an in vitro test using HT-29 cells verified that the proposed patch could be used for the treatment of tumors in the gastrointestinal tract. As a result, the proposed mucoadhesive patch is expected to improve the existing treatment performance of gastrointestinal tumors and can be applied to other types of lesions.
with a diameter of 6 mm and height of 8 mm. In addition, a stainless pipe with a diameter of 1 mm was used for the hitching and guiding pins attached to the magnet jig. The magnetically actuated capsule was operated using an EMA system with six coils, as shown in Figure 3e. The EMA system generates uniform and gradient magnetic fields according to the current applied to each coil, thereby operating the capsule in the region of interest. The patch delivery assembly was moved up and down along the magnetic guide in the capsule using the external magnetic field, and the cover was rotated using the hitching pin of the patch delivery assembly. The capsule could be changed to an open or closed state through the rotation of the cover, as shown in Figure 3c,d, respectively. In the closed state, the cover of the capsule is closed to protect the inner equipped patch, and the capsule can move to the target lesion. In the open state, the cover of the capsule is opened, and the equipped patch is exposed and delivered to the target lesion.

**Morphology Analysis of Mucoadhesive Patch**: The overall appearance and cross-sectional images of the fabricated mucoadhesive patches were observed using CAMScope software (SOMETECH, Korea). In particular, the chitosan–catechol layer in the patch was observed using SEM (UHR FE-SEM, SU8230, Hitachi).

**Adhesion Force Measurement between Proposed Mucoadhesive Patch and Porcine Small Intestine**: The adhesion force between the proposed mucoadhesive patch and porcine small intestine was measured using a motorized stage (KZL06050-N1-FD, Suruga Seiki) and load cell (DBCM-2, Bongshin). First, the porcine small intestine was placed on the motorized stage, with the load cell fixed slightly above. The proposed mucoadhesive patch was connected to the load cell with a thread. Then, after the proposed mucoadhesive patch was in contact with the porcine small intestine for a certain time, the motorized stage was moved downward and the adhesive force was measured through the force applied to the load cell. The adhesive forces were measured for the porcine small intestine with the two mucoadhesive patches fabricated by freeze-drying after freezing in a −4 °C refrigerator and −80 °C deep freezer.

**Temperature Change of Proposed Mucoadhesive Patch Using an AMF Stimulation**: The temperature change of the proposed mucoadhesive patch under an AMF was measured. The patch was immersed in PBS, and then an AMF stimulation (320 A, 272 kHz) was applied for 15 min. In this test, the initial temperatures of the patch were set to room temperature and 37 °C, and the temperature changes of the patch were measured every 1 min using a thermal imaging camera (GTC 400 C, BOSCH).

**Drug Release of Proposed Mucoadhesive Patch**: The drug release of the proposed mucoadhesive patch was measured using UV–vis spectrometer (SPECTROstar Nano, BMG LABTECH) analysis. The patch was immersed in a PBS solution prewarmed to 37 °C. The concentration of the drug released from the patch (DOX) was measured at 490 nm after collecting the supernatant of this solution after a certain time. In this test, two PBS solutions (pH 7.4 and 2.0) were used, and measurements were conducted for the cases where the AMF (320 A, 272 kHz) was on and off. In the case of the AMF, stimulation was applied to the sample for 10 min, and then the same process was followed as for the case without the AMF.

**Ex Vivo Patch Delivery Test**: Ex vivo patch delivery tests were performed in the porcine small intestine and stomach to confirm the feasibility of active delivery of the mucoadhesive patches to various lesions in the gastrointestinal tract using a magnetically actuated capsule. Three target lesions were marked on the porcine small intestine using dye, and the mucoadhesive patches in the magnetically actuated capsule were delivered to the target lesions using the EMA system. Specifically, the magnetically actuated capsule with the closed cover was moved to the target lesion and aligned in the vertical direction (z-axis), and then the capsule was switched to the open state. The patch contacted the target lesion for 2 min by applying a gradient magnetic field in the z-axis direction. Then, the capsule was aligned in the horizontal direction through a horizontal uniform magnetic field, and the first patch was separated and delivered to the first target lesion. The capsule then switched to the closed state and moved to the next target lesion. Similarly, the second and third patches were actively delivered to their respective target lesions.

Next, an ex vivo test using a porcine stomach was performed. First, the porcine stomach was attached to a 3D commercial stomach model. The patches were delivered to multiple lesion sites using a magnetically actuated capsule through the magnetic field generated by the EMA system. Three patches were actively delivered by repeating the process in which the capsule was moved to the lesion site in the closed state, and the patch was sequentially delivered to the lesion sites after switching the capsule to the open state.

**In Vivo Therapeutic Effect Test of Proposed Mucoadhesive Patch Using HT-29 Cells**: The therapeutic effect of the proposed mucoadhesive patch was analyzed through an in vivo experiment using HT-29 cells. First, HT-29 cells (human colorectal cancer cell line, ATCC, USA) were cultured in an RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). HT-29 cells were counted using a cell counting kit-8 (CCK-8) and seeded at a concentration of 1 × 10⁶ per dish in a 35 mm cell culture dish. The cells were then incubated in a 5% CO₂ environment for 24 h. After HT-29 cells were cultured, the proposed patch was placed on the cultured cells, as shown in Figure 5. A therapeutic effect experiment was performed on six groups: control, mucoadhesive patch without DOX and MNPs (MP), mucoadhesive patches including MNPs (MP + MNPs) with and without an AMF stimulation, and mucoadhesive patches including DOX and MNPs (MP + DOX + MNPs) with and without an AMF stimulation. HT-29 cell viability was measured for each group 24 h after applying the experimental conditions to the cell culture dish seeded with HT-29 cells. In the groups with AMF stimulation, the AMF (320 A, 271 kHz) was applied for the initial 10 min. The nuclei of living cells were stained using DAPI to measure cell viability, and fluorescence images of cells were obtained using a fluorescence microscope. In addition, to confirm the overall cell viability of cancer cells, CCK-8 for each group was measured at 450 nm using a UV–vis spectrophotometer (SPECTROstar Nano, BMG LABTECH). Each experiment was performed four times, and statistical analysis was conducted using p-test and one-way analysis of variance. Results between groups were considered statistically significant when p < 0.005.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

Research data are not shared.

**Keywords**

active delivery, gastrointestinal cancer treatment, hyperthermia, mucoadhesive patch, multiple delivery
