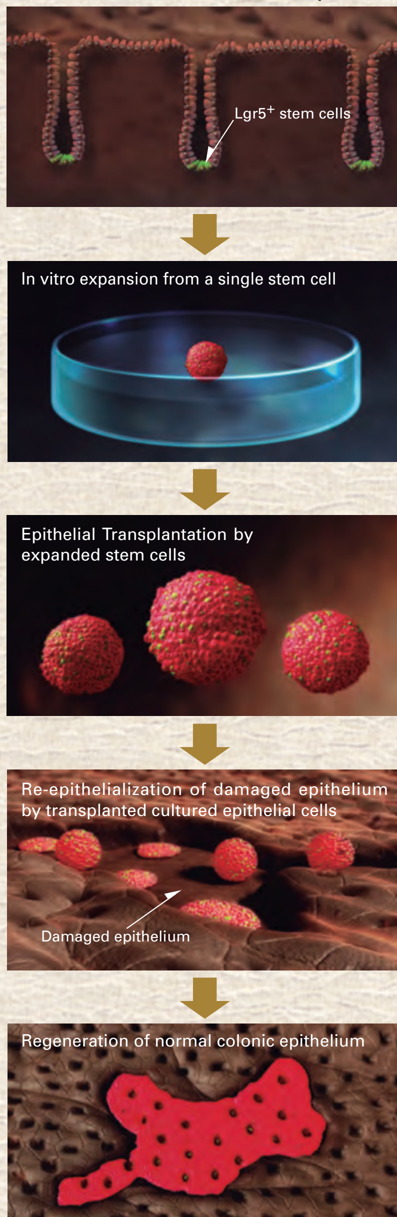
# Functional Engraftment of Colon Epithelium Expanded In Vitro from a Single Adult Lgr5+ Stem Cell

**EPITHELIAL REGENERATION IS** one of the critical steps necessary for the healing of wounds at the surface of small intestine and colon. We have investigated this process intensely and previously reported that damaged epithelia of the human intestinal tract were partly rescued by bone marrow-derived cells.[1] However, regeneration of wounded surfaces mainly depends on the re-expansion of epithelial cells after damage and adult epithelial stem-cell therapy has been considered to hold promise for the treatment of various gastrointestinal disorders such as inflammatory bowel diseases, while it was impossible to evaluate even its feasibility because of the technical difficulties to expand intestinal epithelial stem cells *in vitro*.

***Fig. 1:***
**Colonic Stem Cell Culture and Transplantation**




Our group led by Drs. Shiro Yui and Tetsuya Nakamura recently succeeded to show the first positive evidence for these very challenging issues.[2] In this paper, we firstly reported our original organoid culture methodology, named TMDU protocol for long-term expansion of colonic stem cells, in which cells that are positive for leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5). In brief, we used Type I collagen gel as an extracellular matrix of this three-dimensional culture protocol and the medium that we used contained only five types of recombinant proteins (Wnt3a, R-spondin1, Noggin, EGF and HGF) and BSA. Interestingly, $Lgr5^+$ colonic stem cells appeared to expand unrestrictedly in terms of their proliferative capacity under this defined and completely serum-free condition, forming round cystic structures called colon organoids which maintained their original colonic identity. Secondly, we tested the transplantability of these cultured colonic epithelial cells by reintroducing $GFP^+$ colon organoids into a superficially damaged mouse colon, and found that transplanted donor cells readily integrated into the mouse colon, covering the area that lacked epithelium as a result of the introduced damage by Dextran Sodium Sulfate (DSS) in recipient mice. At four weeks after transplantation, the donor-derived cells constituted a single-layered epithelium, which formed self-renewing crypts that were functionally and histologically normal. Moreover, we observed long-term (>6 months), histologically normal engraftment with

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transplantation of organoids derived from a single adult $Lgr5^+$ colon stem cell after extensive *in vitro* expansion. In addition, engrafted recipient mice had higher body weights than ungrafted controls, implying a beneficial role of epithelial transplantation at least in DSS-induced acute colitis model. These observations clearly confirmed that *Lgr5* marks genuine stem cells that retain their self-renewal and multilineage-differentiation properties even after prolonged culture, and also they exactly revealed the feasibility of colon stem-cell therapy based on the *in vitro* expansion of a single adult colonic stem cell.

Our study provided for the first time a proof of principle that cultured $Lgr5^+$ cells can be used for stem-cell therapy to repair damaged epithelium. The most out-standing message of this paper was strongly highlighted in *Nature* as being "one-step closer to gut repair."[3] Although further optimization is clearly needed, our study strongly implies that *in vitro* expansion and transplantation of gastrointestinal stem cells may be a promising, simple and safe option of regenerative and gene-therapy strategies for patients with severe gastrointestinal epithelial injuries.

References

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