Researchers make significant progress in engineering digestive system tissues

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Researchers at Wake Forest Institute for Regenerative Medicine have reached important milestones in their quest to engineer replacement tissue in the lab to treat digestive system conditions - from infants born with too-short bowels to adults with inflammatory bowel disease, colon cancer, or fecal incontinence.

Reporting today in *Stem Cells Translational Medicine*, the research team verified the effectiveness of lab-grown anal sphincters to treat a large animal model for fecal incontinence, an important step before advancing to studies in humans. And last month in *Tissue Engineering*, the team reported success implanting human-engineered intestines in rodents.

"Results from both projects are promising and exciting," said Khalil N. Bitar, Ph.D., AGAF, senior researcher on the projects, and professor of regenerative medicine at the institute. "Our goal is to use a patient's own cells to engineer replacement tissue in the lab for devastating conditions that affect the digestive system."

Sphincter Project: The lab-engineered sphincters are designed to treat passive incontinence, the involuntary discharge of stool due to a weakened ring-like muscle known as the internal anal sphincter. The muscle can lose function due to age or can be damaged during child birth and certain types of surgery, such as cancer.

Current options to repair the internal anal sphincter include grafts of skeletal muscle, injectable silicone material or implantation of

mechanical devices, all of which have high complication rates and limited success.

"The regenerative medicine approach has a promising potential for people affected by passive fecal incontinence," said Bitar. "These patients face embarrassment, limited social activities leading to depression and, because they are reluctant to report their condition, they often suffer without help."

Bitar's team has been working to engineer replacement sphincters for more than 10 years. In 2011, the team was the first to report functional, lab-grown anal sphincters bioengineered from human cells that were implanted in immune-suppressed rodents. The current study involved 20 rabbits with fecal incontinence. Eight animals were treated with sphincters engineered from their own muscle and nerve cells, eight animals were not treated and four received a "sham" surgery.

The sphincters were engineered using small biopsies from the animals' sphincter and intestinal tissue. From this tissue, smooth muscle and nerve cells were isolated and then multiplied in the lab. In a ring-shaped mold, the two types of cells were layered to build the sphincter. The entire process took about four to six weeks.

In the animals receiving the sphincters, fecal continence was restored throughout a three month follow-up period, compared to the other groups, which did not improve. Measurements of sphincter pressure and tone showed that the sphincters were viable and functional and maintained both the muscle and nerve components. Currently, longer follow up of the implanted sphincters is close to completion with good results..

Intestine Project: The intestine project is aimed at helping patients with intestinal failure, which is when the small intestine malfunctions or is too

short to digest food and absorb nutrients essential to health. Patients must get nutrition through a catheter or needle. The condition has a variety of causes. Infants can be born with missing or dysfunctional small intestines. In adults, surgery to remove sections of intestine due to cancer or other disease can result in a too-short bowel. Intestinal transplant is an option, but donor tissue is in short supply and the procedure has high mortality rates.

"A major challenge in building replacement intestine tissue in the lab is that it is the combination of smooth muscle and nerve cells in gut tissue that moves digested food material through the gastrointestinal tract," said Bitar.

Through much trial and effort, his team has learned to use the two cell types to create "sheets" of muscle pre-wired with nerves. The sheets are then wrapped around tubular molds made of chitosan, a natural material derived from shrimp shells. The material is already approved by the U.S. Food and Drug Administration for certain applications.

In the current study, the tubular structures were implanted in rats in two phases. In phase one, the tubes were implanted in the omentum, which is fatty tissue in the lower abdomen, for four weeks. Rich in oxygen, this tissue promoted the formation of blood vessels to the tubes. During this phase, the muscle cells began releasing materials that would eventually replace the scaffold as it degraded.

For phase two, the bioengineered tubular intestines were connected to the animals' intestines, similar to an intestine transplant. During this six-week phase, the tubes developed a cellular lining as the body's epithelial cells migrated to the area. The rats gained weight and studies showed that the replacement intestine was healthy in color and contained digested food.

The researchers are excited by the results and their next step is to test the structures in larger animals.

"Our results suggest that engineered human intestine could provide a viable treatment to lengthen the gut for patients with gastrointestinal disorders, or patients who lose parts of their intestines due to cancer," said Bitar.

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