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Acta Biomaterialia 5 (2009) 76–83
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Bioactive glass modulation of intestinal epithelial cell restitution

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Received 22 April 2008; received in revised form 17 July 2008; accepted 12 August 2008
Available online 26 August 2008

Abstract

Repair of superficial injury to the gastrointestinal mucosa involves the process of restitution, the rapid migration of epithelial cells across damaged areas. The effect of 45S5 bioactive glass on epithelial restitution was assessed using a novel co-culture model incorporating wounded intestinal epithelial cell monolayers and sub-epithelial myofibroblasts to simulate in vivo conditions that occur during superficial mucosal ulceration. Epithelial wound healing was not increased by culture medium conditioned with bioactive glass, with 1% (w/v) bioactive glass inhibiting cell migration. Epithelial wounds co-cultured with myofibroblasts grown on surfaces coated with 0.1% (w/v) bioactive glass increased wound healing compared with co-cultures containing no bioactive glass. Myofibroblasts grown on surfaces coated with bioactive glass secreted significantly increased amounts of basic fibroblast growth factor but did not increase epithelial cell proliferation, indicating the wound healing observed was due to restitution. These data from a model of superficial mucosal injury suggest bioactive glass may function as a stimulant of paracrine mucosal signaling networks that promotes rapid epithelial repair.

Keywords: Bioactive glass; Epithelial; Restitution

1. Introduction

Epithelium lining the gastrointestinal tract is formed of a highly dynamic population of cells which rapidly proliferate and turnover every 24–96 h [1]. The lining forms an important barrier to a wide range of noxious substances present in the lumen. Injury to this epithelial barrier can occur as a result of inflammatory bowel disease, peptic ulcer disease with resultant erosions or ulcerations, infectious agents and/or their toxins, ischaemia or radiation. Such damage may lead to systemic penetration of toxins and immunogenic factors, causing generalized inflammation and remote organ pathology. Therefore, rapid resealing of breaks in the continuity of the epithelium caused by injury or physiological damage is crucial in order to preserve normal homeostasis.

Mucosal injury can be classified as deep or superficial, deeper injuries being associated with more extensive haemorrhage and tissue necrosis. Superficial injury is limited to the upper region of the mucosa and involves sloughing of epithelial cells but relative preservation of the underlying basal lamina. Repair of these injuries involves a process termed epithelial restitution [2]. This process has been observed both in vivo and in vitro and involves the migration of epithelial cells adjacent to the wound over the denuded basal lamina. Restitution requires epithelial cells to flatten, spread, migrate and finally repolarise [3]. The process is independent of cell proliferation, occurs within minutes to hours and is suggested to be an initial mechanism to prevent deeper mucosal damage [2,4]. Re-establishment of epithelial continuity by restitution may be followed by cell proliferation to replace the decreased epithelial cell population [5]. Subsequently, the undifferentiated epithelial cells undergo maturation and differentiation.

Peptide growth factors are expressed in and modulate the function of intestinal epithelium and lamina propria cell

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doi:10.1016/j.actbio.2008.08.003

populations [6,7]. They act in a paracrine or autocrine fashion, forming a network of interrelated factors within the intestinal mucosa, exhibiting pleiotropism in their cellular sources and targets. Most have multiple functional properties, contributing to the highly redundant nature of the network.

Intestinal sub-epithelial myofibroblasts are present in the lamina propria immediately beneath the epithelium and have been shown to secrete several of the peptide growth factors that promote intestinal epithelial restitution [4,8]. These include acidic and basic fibroblast growth factor (bFGF) [9], epidermal growth factor, interleukin-1β, transforming growth factor (TGF)-α and -β [10], hepatocyte growth factor [11] and vascular endothelial growth factor [12]. TGF-β has been shown to have a central role in modulating the effects of some of these factors on intestinal epithelial restitution [10]. Because of their potent effects, there is growing clinical interest in the use of recombinant peptide therapies that can modulate epithelial repair and protection [13,14].

Silicate bioactive glasses are a group of surface-reactive glass ceramics in the four-component system of oxides of silicon, calcium, sodium and phosphorus that were originally developed to promote tissue adhesion of skeletal prostheses [15]. The original composition is now referred to as 45S5 Bioglass® and is composed (in wt.%) of 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5. The biological activity of bioactive glass has been studied extensively in relation to its ability to form stable and mechanically strong bonds to bone and soft connective tissues [16]. More recently, 45S5 bioactive glass has been shown to stimulate a significant increase in the secretion of angiogenic growth factors from human fibroblasts in vitro [17,18] and significantly increase vascularisation into tissue engineering scaffolds in vivo [19].

The current study aimed to investigate whether bioactive glass can modulate intestinal epithelial restitution using a novel in vitro wound healing model of confluent monolayers of intestinal epithelial cells.

2. Materials and methods

2.1. Cell culture

Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC, Wiltshire, UK) and studied between passages 10 and 20. The CCD-18Co cell line, which exhibits most of the known characteristics of intestinal sub-epithelial myofibroblasts [20], was obtained from the American Type Culture Collection (ATCC, MD, USA) and studied between passages 10 and 20. Both cell lines were cultured in Eagle's minimum essential medium (Sigma, UK) supplemented with 10% foetal calf serum (Gibco), 2 mM L-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 1% non-essential amino acids (Sigma), antibiotics (penicillin, 50 U ml^{-1}; streptomycin, 50 μg ml^{-1}) and antimycotic (amphotericin B, 0.05 μg ml^{-1}) (Gibco, Paisley, UK). All incubations were conducted in 5% CO_2 at 37 °C.

2.2. 45S5 bioactive glass coated surfaces and conditioned culture medium

45S5 bioactive glass particles (mean particle size of 2 μm and identical in composition to 45S5 Bioglass® [15]; a kind gift from Schott Glass, Germany) were used to prepare slurries (0.01%, 0.1%, 1% (w/v)) in deionized and distilled water, as previously described [21]. One hundred microliters, 594 μl or 1.32 ml of each slurry was added to the wells of a 96- or 24-well tissue culture plates (Sigma, UK) or to 30 mm tissue culture plate inserts (0.4 μm pore size) (Millipore, Herts, UK), respectively, to produce an equal surface coating of 45S5 bioactive glass particles (3.125, 0.3125 and 0.03125 mg cm^{-2} for 1, 0.1% and 0.01% (w/v), respectively). The plates and inserts were allowed to air dry in a laminar airflow cabinet, producing a stable adherent coating of 45S5 bioactive glass particles.

Bioactive glass conditioned medium was collected after incubating culture medium in the 24-well tissue culture plates coated with bioactive glass particles for 24 h at 37 °C. The pH of the conditioned medium was measured immediately after collection. The conditioned medium was filtered through a 0.2 μm filter and stored at −70 °C. Conditioned medium derived from myofibroblasts stimulated with bioactive glass was produced by seeding 3.8×10^4 CCD-18Co cells onto surfaces coated with the different concentrations of bioactive glass particles. After 24 h the conditioned culture medium was collected and stored as outlined above. Control conditioned medium was also collected from CCD-18Co cells grown on uncoated surfaces or from culture medium not exposed to CCD-18Co cells or bioactive glass. All types of conditioned medium were collected in replicates of five.

2.3. Wounding assays

Wound assays were performed by modifying a previously described method in [9]. Confluent monolayers of Caco-2 cells in 60 mm tissue culture treated dishes (Falcon, NJ, USA) were wounded using a razor blade to imprint a line on the surface of the dish. Cells in front of the wound margin were removed by sliding a foam sponge along the edge of the razor blade. The imprinted line was used as the wound margin and two wounds, approximately 35–40 mm in length, were made in each dish, separated by approximately 10–15 mm. Cells were washed with serum-free medium and the wounded monolayers incubated with 3 ml culture medium under the various conditions for a further 24 h.

A novel co-culture wound model was tested consisting of tissue culture inserts coated with different concentrations of bioactive glass and/or seeded with CCD-18Co intestinal myofibroblasts (3×10^5 cells $insert^{-1}$) at the time of placing the inserts over the wounded monolayers (Fig. 1).

Six areas along the wound margins in each dish were marked for identification after the incubation period. After incubation the wounded monolayers were washed with phosphate-buffered saline (Sigma) and fixed with methanol/

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