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The role of phosphoinositide 3-kinase in adhesion of oral epithelial cells to titanium

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ABSTRACT

Background: Oral epithelial cells (OECs) adhesion to titanium may improve the success rate of implant restoration.

Purpose: We investigated the mechanism by which OECs adhere to titanium dental implants.

Materials and methods: (1) After culturing rat OECs on titanium plates (Ti) or culture dishes in the presence or absence of a phosphoinositide 3-kinase (PI3K) activator or inhibitors and/or growth factors, and OEC morphology under these conditions were analyzed. (2) Right maxillary first molars were extracted and replaced with experimental implants. The rats were treated with or without growth factors.

Results: (1) Cell adherence was lower of OECs on Ti than in those on culture dishes, as were the levels of integrin β4 and the continuity of F-actin structures. After PI3K inhibition, markedly reducing adherence to both substrates. In contrast, PI3K activation with activator or insulin-like growth factor restored the OEC adherence and the expression of adhesion molecules on Ti to the levels seen in OECs cultured on dishes. Cell migration was inhibited by PI3K activation. (2) High expression of integrin β4 was observed in the peri-implant epithelia of PI3K-activated rats.

Conclusion: These findings suggest that PI3K plays an important role in the adhesion of OECs to Ti.

1. Introduction

Dental implants are rapidly becoming an important treatment modality for edentulism, and the biological techniques for achieving stable fixation of implants in the alveolar bone are becoming highly sophisticated. As important as the oseointegration, the soft tissue healing around the implant which forms the biological seal is crucial in the long-term prognosis of the implant. Two studies have reported an improvement of epithelial sealing around the dental implant, in one case by making microgrooves on the surface of the implant[1] and in another by adopting a scalariform implant shape.[2] Dental implants penetrate the oral mucosa, creating a peri-implant epithelium (PIE)—implant interface with an associated risk of inflammation. Elucidation of the mechanisms underlying local defense and PIE-to-implant adhesion is therefore important.

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Integrins mediate cell adhesion to the extracellular matrix,[3] which is an important stage in the regulation of epithelial cell differentiation and epidermal stratification.[4] Laminin-5 (Ln-5) is a major adhesive ligand for integrins in the mature cutaneous basement membrane.[5] Whereas, plectin acts to link integrins (notably integrin α6β4) to intracellular actin filaments.[6] It has been reported that integrin α6β4, Ln-5 and plectin are central in the function of hemidesmosomes, the structures within the internal basal lamina that link the intracellular intermediate filament network with the extracellular matrix (basement membrane), thus providing stable anchorage.[7–11] This is of importance to the current study because the natural junctional epithelium and the PIE are both connected to the substrata by hemidesmosomes in the internal basal lamina.

Integrin α6β4 is found in hemidesmosomes both *in vivo* and *in vitro*, and regulates the cell cycle, cell invasion and cell adhesion,[12,13] often (but not exclusively) *via* the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways.[14–16] It has a distinctive structure and subcellular localization, with its β4 subunit containing a large and unique (among integrin β subunits) cytoplasmic domain that interacts with the cytoskeletal elements of hemidesmosomes and, *via* plectin, links integrin α6β4 to the actin filament system.[11,17] This domain is also a key determinant in the activation of PI3K, MAPK and other signaling pathways activated by this integrin.[18]

The epithelium–implant interface has both defense and bonding/mechanical closure functions.[19] However, bacterial accumulation around the implant induces inflammatory destruction more easily than around the natural tooth,[20] leading to damaged collagenous tissue through which epithelial cells can migrate,[21,22] leading to pocket formation. Additionally, Haapasalmi et al. reported that the loss of integrin α6β4 expression leads to uncontrolled epithelial growth in the gingival pocket during chronic periodontal infection.[23] This epithelial proliferation and migration (known as 'epithelial down-growth') is halted by confrontation with a healthy collagenous matrix.[21,22] It is therefore critical to prevent epithelial down-growth by promoting adherence and stabilizing the epithelial soft tissue seal.[2]

Dental implants are generally manufactured from medical-grade titanium (Ti), and thus the adherence of epithelial cells to this material is the main factor determining the strength of the PIE-to-implant bond. Expression of cell adhesion molecules is reduced in cells cultured on Ti, which may be a consequence of micro-environmental factors, such as the lack of coating on Ti surfaces, the negative surface charge, or liquation.[24–26] However, the relevance of these factors is poorly understood. We investigated the mechanisms by which oral epithelial cells (OECs) adhere to Ti implants and to natural teeth, and found that PI3K signaling is vital for the adhesion of OECs to Ti.

2. Materials and methods

2.1. Titanium plates

The substrates used were smooth commercially available pure wrought titanium plates (Ti) (diameter, 30 mm; thickness, 1 mm; Japan Industrial Specification H 4600, 99.9 mass% Ti) and polystyrene culture dishes (dishes) (Falcon Labware, Oxford, UK). The surface topographies were determined using a profilometer (Handy Surf E-30A; Tokyo Seimitsu, Tokyo, Japan). The Ra parameter, which was the centre-line average, was measured with a cut-off value of 0.8 mm and a length of 3.0 mm. The method for growing OECs on these surfaces was described previously.[27]

2.2. Cell culture

Isolation and culture of rat OECs was performed following the method of Shiraiwa et al.[28] Briefly, oral mucosa derived from 4-day-old Wistar rats was incubated with dispase (1×10^3 IU/ml) in Mg^{2+} and Ca^{2+}-free Dulbecco's phosphate-buffered saline (PBS) for 12 h at 4 °C. The oral epithelium was then separated from the connective tissue using two pairs of forceps. The epithelium was dispersed by triturating (10×), then seeded either directly onto culture dishes (dishes) or onto Ti. OECs were cultured in Defined Keratinocyte Serum-Free Medium (DK-SFM; Invitrogen Corporation, New York, US) containing gentamycin (50 μg/ml) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

2.3. Adhesion assays

The adhesive capacity of OECs was evaluated by adhesion assays. Cells (5×10^5 cells/dish) were plated on each substrate and cultured in DK-SFM for one week. Prior to adhesion assays, cells were incubated with or without the following additives: (1) the PI3K inhibitor, wortmannin (WN; 10 μM; 30 min); (2) the structurally- and mechanistically distinct PI3K inhibitor, LY294002 (LY; 20 μM; 30 min); (3) the PI3K activator, PA (10 μg/ml, 30 min); (4) insulin-like growth factor (IGF)-I (10 ng/ml, 6, 12 or 24 h); or (5) epidermal growth factor (EGF; 10 ng/ml, 6, 12 or 24 h). The number of adherent cells was measured using the method of Goodwin and Pauli.[29] Briefly, after three washes with culture medium, non-adherent or weakly attached cells were removed by shaking at 75 rpm in α-MEM for 5 min using a rotary shaker (NX-20, Nissin, Tokyo, Japan). This process was repeated for three times. Any remaining adherent cells were counted and calculated as a percentage of the initial count, which was used as a measure of the adhesive capacity of these cells.[30] To measure the number of attached cells, WST-8 kit (Dojindo, Kumamoto, Japan) was used. After WST-8 solution addition and incubation at 37 °C for 2 h, the solution was retrieved from Ti or dishes to a 96-well plate and absorbance at wavelength 450 nm was measured using a spectrophotometer (NJ-2300, Biotech, Tokyo, Japan).

2.4. Immunofluorescence staining for adhesion proteins

OECs were cultured for 7 day, and then incubated for 30 min with or without either 1 mM wortmannin (Sigma Chemical Co., St. Louis, MO) or 50 mM LY29002 (Sigma Chemical Co.) then washed three times with PBS (Fig. 3A). In other experiments, OECs were incubated for 6, 12 or 24 h with or without either IGF-1 (Sigma Chemical Co.), PA (Santa Cruz Biotechnology, CA, USA), or EGF (Sigma Chemical Co.) (Fig. 4A). These cells were washed three times with PBS, fixed in 4%