FEBS Letters 588 (2014) 1178–1183

journal homepage: www.FEBSLetters.org

Quantitative investigation of calcimimetic R568 on beta cell adhesion and mechanics using AFM single-cell force spectroscopy

Eleftherios Siamantouras [a], Claire E. Hills [b], Mustafa Y.G. Younis [b], Paul E. Squires [b], Kuo-Kang Liu [a,*]

[a] School of Engineering, University of Warwick, CV4 7AL, UK
[b] School of Life Sciences, University of Warwick, CV4 7AL, UK

ARTICLE INFO

Article history:
Received 20 December 2013
Revised 14 February 2014
Accepted 24 February 2014
Available online 5 March 2014

Edited by Lukas Huber

Keywords:
AFM-SCFS
Cell adhesion
Indentation
Elasticity
CaSR
Calcimimetic

ABSTRACT

In this study we use a novel approach to quantitatively investigate mechanical and interfacial properties of clonal β-cells using AFM-Single Cell Force Spectroscopy (SCFS). MIN6 cells were incubated for 48 h with 0.5 mM Ca^{2+} ± the calcimimetic R568 (1 μM). AFM-SCFS adhesion and indentation experiments were performed by using modified tipless cantilevers. Hertz contact model was applied to analyse force–displacement (*F–d*) curves for determining elastic or Young's modulus (*E*). Our results show CaSR-evoked increases in cell-to-cell adhesion parameters and *E* modulus of single cells, demonstrating that cytomechanics have profound effects on cell adhesion characterization.

1. Introduction

The role of the calcium-sensing receptor CaSR in the systemic circulation is to sense changes in extracellular Ca^{2+} and evoke appropriate counter-regulatory responses to regain normocalcaemia [4]. The functional link between the receptor and regulation of systemic calcium in normal physiology and disease has been extensively studied [4]. However, CaSR expression is not restricted to the cells involved in the control of systemic Ca^{2+} [5]. It has been well recognized that CaSR activation affects function in disparate tissue types, including pancreatic beta-cells [29,12,19,21].

Epithelial (E)-cadherin is a surface adhesion protein involved in tethering adjacent cells and ensuring close cell–cell interaction. E-cadherin ligation mediates beta-cell-to-beta-cell coupling and regulates intercellular communication within islets [3]. A study by Rogers et al. [27] suggested that E-cadherin mediated cell adhesion contributes to the enhanced secretory function of beta-cell clusters. Neutralization of E-cadherin reduced glucose-evoked synchronicity in calcium signals between adjacent cells and reduced insulin secretion [27]. These data imply that E-cadherin mediated cell adhesion has important repercussions for the islet function in terms of glucose responsiveness and insulin secretion.

We have previously demonstrated that the activation of CaSR using the calcimimetic R568, increased the expression of E-cadherin, which in turn increased functional tethering between beta-cells [15]. In the current study we quantitatively monitored changes in cell elasticity induced by activation of the CaSR by the calcimimetic R568. Atomic Force Microscopy based Single Cell Force Spectroscopy (AFM-SCFS) was used to perform cell-to-cell adhesion and single cell indentation experiments. The SCFS system incorporates an improved positioner to allow for longer displacement measurements up to 100 μm for separating two adherent cells, in a high force resolution (∼pN) over a large dynamic range (∼5 pN to ∼100 nN). This system provides sufficient force and displacement ranges to ensure accurate detection of maximum unbinding force of ligand-receptor interactions in cell-to-cell adhesion measurement [2,26,9,14], while it has been used extensively for studying cadherin mediated adhesion [1,25,22]. In the current study the instrument was also fitted with a spherical bead-attached cantilever beam to indent single cells and thereby calculate cell elasticity, i.e. Young's modulus, from the measured force–displacement curves using Hertzian contact model [32]. The novel use of this improved AFM-SCFS system permits us to examine cellular adhesion, tethering of cells and cell elasticity and more importantly to elucidate the intricate interplay between these factors.

* Corresponding author. Address: School of Engineering, University of Warwick, Coventry CV4 7AL, UK.
E-mail address: I.K.Liu@warwick.ac.uk (K.-K. Liu).

http://dx.doi.org/10.1016/j.febslet.2014.02.058
0014-5793/

2. Materials and methods

2.1. Materials

MIN6 cells were obtained from Dr. Y. Oka and J.-I. Miyazaki (Univ. of Tokyo, Japan). Fibronectin, Dulbecco's Modified Eagles Medium (DMEM), Hams-F12, glutamine, penicillin-streptomycin and phosphate buffered saline (PBS) were from Sigma–Aldrich (Poole, Dorset, UK). The calcimimetic R568 was from Amgen Inc. (Thousand Oaks, CA, USA). Tissue culture plastic-ware was from Invitrogen Life Technologies (Paisley, UK).

2.2. Maintenance of MIN6 cells

MIN6 cells (passage 35–40) were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air in DMEM supplemented with 15% FCS, glutamine (2 mM) and penicillin/streptomycin (100 U/ml/0.1 mg/ml). Prior to treatment, cells were seeded onto 40 mm petri-dishes and serum starved overnight. Cells were then placed for 48 h in DMEM containing both low glucose (5 mM) and low calcium (0.5 mM) +/− the calcimimetic R568 (1 μM) [15]. Suspended (free) cells were prepared under identical conditions before being physically scrapped off the T25 flasks with gentle agitation and re-suspended in fresh DMEM.

2.3. Atomic Force Microscopy

Experiments were performed using the CellHesion®200 module (JPK Instruments, Germany) installed on an Eclipse TE 300 inverted microscope (Nikon, USA). During each experiment, cells were maintained at 37 °C using a BioCell™ temperature controller (JPK Instruments, Berlin, Germany). All experiments were performed in CO_2 – independent media. Phase microscopy images were acquired using a CCD camera connected on the side port of the microscope. The entire set-up was supported on an anti-vibration table (TMC 63-530, USA).

Tip-less silicon nitride cantilevers (Arrow TL-1, NanoWorld, Switzerland), with force constant 0.03 N/m, were used for conducting cell-to-cell adhesion and single cell indentation experiments. The actual spring constant of the cantilever was determined before experiments by using the manufacturer's software (JPK instruments, Germany) based on the thermal noise method [17]. Since the resonance of soft cantilevers in fluid is much lower and very susceptible to noise a correction factor of 0.251 was used [6].

2.4. Cell-to-cell adhesion experiments

The tip-less cantilevers were chemically functionalized for a single suspended cell to be attached. Initially the cantilevers were sterilised by UV treatment (15 min), before being incubated in poly-L-lysine (25 μg/ml in PBS, 30 min, RT) and then fibronectin (20 μg/ml in PBS, 2 h, 37 °C) [16]. After functionalization cantilevers were stored in PBS solution at 4 °C and used within 3 days. To record a force curve for calibration, the cantilever was configured to approach the base of a cell-free petri-dish once, to minimize the loss of coating (set-point 2 V). Suspended cells were dispensed into the petri dish using a pipette. Free cells stick on the substrate within 5 min, hence the cell-cantilever attachment procedure was performed rapidly (2 min). With the aid of optical microscope the cantilever was pressed against a single free cell by performing a force curve. The set-point force and contact time was 0.8 nN and 5 s, respectively. During the contact period, the instrument was set in a constant force mode, in which force is kept constant by adjustments of the piezo-actuator height. Once a single cell was attached to the cantilever, it was left to recover for at least 5 min to form strong binding with the functionalized surface [11].

The cantilever-attached cell was brought in contact with another cell adhering on the substrate, until a preset contact force of 0.8 nN was reached. The cells remained in contact for 5 s, in which surface bonding was formed. Next, the cantilever was retracted and force versus displacement measured until the two cells were completely separated. The procedure was repeated three times for each cell tested, with 30 s intervals between each measurement. The attached cell was used to perform measurements on approximately 3–5 cells for each dish, using multiple dishes from at least 3 separate samples of cells in each experiment ($n = 3$).

2.5. Cell indentation experiments

Using a small amount of two-part fast setting epoxy glue (5 min), colloidal probes were prepared by gluing an 11 μm polystyrene microsphere (Polybeads®, Polysciences, USA) on a tipless TL-1 cantilever. The attachment procedure was performed on the stage of AFM with the aid of the inverted optical microscope. The microsphere was attached immediately by performing an approach curve directly above the sphere. Fig. 1(a) shows optical images of polymeric bead attached to a tipless cantilever.

Each substrate cell was indented 5 times with an interval pause of 60 s, while force–displacement (F–d) curves were recorded simultaneously. For consistency, all cells were indented immediately

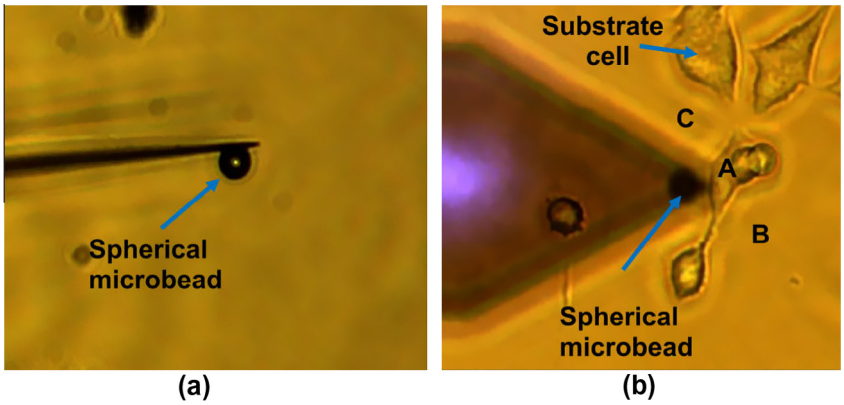

Fig. 1. (a) An optical image showing the side view of a 10 μm silica microsphere attached to the end of an arrow TL1 tipless cantilever. (b) An optical image showing the top view of the cantilever-bead and MIN6 cells on the substrate. The determination of cell height prior indentation experiments is also demonstrated; a low set-point force (0.2 nN) was used for the AFM cantilever to touch a point in a clean area, such as B and C, next to a measured cell (A area). The displacement difference between B (or C) and A was used to determine the height of the cell.