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Effect of soft foods on primary human gingival epithelial cell growth and the wound healing process

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ABSTRACT

Objectives: Investigate the effect of soft diet foods on gingival epithelial cell growth, migration, and mediator secretion.
Methods: Human gingival epithelial cells were stimulated for various time periods with the following soft diet foods: orange juice, drinkable yogurt, and a nutritional drink. Cell growth was determined by an MTT assay and cell migration was investigated by a scratch assay and F-actin filament staining. Keratin production was analyzed by Western blot and wound healing mediators IL-6 and human β-defensin 2 were quantified by ELISA.
Results: We demonstrate, for the first time, that certain soft diet foods increased the production of keratin 5, 14, and 19 by gingival epithelial cells. These proteins were known to be produced by proliferating cells. The soft foods tested also stimulated gingival epithelial cells to produce IL-6 and human β-defensin 2. Soft foods are capable of promoting gingival epithelial cell migration by increasing F-actin production, which is part of the wound healing process. Results varied depending on the foods tested.
Conclusion: Gingival epithelial cells interacted with the soft diet foods under study. This interaction was shown to upregulate keratin expression, as well as IL-6 and human β-defensin 2 secretions. Furthermore, following cell wound, the soft foods upregulated post-scratch cell migration and F-actin production. Overall data suggest that the choice of foods in soft diets following oral surgery may influence the wound healing process of gingival epithelial cells.

1. Introduction

The oral cavity plays a major role in human health and well-being. However, it is quite often subjected to minor as well as major wounds caused by periodontal diseases, dental implant surgeries, cancer resection, *etc.* (Lau, Johnston, Fritz, & Ward, 2013).

Oral surgeries typically involve invasive procedures with possible post-operative complications that may prolong healing time and affect the successful clinical outcome (Chanavaz, 1999; Shigeishi, Ohta, & Takechi, 2015). The speed of the healing process is linked not only to the length of the surgical procedure and the clinician's skill, but also to the patient's health condition, smoking status, *etc.* (Lau et al., 2013). Another key factor affecting healing is the patient's diet. To increase oral tissue healing following invasive surgery, it is important that the patient adopt safe dietary strategies. Indeed, inappropriate foods may increase patient-mediated healing complications. It has been suggested that specific dietary strategies before as well as after surgery should be part of a global approach to optimize healing following dental implant and periodontal surgeries (Charoenkwan & Matovinovic,

2014). Selecting the appropriate foods and controlling food temperature may contribute to minimizing post-surgical edema and hemorrhages (Alissa, Esposito, Horner, & Oliver, 2010). Diet consistency must therefore be controlled to avoid trauma at the sites of surgical incisions. Whether the foods are hard or soft, hot or cold, spicy or mild, they will inevitably come in close contact with the oral epithelium. If food consistency and temperature is not monitored, the wound healing process can be delayed.

Because they come in direct contact with foods, epithelial cells are an important cell type in the oral cavity. It is well known that epithelial cells are interconnected, forming layers that constitute an efficient protective barrier (Presland & Dale, 2000; Rouabhia & Allaire, 2010). The basal layer consists of proliferating cells, while the suprabasal layer is formed of differentiated cells. The cells in these different layers express various keratins (K) to form intermediate filaments that contribute to cell cohesion. Highly proliferative epithelial cells comprising the basal layer of the epithelium express specific keratins such as K5, K14, and K19 (Alam, Sehgal, Kundu, Dalal, & Vaidya, 2011), while suprabasal cells express keratins K1 and K10 (Simpson, Patel, & Green, 2011).

In addition to its function as a protective physicochemical barrier against the outside environment, the oral epithelium has many metabolic and immunological roles. These include fluid and ion transport regulation, mucus production/elimination, participation in innate and adaptive immunity, modulation of inflammation, cell migration, and repair processes (Naglik & Moyes, 2011; Politis, Schoenaers, Jacobs, & Agbaje, 2016). In response to microbial infections, oral epithelial cells produce significant amounts of cytokines, including IL-6, IL-8, and TNFα (Bahri, Saidane-Mosbahi, & Rouabhia, 2010; Derradjia et al., 2016; Dongari-Bagtzoglou & Fidel, 2005). These cytokines are key players in controlling oral infections and promoting wound healing. Epithelial cells are also a source of peptides displaying broad-spectrum antimicrobial activity against a wide range of pathogens (Bahri, Curt, Saidane-Mosbahi, & Rouabhia, 2010). The antimicrobial peptide family includes β-defensin family produced by all human epithelial cells (Hans & Madaan Hans, 2014). In the oral cavity, β-defensins were found in oral mucosa, gingiva, and tongue epithelium along with salivary glands (Gomes & Fernandes, 2010). Several *in vitro* assays have revealed β-defensins to be active against a broad range of microbes including Gram-positive and Gram-negative bacteria, enveloped viruses, and fungi (Mineshiba et al., 2003). However, following oral surgery, epithelial cell functions may be deregulated. One clinical recommendation is to adopt a soft food diet to prevent the harmful contact of solid foods with tissue edges at surgical sites (Charoenkwan & Matovinovic, 2014). The soft foods often recommended following oral surgery include juice, drinkable yogurt, and soups. Thus far, no study has investigated the effect of these different soft foods on gingival epithelial cell behaviors following surgery.

We investigated the effect of three different soft foods on human gingival epithelial cell growth, cell migration, and wound healing as well as on the expression of keratins (K5, K14, and K19) and the secretion of IL-6 and human β-defensin-2.

2. Materials and methods

2.1. Soft foods used in this study

Orange juice (Oasis®, Industries Lassonde, Rougemont, QC, Canada), drinkable yogurt (Yop, vanilla, Yoplait®, Yoplait Canada, Mississauga ON, Canada), and a nutritional drink (Ensure® High Protein, Abbott Laboratories, Montréal, QC, Canada) were purchased from local stores (Québec City, QC, Canada). According to the manufacturers, the orange juice constituents are: vitamin C (150%), carbohydrates (9%), calcium (4%), iron (2%) and sodium (1%). The drinkable yogurt constituents are: saturated + trans (17%), carbohydrates (9%), sugars (26 g), sodium (4%), protein (7 g), calcium (20%), vitamin A (2%) and vitamin D (25%). The nutritional drink constituents are: protein (20 g), fat (11 g), carbohydrate (44.2 g), fibre (3 g), calcium β-hydroxy-β-methylbutyrate (1.5 g), choline (82 mg). The nutriments were diluted (v/v) with a sterile saline solution before adjusting the pH to 7.00–7.20 using either 2 N HCl or 2 N NaOH under sterile conditions, and were subsequently stored at 4 °C before being used for the experiments. Fresh solutions were prepared weekly.

2.2. Primary human gingival epithelial cells

Normal human gingival epithelial cells were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA). According to the manufacturer, the oral keratinocytes/epithelial cells (Catalog#2610) were extracted from human oral mucosa and characterized by immunofluorescence using antibodies specific to cytokeratine-8, -18 and -19. Cells were negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. Epithelial cells were cultured in Dulbecco's modified Eagle's (DME)–Ham's (3:1) medium (DMEH) supplemented with 5 μg/mL of human transferrin, 2×10^{-9} M of 3,3′,5′-triiodo-L-thyronine, 0.4 μg/mL of hydrocortisone, 10 ng/mL of epidermal growth factor,

100 IU/mL of penicillin G, and 10% fetal bovine serum. The medium was changed three times a week. When the culture reached 90% confluence, the cells were detached from the flasks with a 0.05% trypsin–0.1% ethylenediaminetetraacetic acid (EDTA) solution, washed twice, and resuspended in DMEH-supplemented medium at a final concentration of 10^6 cells/mL. Cells at the third passage were used to perform the experiments.

2.3. Effect of soft foods on gingival epithelial cell growth

Gingival epithelial cells were seeded into 6-well plates at 3×10^5 cells/well in DMEH medium and were incubated thereafter in a humidified atmosphere containing 5% CO_2 at 37 °C. When the cultures reached 80% confluence, they were covered with a thin layer (2 mL) of water bath-warmed solutions of orange juice, yogurt, or nutritional drink. The contact between the cells and the nutriment solutions was maintained for 5 or 10 min. The control groups consisted of cultures overlaid with 2 mL of DMEH supplemented with 10% FBS. After each contact period, the epithelial cultures were washed to remove the foods. DMEH (5 mL) medium was then added to each well, followed by an incubation for 24, 48, or 72 h, after which time cell growth was assessed by means of the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT; Sigma, St. Louis, MO, USA) (Denizot & Lang, 1986). Briefly, a stock solution of MTT (5 mg/mL) was prepared in phosphate-buffered saline and added to each culture well at a final concentration of 1% (v/v). Gingival epithelial cultures were incubated for 3 h at 37 °C with MTT, after which time the supernatant was removed, and the adherent cells were washed twice with warm culture medium. Following the final wash, 1 mL of 0.04 N HCl in isopropanol was added to each culture well, with incubation extended for an additional 15 min. At this step, 200 μL of the reaction mixture was transferred to a 96-well flat-bottom plate, with the absorbance (optical density) measured at 550 nm by means of a microplate reader (Model 680; Bio-Rad Laboratories, Mississauga, Ontario, Canada). Results are reported as the means ± SD, n = 4.

2.4. Effect of soft foods on K5, K14, and K19 production

Following gingival epithelial cell stimulation with orange juice, yogurt, or nutritional drink for 10 min and cell cultivation for 24 h, total proteins were extracted by adding 200 μL of a lysis buffer (25 mM of Tris–HCl, pH 8.0, 0.15 M of NaCl, 1 mM of EDTA, 10% glycerol, 0.1% SDS, 0.05% sodium deoxycholate, and 1% Triton X-100) to the cultures. The lysis buffer was supplemented with 2 μL of a ready to use protease and phosphatase inhibitor solution (Sigma-Aldrich, Oakville ON). Protein concentration in each sample was determined by performing a Bradford assay. The samples were then used to evaluate keratin K5, K14 and K19 expression by Western blot analyses, as previously reported (Alharbi & Rouabhia, 2016). The proteins (20 μg) were separated by SDS-PAGE using 4% stacking gel and 10% separating gel in a Bio-Rad Mini-PROTEAN II vertical slab gel apparatus. The gel was then transferred onto a poly(vinylidene difluoride) membrane for 1 h at 100 V with a refrigerated Tris-glycine buffer (25 mM of Tris, 19.2 mM of glycine, 20% methanol, and 100 mM of Na_3VO_4). The membrane was then incubated with 5% non-fat milk in 0.1% Tween-20–Tris-buffered saline (TTBS) for 1 h, then overnight with a primary antibody (anti-K5 at 1/1000, anti-K14 at 1/1000, or anti-K19 at 1/1000), after which time the membrane was washed 4 × 10 min with TTBS buffer. The peroxidase-conjugated secondary antibodies were then applied onto the blot for 1 h, with the membrane washed thereafter 4 × 10 min with TTBS buffer. Finally, detection was performed using the chemiluminiscence-based horseradish peroxidase substrate system (Millipore, Billerica, MA, USA), according to the manufacturer's instructions. Visualization was carried out by means of the VersaDoc™ MP 5000 System (Bio-Rad Laboratories). The protein band analyses were performed with Quantity One® Version 4.6.3 (Bio-Rad Laboratories). The software