Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model

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Endoscopic transplantation in oesophageal ulceration

The late transplantation pioneer, Norman Shumway, MD, was fond of saying, "The future of transplantation is xenotransplantation. And, it always will be." Tissue engineering, the *in vitro* creation of functional replacement tissues, has been proposed as a way to replace lost or damaged tissues due to acute or chronic disease for nearly 20 years.¹ Despite the tremendous interest, there are a few tissue-engineered constructs that have gained considerable acceptance in clinical use, although progress is being made. Ohki *et al*,² demonstrate recent progress of the potential application of tissue engineering in endoscopy.

The complexity of organs and the lack of knowledge of how these organs are patterned in their development have made tissue engineering of solid organs like the liver difficult.³ In the case of the liver, the construct must have two separate vascular inflows, a single vascular outflow and a separate biliary drainage system. This also necessitates a particular polarity in the organisation of hepatocytes. Thus, despite the desire and need to replace liver allografts as a treatment for end-stage liver disease, there is no solution in sight. Because of the complexity of many solid organs, tissue engineering has turned to relatively simpler structures like bone, cartilage and skin, and it is likely that the first clinically successful tissue engineered construct will be one of these tissues.

In the study by Ohki *et al*,² the authors are not engineering an entire oesophagus, but rather are attempting a simpler task. Their goal is the restoration of the protective stratified epithelium after endoscopic submucosal dissection using cultured sheets of cells harvested from the buccal mucosa. They report short-term survival of these grafts with improved healing or the areas of resected oesophageal mucosa.

Their study is consistent with what is known about skin wound healing. It is known from the treatment of burn patients that more rapid re-epithelialisation leads to decreased scarring and contracture.⁴ By overlaying an epithelial sheet over the area of resected oesophageal mucosa, the authors are potentially minimising the scarring that can lead to stenosis. The proposed mechanism for the decrease in fibrosis and contracture in the burn model is an epithelial-mesenchymal interaction. A major technical obstacle to overcome would be to prevent dislodging of the graft by normal peristalsis in the oesophagus, but the authors report no problems with this in their small numbers of animals. The only method they describe to minimise this problem is to limit the animals to water intake alone during the first 2 days after the procedure.

A number of parallels exist between the reported study and what has been attempted for skin wound healing. A number of studies reported injection of cells into a chronic, non-healing wound. The cells can be fibroblasts or keratinocytes. More recently, there has been a surge of interest with the use of adult multipotent cells or mesenchymal stem cells. In all of these cases, the use of a cellular graft has led to improved wound healing, regardless of the cell type used. Although there has been no direct comparison of the different cell types, the data would suggest that each aids wound healing to a similar degree. What has also been clear is that there is no long-term persistence of the cells that have been grafted in place. Short-term survival of transplanted cells up to 2 weeks has been described by some reports, but there is no long-term persistence.⁵ Because the type of cell does not seem to matter, nor is there long-term engraftment, the interpretation has been that these cells func-

tion more as factories providing a number of growth factors that aid in wound healing rather than actually incorporating in the eventual repaired tissue. In their paper, Ohki *et al*² have shown persistence of their transplanted cells up to 8 days, and this is consistent with wound-healing data. It would be informative if they follow these animals to the point of complete resolution of mucosal healing and then assay for the presence of transplanted cells.

The importance of epithelial-mesenchymal interactions has been shown in the short history of attempts to create tissue-engineered bowel. The initial studies consisted of simply seeding cultured enterocytes to a scaffold.⁶ Although the authors were able to grow these cells appropriately, they did not form villous structures as would be required to create enough absorptive surface area in engineered intestine. Later studies by this group showed that the use of what they termed "epithelial organoid units" led to improved morphology and histology with the formation of crypt and villous structures.⁷ The key addition in this preparation was the smooth muscle cells of the muscularis mucosa that normally form the mesenchymal layer underneath the intestinal epithelium. Other studies have just transplanted smooth muscle cells in intestinal grafts that were then sewn as patches on normal intestine and found that crypt/villous architecture is better with the presence of the smooth muscle cells.⁸ In this case, the epithelial cells were believed to have migrated to the graft from the surrounding normal intestine.

What is still unclear is whether the presence of the crypts and villi in the engineered intestine that histologically seems similar to normal bowel epithelium translates into adequate absorptive function. These authors have shown the expression of appropriate enzymes for processing carbohydrates and of transport molecules for the absorption of glucose.⁹ They have used an Ussing chamber model to demonstrate that the neo-mucosa is capable of generating the same trans-epithelial electrophysiological gradient as normal ileal mucosa, suggesting that electron and fluid transport in the neo-mucosa is adequate. However, it remains to be seen whether a section of engineered intestine placed in continuity with the intestinal tract is capable of adequate absorption of fluid, nutrients and electrolytes.

An added problem is the creation of a vascular supply. Although a tubular construct can be bathed in culture media *in vitro*, implantation of any significant length would require developing a vascular supply. An ingenious method of using

the body as a bioreactor was devised, where the tissue-engineered construct would be seeded with cells in vitro, but then transplanted into the animal for further growth.⁷ After implanting the construct into the omentum, angiogenesis would lead to vascularisation of the construct. Because the omentum has a vascular tree, implantation of the construct in the distal vascular tree could allow harvest of the graft on a vascular pedicle for anastomosis to the native intestine.

In normal intestine, there exists an extensive lymphatic system that is important in normal absorptive processes as well as for immune functions. A similar lymphatic system would be required in engineered intestine. Specific attempts to engineer lymphatics have not been successful, but in a study of tissue-engineered small intestine that was created using intestinal organoid units on a scaffold transplanted into the omentum, Duxbury *et al* report that they detected structures in the intestine that are negative for markers of vascular endothelial cells but positive for markers of lymphatic endothelium.¹⁰ As the authors are using a heterogeneous mix of cells in their intestinal organoid units, it is possible that the cells or signals to create a lymphatic structure are present as well. It remains to be seen by which mechanism these lymphatics are created, and whether they are adequate to perform the functions required of them.

An important final requirement for tissue-engineered intestine is the ability to undergo peristalsis in a co-ordinated manner with the rest of the gastrointestinal tract. Failure of proper peristalsis

would lead to a partial or complete functional bowel obstruction depending on the degree to which the intestinal contents are able to pass through that segment. The presence of ganglion cells has been reported in engineered intestine, but there are no data on whether these are capable of co-ordinating with the surrounding bowel to allow for peristalsis.¹¹

It is clear that there are considerable obstacles to creating functional tissue-engineered intestine that can be clinically used. Several groups have tackled this problem in a stepwise manner to create structures that histologically seem similar to a normal bowel. Tissue-engineered oesophagus has been reported.¹² In an animal model, use of engineered oesophagus as an onlay patch led to a normal-appearing oesophagus by fluoroscopy. However, use of the engineered oesophagus as an interposition graft led to a dilated sac. The next major step in the experimental work is to show that these constructs can be functional as an interposition graft. The work of Ohki *et al*² shows how the knowledge that has been gained about tissue engineering in gastrointestinal tract can be used in some more limited applications.

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Therapeutic potential of fractalkine

Therapeutic potential of fractalkine: a novel approach to metastatic colon cancer

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Fractalkine is involved in the pathogenesis of different types of cancer and in various clinical disease states

Several experimental approaches have shown that a variety of chemokines have anti-tumour activity either by chemoattracting natural killer cells, monocytes and macrophages, or by

accumulating dendritic cells.¹ Accumulating evidence has shown that fractalkine (CX3CL1), the unique member of the CX3C chemokine subfamily, is involved in the pathogenesis of different

types of cancer^{2–3} and in various clinical disease states beyond cancer, such as atherosclerosis, glomerulonephritis, rheumatoid arthritis, HIV disease and sepsis.^{4–6} In contrast with other chemokines, fractalkine exists in two forms, each mediating distinct biological actions.⁷ The membrane-anchored protein, which is expressed primarily on the endothelium, serves as an adhesion protein promoting the retention of monocytes and T cells.⁸ The soluble form originates from extracellular proteolysis by proteases, such as tumour necrosis factor- α converting enzyme (also known as ADAM17) and ADAM10.⁹ The secreted form resembles more a conventional chemokine and strongly induces chemotaxis and causes migration of natural killer cells, cytotoxic T lymphocytes and macrophages. Both chemotaxis and adhesion are mediated by the G protein-coupled receptor CX3CR1,¹⁰