Blood, Guts, and Hope

It is 2AM. You wake up with searing pain in your abdomen and you have the intense and urgent need to use the bathroom. But you go back to bed thinking it was probably something you ate.

However, you are woken up again at 6AM with the same mind-numbing pain and again race straight to the bathroom. You now know something is wrong. And it is completely unexpected, because you are generally healthy. You exercise and eat right, you get enough sleep. What could it possibly be? You decide you have to make an appointment with your doctor in the morning.

In the doctor's office, you explain the situation but insist you are healthy and take good care of yourself. The doctor begins some tests: poking, prodding, listening, and looking. You are uncomfortable and nervous.

The doctor informs you they need to perform a colonoscopy, which will require preparation, light anesthesia, and someone to drop you off and pick you up. The procedure is scheduled for Friday. All week, you experience the same nightly ordeal and now it also has begun happening during the day while you are at work. You have to sheepishly run out of an 11AM meeting to use the bathroom. Only now, you are also noticing a little blood in the toilet.

The day before the colonoscopy, you have to prepare by drinking liters of a salty, horrible-tasting solution. The doctor explained that this would help empty your bowels so that the physicians will be able to examine your intestine. You are doing your best to choke down the solution but it is the worst tasting drink you have ever had. And you still have two liters to go.

In the morning your best friend drops you off at the hospital. Before you know it, you are lying on a cold, hard table, in only a gown, and feeling sleepy from the anesthesia.

When you wake up you are lying in a room. A few moments later, the doctor enters and begins reviewing your colonoscopy with you. They found significant inflammation of your colon and that is what is causing your abdominal issues.

You, along with almost 60,000 individuals in the United States each year, were just diagnosed with inflammatory bowel disease. This chronic disease affects almost two million individuals in the United States alone. The disease is characterized by inflammation of the gastrointestinal (GI) tract. And the inflammation leads to injury of the organ, impairing its function. As a result, symptoms can include significant abdominal pain, diarrhea with the presence of blood, and the need for frequent bowel movements. Even worse is the social stigma associated with such a disease, which makes living a normal lifestyle almost impossible.

Living with a Chronic Disease

Inflammatory bowel disease encompasses two subtypes based on where the inflammation is located in the GI tract. The most prevalent subtype is *ulcerative colitis,* in which the inflammation is present in the distal colon and rectum and affects the outermost layer of the tissue (an inflammation of this region with similar symptoms, called *acute colitis,* can be brought on by a bacterial or fungal infection, but this illness usually clears up after one round of treatment). Crohn's Disease, the second type, is characterized by inflammation affecting the entire thickness of the GI tissue and can occur in any part of the GI tract from the mouth to the anus. Ulcerative colitis and Crohn's disease are not mutually exclusive, with many patients having both types.

Patients with this disease experience life-altering symptoms that have a tremendous negative effect on their quality of life. Worse, onset and diagnoses typically occurs in one's 20s, meaning patients are living with these symptoms their entire adult life. Our colons are responsible for absorbing a significant amount of water. When this water is not absorbed, it leads to diarrhea, incontinence, and frequent bowel movements, potentially up to 20 times a day. It is difficult to imagine being able to live one's life the same way after diagnosis, with patients having to constantly manage their symptoms and plan activities around their disease.

Like most inflammatory conditions, the first line of treatment options involves trying to get large doses of anti-inflammatory medications directly to the affected site. In the case of ulcerative colitis, where the inflammation is present in the distal colon and rectum, treatment regimens often include the use of medicated enemas and suppositories, which are administered at home by the patients themselves. If these treatments are strictly adhered to, they can be effective. But there is a catch: The enema or suppository has to be retained in the colon overnight.

Our GI tract is covered in a thick mucus layer that helps to protect the tissue. When drugs are administered to the GI tract, the mucus layer can be somewhat of a barrier to the drugs. The drugs must slowly make their way through the mucus layer before they get to the tissue. That's why enemas and suppositories must be retained for many hours to give the drugs enough time to get through the mucus and start entering the inflamed tissue before they can have a beneficial effect and reduce the inflammation. Because of the need for extended retention, patients are typically instructed to administer the enema before bed, because lying down can make retaining the enema easier. After emptying their bowels and lying down, patients then take a small applicator that looks like a large eyedropper and inserts the tip in the rectum so that they can infuse the fluid. They must then stay lying down for as long as possible. The entire regimen is a precarious and uncomfortable experience that must be endured every night for weeks or even months at a time. And when patients are suffering from active disease and are experiencing urgency and frequent bowel movements, they need the medication the most, but that's when retention is almost impossible. As a result of ineffective treatment, 70 percent of patients are diagnosed as having uncontrolled disease. This sustained, recurring injury and inflammation eventually destroys the colon, and 20 percent of patients have to have parts of their colon surgically removed.

The increasing severity of the disease over time is enabled by poor control of the disease when it is first diagnosed, which is predominantly a result of difficulties with retention of the enema, resulting in little therapeutic delivery to the site of inflammation. What these patients need is a method to deliver the anti-inflammatory drugs directly to the site of inflammation without the need for retaining the enema. Such an option could potentially enable greater control of their disease, and allow self-administration of their treatment on the go instead of being limited to at-home application when lying down, and thus lead to a significant improvement in quality of life.

Speedy Delivery

The problem of ultra-rapid delivery is an interesting and challenging question that we have been tackling in our laboratory at the Massachusetts Institute of Technology. Our research has focused on the use of *physical enhancers* to potentially enable ultra-rapid delivery of drugs locally to the GI tract. Physical enhancers are technologies that help drug delivery using mechanisms not limited to passive diffusion. A traditional enema, for example, is delivered by the natural diffusion of the drug through the thick mucus layer lining the GI tract. Physical enhancers could actually "propel" the medication into the tissue.

Our work has focused specifically on the use of ultrasound to achieve this ultra-rapid delivery. Ultrasound is a pressure wave, similar to sound waves, but with a frequency above our limit of detection, which is approximately 20 kilohertz.

Most individuals are familiar with the use of ultrasound in the clinic for imaging. Ultrasonic imaging uses very high frequencies, typically above 1 megahertz, to visualize structures within the body. For drug delivery applications, our group and others have shown that low-frequency ultrasound, with a frequency below 100 kilohertz, is most useful, because of a phenomenon known as *acoustic cavitation.* When ultrasound waves travel through fluid, the varying pressure gradient can spontaneously grow small bubbles in the fluid. These bubbles oscillate and grow in size because of the oscillating pressure field produced by the ultrasound. Some bubbles actually grow so large that they can no longer support themselves and they implode in a phenomenon known as *transient cavitation.* In this case, the surrounding fluid rushes into the previously empty space of the bubble, creating a *microjet.*

These microjets are tiny, powerful streams of fluid that travel at very high velocities. When these jets hit tissue, they can painlessly and temporarily make it more permeable and propel therapeutics directly into the tissue. As a result, we have investigated the use of ultrasound for ultra-rapid drug delivery in the rectum.

Ultrasound Enablers

We created a prototype device capable of administering an enema while simultaneously emitting low-frequency ultrasound through the enema, to induce transient cavitation within the rectum.

We have tested this device in multiple animal models, including both rodents and pigs. Rigorous safety and tolerability studies were performed to ensure that tissues would abide the treatment well. We have tested both single administration, and daily administration for up to five weeks. We then analyzed issue health by observing tiny tissue samples imaged using a microscope. Our observations found no evidence of damage to the tissue locally. Moreover, we have looked at potential injury to all internal organs and have found no evidence of any adverse effects. We also analyzed the potential for bacteria in the gut to inadvertently cross the tissue layer while it was made more permeable by the ultrasound, and get into the blood stream, but we found no evidence of this happening. Indeed, the microjets that are formed as a result of the ultrasound treatment are much smaller than even the tiniest bacteria. In addition, even more aggressive procedures—such as tissue biopsy collection over the course of a routine colonoscopy, which can result in significant tissue injury—are well tolerated and rarely result in complications. In mice, we further profiled cytokines in tissue, molecules that can act as danger signals to immune cells, and observed no change in type or amount of cytokines present after ultrasound treatment.

We tested the therapeutic benefit of the treatment in a mouse model of GI inflammation, where we examined treatment with standard enemas using the anti-inflammatory drug mesalamine, compared to the mesalamine enema in combination with ultrasound. Ultrasound was found to significantly enhance the benefit of treatment, and even completely removed signs of the disease in those animals receiving ultrasound. Mesalamine enemas alone were found to have no therapeutic benefit compared to animals receiving no treatment, an observation that has previously been reported in mice because of very rapid gastric exit times, reducing the time of absorption. The tremendous benefit observed through the simultaneous use of ultrasound further underscores the rapidity of delivery of the mesalamine into the tissue, where it can begin reducing inflammation.

More recently we have begun exploring the delivery of more complex therapeutics, such as DNA and RNA. These molecules hold great promise but have posed significant challenges to creating therapies. In part the limitations include the delicate nature of these molecules and their susceptibility to degradation, as well as the need to not just get this material into tissue, but into specific cells within the tissue. We have observed promising results on the delivery of naked, unencapsulated RNA locally into the GI tract, something that has not been reported to our knowledge. The ability to potentially deliver a broad-range of therapies without the need for tedious formulation development might enable a new treatment paradigm for GI-based diseases. Enabling more efficacious treatments that can successfully be used by patients would open a new door in an often-overlooked set of diseases.