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Novel quartz crystal microbalance based biosensor for detection of oral epithelial cell–microparticle interaction in real-time

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Abstract

Recent applications of quartz crystal resonant sensor technology to monitor cell adhesion and specific ligand interaction processes has triggered the development of a new category of quartz crystal microbalance (QCM) based biosensors. In this study human oral epithelial cells (H376) were cultured on quartz sensors and their response to microspheres investigated *in situ* using the QCM technique. The results demonstrated that this novel biosensor was able to follow cell–microsphere interactions in real-time and under conditions of flow as would occur in the oral cavity. Unique frequency profiles generated in response to the microspheres were postulated to be due to phases of mass addition and altered cellular rigidity. Supporting microscopic evidence demonstrated that the unique frequency responses obtained to these interactions were in part due to binding between the cell surface and the microspheres. Furthermore, a cellular uptake process, in response to microsphere loading was identified and this, by influencing the rigidity of the cellular cytoskeleton, was also detectable through the frequency responses obtained.

Keywords: Quartz crystal microbalance; Biosensor; Oral epithelial cells; Cell culture; Real-time

1. Introduction

The efficacy of conventional dosage forms employed to enhance oral hygiene is limited by the rapid decline in the concentration of actives after administration. Strategies to improve retention of pharmacological actives within the oral cavity have included the incorporation of bioadhesives and a number of methods exist to evaluate the ability of such compounds to interact with oral mucosal substrates for prolonged periods (Chickering and Mathiowitz, 1995 and Kockisch et al., 2003). Studies more pertinent to human oral epithelial cell behaviour include the use of buccal cell isolates (Kockisch et al., 2001) and cultured cells (Doucet et al., 1996; Lawrence. et al., 1996). Few studies currently allow dynamic assessment of such interactions under conditions of flow as would occur in the oral cavity (Farokhzad et al., 2005) and none currently utilise human oral epithelium under these conditions.

The quartz crystal microbalance (QCM) was originally developed to follow molecular adsorption at the gas–solid interface after Sauerbrey (1959) identified the existence of a linear relationship between mass addition at the piezoelectric substrate surface and change in the recorded resonant frequency. Development of the technique to monitor events in the liquid phase resulted in the application of the technique to a number of processes pertinent to biological research such as ligand–receptor interactions (Janshoff et al., 1997) characterisation of protein adsorption (Höök et al., 1998), nucleic acid hybridisation (Furtado and Thompson, 1998) and QCM based immunoassay (Aizawa et al., 2001).

The QCM technique is particularly attractive to the field of cell biology as it has the potential to monitor cell–surface interactions in a dynamic and non-invasive way. Thus it allows the generation of kinetic data, and as cumbersome labelling techniques are negated, the investigation of cellular responses without compromise to cellular architecture or protein structure. Such advantages have resulted in several pioneering studies which demonstrate the ability of the technique to monitor the adhesion and proliferation of a number of mammalian cell types *in situ* on the QCM surface (Matsuda et al., 1992; Redepenning et al., 1993; Gryte et al., 1993; Janshoff et al., 1996; Wegener et al., 1998, 2000). These preliminary studies identified that only specific, integrin-mediated cell-adhesion is detectable at the sensor

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surface. Furthermore, exploitation of this phenomenon revealed that the technique was sufficiently sensitive to discriminate between various substrates (as crystal surface modifications) and their impact on the cell adhesion process (Fredriksson et al., 1998a,b; Nimeri et al., 1998; Wegener et al., 2001; Cavic et al., 2001) resulting in the adoption of QCM technology as a routine tool in the field of biomaterials (Lord et al., 2006), and microbial biofilm research (Reipa et al., 2006).

Recent studies have demonstrated that the technique can detect cellular responses to chemical or biological stimuli as mediated through alterations in the cells' cytoskeletal architecture (Zhou et al., 2000; Cans et al., 2001; Marx et al., 2001, 2003). These studies highlight the potential to incorporate viable cells as signal transduction elements in combination with acoustic signal detection to investigate dynamic cellular responses to environmental modifications. To date, no studies have exploited these sensitive capabilities to investigate the response of oral keratinocytes to model microparticles that may ultimately be utilised in oral formulations, under conditions of flow as would occur in the oral cavity. Thus, the aims of this study were to develop a biosensor that incorporated a viable monolayer of human oral keratinocytes *in situ* on the quartz crystal surface to investigate cellular response to model microparticles, as might be used in oral healthcare formulations.

2. Experimental

2.1. Instrumentation

The QCM device was based on a novel surface mounted oscillation circuit with automatic gain control (AGC) designed to give extremely high amplitude oscillation with low noise, under liquid loading (Paul et al., 2001; Pavey et al., 2003). Gold-coated (100 nm thickness) 10 MHz AT-cut quartz crystals (8.6 mm diameter) were supplied with customised electrode configurations to allow both electrode contacts to be made on the crystal underside (Spartan Europe, UK). Crystals were mounted within a specially designed flow-cell and connected via flexible shielded electrodes to the oscillator head unit. Crystal oscillation data were recorded at 1 s intervals using a Fluka PM6685 frequency counter, connected via a PCIIa GPIB card to a personal computer (PC) running Fluka Timeview™ data acquisition and control software. The crystal oscillator and flow cell were housed in a thermostatically controlled oven set to 37 ± 0.1 °C (Denley, UK), and ambient and flow cell temperatures monitored using a 5-channel data logging electronic thermometer (PICOlog), K-type thermocouples (Radio Spares,UK) and dedicated software running on a second PC. Liquid delivery to the crystal surface (2–10 μL/min) was via PEEK® HPLC tubing using a Harvard syringe driver and glass gas-tight syringes (SGE Europe, UK). Samples were added to this mobile phase via a Rheodyne 9725 MBB™ HPLC injection valve fitted with a 20 μL PEEK® injection loop.

2.2. Cell culture

The oral epithelial cell line, H376, derived from a human squamous cell carcinoma (Prime et al., 1990) was employed throughout. Cells were cultured at 37 °C and 5% (v/v) CO_2 in Dulbecco's modified Eagle's medium (DMEM)/Hams F12 nutrient mix with 1 g/L glucose (Sigma–Aldrich, UK) supplemented with 10% (v/v) heat-inactivated foetal calf serum, 20 mM L-glutamine, 0.5 μg/mL hydrocortisone (Sigma–Aldrich, UK) and 2500 IU/mL penicillin and streptomycin (Invitrogen, UK). The culture media was exchanged on the first day after passage and then every 2 days thereafter. Routine subculturing of confluent cell layers was performed using standard trypsinisation (0.05% (w/v)/1.5 mM EDTA) after which the cells were seeded into fresh flasks at a density of 3×10^3 cells/cm^2. The cells were maintained in culture for no longer than ten passages during all experimental work to ensure that the neoplastic phenotype did not change as a result of prolonged passage *in vitro*.

2.3. Preparation of biosensors

Quartz crystals were cleaned by immersion in ethanol, spin-coated with polystyrene (0.5% w/v in toluene) as previously described (Fredriksson et al., 1998a) and treated in an oxygen-plasma for 2 min. This was shown to provide a suitably hydrophilic surface (receding water contact angle of less than 5°) designed to mimic that of tissue culture plastic and thus promote cell adhesion. Suspensions of H376 cells were prepared from near confluent monolayers using standard trypsinisation, 0.05% (w/v) trypsin/1.5 mM EDTA for 10 min at 37 °C. Petri dishes containing prepared crystals after their sterilisation by immersion into 70% ethanol were inoculated with cells at a density of 6×10^3 cells/cm^2. Control sensors to investigate frequency responses in the absence of cells were prepared by incubating modified quartz crystals in complete culture medium only.

2.4. Biosensor/microsphere interaction assay

DMEM/F12 nutrient mix with 15 mM HEPES (Sigma–Aldrich, UK) was equilibrated overnight at 37 °C and 5% (v/v) CO_2, syringe filtered (0.2 μm pore, Nunc) into a glass gas-tight syringe and used as the mobile phase. Biosensors were removed from cell culture on day 5 after cell seeding (the time taken to establish confluent monolayer coverage across the sensor surface) and installed within the flow cell. After connection to the oscillator circuit, the frequency response was monitored until a stable frequency baseline was established for the sensor under liquid loaded conditions (flow rate 5 μL/min). Fluorescent polystyrene microspheres (0.5 μm, Duke Scientific, Brookhaven International, UK) were diluted (0.05%w/v) in media and introduced to the mobile phase via the Rheodyne™ injection loop. The biosensor frequency response to the microspheres was monitored at 1 s intervals for 30 min. For each assay biosensors were prepared in triplicate to allow for comparison of cell viability between control sensors and those exposed to shear-wave excitation/serum depletion during the time course of experiments. Cell-free sensors were also used as controls to investigate the frequency response of the system to microsphere interactions in the absence of viscoelastic contribution from the cellular monolayer.