Benchmarks

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Separation of intact intestinal epithelium from mesenchyme

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BioTechniques 55:42-44 (July 2013) doi 10.2144/000114055
Key words: intestine; epithelium; mesenchyme; separation

Current protocols for separating adult intestinal epithelial cells from the underlying muscular and mesenchymal tissues typically involve extended incubations, harsh mechanical treatment, and exposure to either proteases or chelating agents. The drawbacks of these approaches include fragmentation, contamination with other cell types, reduced viability, and under-representation of crypt cells. Here we describe a gentle procedure that allows harvesting of pure, fully viable sheets of murine intestinal epithelium, with intact crypts and villi, without enzymes or EDTA. The mesenchyme retains intact villus core projections, is virtually free from epithelial cells, and can be cultured in vitro.

Recent advances in intestinal stem cell biology, gastrointestinal carcinogenesis, and host–microbiota interactions have depended on isolation and in vitro culture of intestinal epithelium. Protocols for separating epithelial cells from the connective tissue of the lamina propria (1–8) typically rely on mechanical agitation of the tissue alone or in combination with proteolytic enzymes, chelating agents, or altered osmolarity. As a result, neither epithelial nor mesenchymal preparations are free from contamination by the other cell type. Detached cells are recovered by centrifugation, which inevitably results in lysis of some cells and reduces the yield of, for example, intact goblet cells. Isolation of intact epithelium requires perfusion of the animal with a high concentration of EDTA and release of the epithelium by vibration (9). Perreault and co-workers used BD Cell Recovery Solution (or "MatriSperse"; BD Biosciences, Franklin Lakes, NJ), a non-enzymatic solution that depolymerizes the extracellular matrix of the basement membrane, to obtain pure epithelial preparations (10–12). However, that method requires extended incubations (24 h for adult cells) followed by shaking to release the epithelium, generating tissue fragments that are recovered by centrifugation.

The method described here involves neither enzymes nor EDTA, is performed at low temperature, and completed in only ~30 min. It takes advantage of the ability of BD Cell Recovery Solution to dissolve the basement membrane and uses air pressure inside an inverted intestine to push the epithelial crypts out of the mesenchymal crypt beds. Since perfusing the animal is not required, the inverted intestine can be washed thoroughly in saline without damage to the tissue. Recovery of the detached epithelium as a coherent sheet with intact crypts and villi eliminates the need for centrifugation and ensures excellent tissue integrity and cell viability. When a single cell suspension is desired, for example for FACS sorting or organoid culture (13), this is easily achieved by brief exposure to trypsin. Since tryptic dissociation is performed on the isolated epithelium, there is no contamination by mesenchymal cells. We demonstrated this protocol on murine intestine, but with minor adjustments it should be applicable from other vertebrates.

The dissected intestine is cut into pieces of 4–5 cm in length and flushed several times with ice-cold phosphate buffered saline (PBS). Each piece is inverted and washed thoroughly by pipetting cold PBS over the tissue to completely remove mucus and gut contents. Inversion is most easily performed by inserting a hooked or dented plastic or stainless steel rod such as a fine steel crochet hook into the gut lumen, securing the rod end with suture, and pulling the rod back (Figure 1A).

Following inversion, one end of an inverted piece of intestine is ligated with suture (Vicryl 3-0; Ethicon Inc, Somerville, NJ). The other end is fitted to the tip of an Eppendorf Combitip Plus (Eppendorf, Hamburg, Germany) with the plunger pulled out and the barrel filled with air and tightly secured with suture (Figure 1A, B). The optimal Combitip size for murine small intestine is 0.5 mL (Eppendorf catalog number 022496042), and for the colon 1.25 mL (Eppendorf catalog number 022261100). To prevent the suture and intestine from slipping off the plastic, a groove may be carved in the end of the Combitip by rotating it in the jaws of surgical scissors.

The tissue is submerged in ice-cold BD Cell Recovery Solution (BD Biosciences, Franklin Lakes, NJ) in a 15 mL

Method summary:

Intact, contamination-free sheets of epithelium from small intestine or colon are isolated in only 30 minutes on ice, without enzymes or EDTA, by application of air pressure on inverted intestine submerged in a solution that dissolves the basement membrane. Both epithelium and mesenchyme retain tissue integrity and can be cultured in vitro.

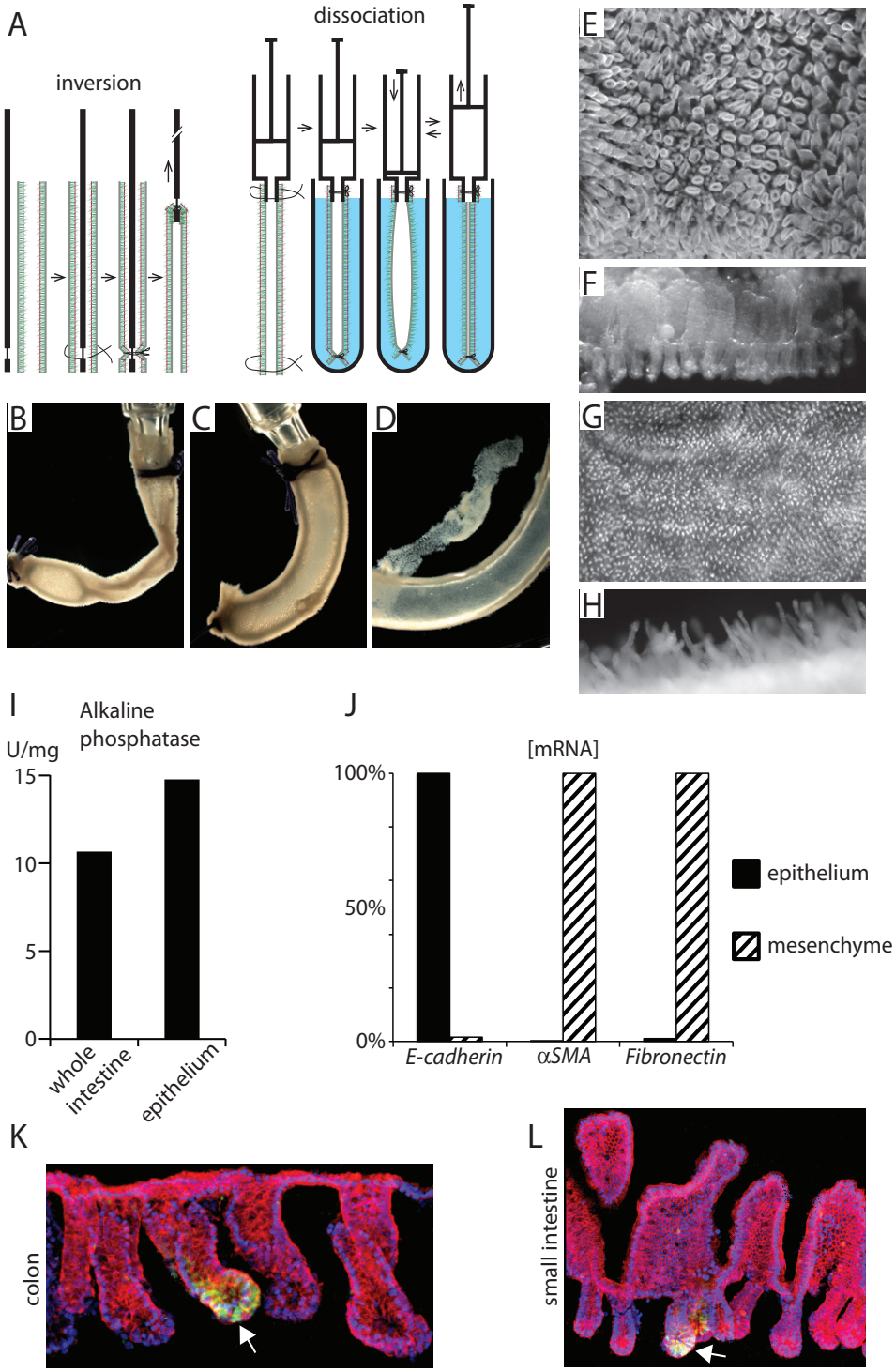

Figure 1. Separation of intact intestinal epithelium from mesenchyme. (A) Schematic of the procedure. A plastic or stainless steel rod, with a dent or hook at the distal end, is inserted into a piece of intestine, secured with suture, and pulled back to invert the intestine, which is then thoroughly washed in saline. The inverted intestine is fitted on the tip of an air-filled Combitip Plus, secured with suture, and sealed at the distal end with another piece of suture. The intestine is submerged in ice-cold BD Cell Recovery Solution, inflated with air from the Combitip and left on ice for 20 min with occasional deflation/re-inflation. (B) Inverted piece of small intestine fitted on Combitip. (C) Intestine inflated with air. (D) Removal of epithelium as a coherent sheet from inflated intestine. (E) Isolated epithelium viewed from the luminal side showing intact villi. (F) Edge of epithelial sheet in profile, with villi above and crypts below. (G) Luminal surface of mesenchyme after removal of epithelium, with projecting villus cores seen as whitish specks and empty crypt beds as dark spots in between. (H) Profile view of mesenchyme after removal of epithelium, showing the intact, bristle-like villus cores. (I) Alkaline phosphatase activity measured on fresh whole intestine and isolated epithelium immediately after separation from mesenchyme, expressed as units/mg protein. (J) Relative abundance of mRNA encoding markers for epithelium (E-cadherin) and mesenchyme (α-smooth muscle actin and fibronectin) in isolated epithelium and mesenchyme, normalized against the 36B4 transcript (14). (K and L) Fluorescence micrographs of vibratome sections of isolated murine colon (K) and small intestine (L) epithelia from adult *mTmG*; *Lgr5-EGFP-ires-CreERT2* mice (15,16). The Lgr5+ stem cells at the bottom of crypts express *EGFP* (green fluorescence, white arrow; expression of the *EGFP* transgene is variegated and most crypts do not express it). The *mTmG* transgene labels all cells red, and nuclei are stained blue with DAPI.

tube and placed on ice. The intestines are then inflated by pushing down on the plunger (Figure 1A, C). The submerged intestine should be deflated and re-inflated with the Combitip plunger every 5 min. During this time, the BD Cell Recovery Solution dissolves the basement membrane and the air pressure pushes the crypts out of the crypt beds. After 20 min, the intestine is rinsed by dipping it in cold PBS. The epithelial layer, which has now detached from the underlying mesenchyme, is recovered with either a tungsten needle or very fine tweezers (Figure 1D).

As shown in Figure 1E, F, K, and L, sheets of epithelium isolated by this procedure retained their 3-D structure, with crypts and villi intact. The muscular/mesenchymal layer left behind also preserved its in vivo structure, with the mesenchymal cores of the villi sticking out like bristles on a brush and the crypt beds visible as tiny holes in between (Figure 1G, H). The alkaline phosphatase activity of the epithelium did not decrease during the procedure (Figure 1I). qPCR quantification of mRNAs expressed specifically in the epithelium (E-cadherin) or the mesenchyme (α-smooth muscle actin and fibronectin), showed that each preparation was essentially free from contamination by the other cell type (Figure 1J). No cell lysis or cellular debris was detected, and neither epithelial nor mesenchymal cells took up Trypan blue. A brief exposure of the epithelial sheet to trypsin (2 min, 37°C, 2.5 mg/mL) (Sigma-Aldrich, St Louis, MO) produced a single-cell suspension that retained >95% viability (Countess, automated cell counter; Invitrogen, Carlsbad, CA). The intact mesenchyme was cultured for ten days in vitro in conventional cell culture medium (Dulbecco's modified Eagle's medium, 10% fetal bovine serum, penicillin-streptomycin; Invitrogen), during which it grew in thickness and assimilated the villus cores into a smooth surface. Epithelial cells remain alive in conventional in vitro culture (10), but do not proliferate, whereas Lgr5+ stem cells from crypts can form crypt organoids in Matrigel (BD Biosciences) with appropriate additives (13).

BD Cell Recovery Solution is widely used to isolate cells cultured in Matrigel and its compatibility with subsequent in vitro culture is well established. According to the manufacturer, it has no enzymatic activity and contains no EDTA but instead depolymerizes the extracellular matrix, thereby disrupting the basement membrane.

The procedure described here provides a rapid and efficient way to isolate epithelium and mesenchyme as starting material for in vitro culture, gene expression analysis, and biochemical characterization. The integrity of the epithelium offers exciting new possibilities for organ culture and tissue recombination.

Acknowledgments

We thank Mozhgan Ghiami for technical assistance. This work was supported by grants to PC from the Swedish Medical Research Council and the Swedish Cancer Foundation.

Competing interests

The authors declare no competing interests.

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Received 28 February 2013; accepted 16 May 2013.

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