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Project: Growing enteroids to study bowel disease

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Introduction:

Crohn's disease is a chronic inflammatory condition that affects the whole gastrointestinal tract. The exact cause is unknown but the result is an exaggerated and self-perpetuating immune response against intestinal bacteria. This causes severe abdominal pain, diarrhoea, fever and weight loss, symptoms which increase in severity in 'flares' and then get better in periods of remission.

The majority of sufferers are treated with medications that suppress the immune system when symptoms are bad, such as steroids and immuno-modulators, but these are stopped or given at a reduced dose in times of remission due to their undesirable side effects.

A nutritionally complete liquid diet, referred to as polymeric formula (PF), is sometimes used as a treatment for acute flares and has been found to be just as effective as immunosuppression in inducing remission. However its effects on the intestinal environment are poorly understood.

Intestinal epithelial cell lines grown in the laboratory are often sourced from cancerous tumours. They are relatively easy to manipulate and because they are selected to be genetically identical, results are consistent. However these cells are markedly different from those that would be found in a real, functioning gut and the results generated may not be clinically useful.

Enteroids are a recently developed, laboratory grown, model of the intestinal tract. They are derived from live intestinal tissue obtained during a colonoscopy or animal dissection. The cells that line the inside of the intestinal tract, called enterocytes, propagate in an environment that closely mimics the conditions in vivo.

The cells are immersed in a gelatinous matrix of basement membrane components such as laminin and collagen IV. Growth factors that would normally be provided by other cell types and nutrients that would have been supplied by the blood are instead added to the liquid medium surrounding the matrix.

This allows researchers to observe directly how the gut responds to various stimuli, without needing to harm the person from which the cells were derived and also generates results that give a more accurate approximation of what really happens in vivo. Change can be determined by observing differences in enteroid appearance that reflect increased or decreased rates of cell division and by change in expression of a commonly used marker of cell maturity called intestinal alkaline phosphatase.

Aim:

1. To grow mouse enteroids and observe normal development over time.
2. To determine the effect the polymeric formula has on enteroid cell differentiation and function through expression of intestinal alkaline phosphatase.

Method:

Live tissue was harvested from wild-type laboratory mice that have been humanely euthanised and dissected.

Enterocytes were first separated from the surrounding connective tissue and muscle, followed by the removal of larger cell wall fragments, leaving fractions of stem cell-rich intestinal crypts. These cells were immersed in a specialised matrix called Matrigel and incubated with sufficient growth factors and nutrients, enabling them to propagate and differentiate over time. New growth factors were added every second day to keep the stem cells dividing and to assist enteroid development. Polymeric formula brand Osmolite was diluted to 20% (v/v) in the enteroid liquid medium. Changes in enteroid structure were recorded by taking images at 4x, 10x and 20x magnification over 24 hours of treatment. At this point the enteroids were fixed and stained with an anti-intestinal alkaline phosphatase antibody.

Results:

The mouse enteroids formed cysts within the first 24 hours and increased in size over the first 10 days. They developed a polarised barrier with the absorptive side facing the hollow interior. New stem cell containing crypts were formed from the fifth day onwards. Enterocytes, Paneth cells and Goblet cells were produced by these stem cells and could be identified by their position, size and opacity. Addition of a physiological concentration of polymeric formula (20% v/v) resulted in a rapid dissociation of enteroid structure over 24 hours, but not significant cell death.

A similar effect was seen when untreated enteroids were deprived of essential growth factors. Doubling the amount of growth factors delayed, but did not prevent the effect that PF has on enteroid structure. Fat soluble and aqueous fractions of PF also caused the enteroids to fall apart, which suggests that more than one component is capable of causing enteroid failure.

There was no discernible difference in IAP expression between cells that were part of the enteroid structure and cells that had dissociated, in both untreated and polymeric formula treated cells. This indicates that despite a loss of structural integrity, PF-treated enteroids still exhibited a similar level of IAP staining when compared to untreated controls.

Conclusion:

These results suggest that experimental use of enteroids may not be suited to experimentation with multiple ingredient stimuli. Instead, this model may be limited to evaluating stimuli such as drugs or single dietary agents where the risk of interference is much lower. Additionally, enteroids seem especially vulnerable to any inhibition of growth stimulating cellular pathways.