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Preventing damage of germanium optical material in attenuated total reflection-Fourier transform infrared (ATR-FTIR) studies of living cells

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ABSTRACT

Germanium is an important element for ATR-FTIR spectroscopy. It has often been selected as a substrate for live cells FTIR spectroscopy because of its low toxicity for most human cells. However, there is a lack of study on the physical interactions between germanium and the living cells. In this study, we have shown that germanium surfaces are easily damaged when living cells are grown directly in contact for more than 20 h on the element surface. We then proposed a methodology to reduce the damage by coating the surface of the element with a thin layer of biological materials and tested for their capacities to protect polished germanium surfaces from living cell cultures. Fibronectin, collagen and gelatine coated and non-coated, newly polished Ge plates were subjected to 48 h culture of HeLa cells for six times and the degree of erosion by the cell was monitored using optical and atomic force microscopy (AFM). The cells showed normal attachment and morphology to either the treated or untreated surfaces and the viability of the cells were confirmed by trypan blue assay. Furthermore we have tested with an ATR FTIR measurement with a multi-bounce Ge element and found that the coatings were thin enough not to interfere with the measurements. However the degree of erosion (measured by the roughness of the Ge plate after cell culturing) was found to depend on the types of coating used where gelatine coating showed the best protection.

1. Introduction

Fourier transform infrared (FTIR) spectroscopy is a powerful label-free analytical technique. It is an ideal method for analysing biological materials [1]. Infrared radiation is a low energy wave so that the measurement can be non-destructive and without any influence on the sample. Several studies of FTIR imaging of fixed biological material have shown significant differences between different cell types [2], cancer cells and normal cells [2,3], as well as cancer cells response to chemotherapy drugs [4–6]. However, using living cells not only avoids artefacts due to the fixation process, but also enables to study the living material through time. To measure living cells, the issue about the strong absorbance of water in the mid-IR region has to be overcome. For this reason, transmission cells with very small path lengths have been designed [7–9] and synchrotron radiation is often employed for the measurement of living cells [10–15]. Attenuated total reflection (ATR) was also found to be a suitable measurement mode for this purpose [16,17]. In ATR mode measurements, samples are probed by the evanescence wave which is generated on the measuring surface when the infrared light undergoes an internal reflection (or multiple internal reflections for a multi-bounce ATR) through the high refractive index ATR element [18]. The ATR FTIR measurement of live cells was achieved by seeding and culturing cells directly on the measuring surface of the ATR element such that the cells are measured in a similar geometrical environment as in a typical cell culturing flask [19]. The path length are small and easily controlled and the sampling depth, which is ~2–3 time the depth of penetration, is approximately 0.5–1.5 μm which probes deep into the attached living cell but without significant interference from the bulk medium above the cells.

The development in the ATR FTIR measurement of live cells allows their study under treatment of drugs *in situ* for time-resolved FTIR spectra of the living cells to be recorded [20]. One of the promising applications of ATR FTIR study is the imaging of living cells in micro ATR mode [21]. Imaging in micro ATR mode has the added advantage of the increased numerical aperture where the light is further focused when approaches the sample through the high refractive index ATR element. A four times improvement in spatial resolution has been demonstrated when using Ge, which has a refractive index of 4, as the ATR element of the objective [22].

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Using this approach, it has been shown that major cell organelles such as nucleus and endoplasmic reticulum can be resolved [22]. Ge is an important ATR element and it is universally used for micro ATR imaging. The surface properties of Ge are different to the standard cell culturing flasks which living cells may interact differently. A previous study has shown that cells can be cultured on Ge surface with no observable difference in morphology when compared to cell grown in tissue culture flask [23] and the attachment of cells was improved when the ATR surface was coated with a thin layer of collagen [21]. However, the coating of Ge appears to be more important than simply improving the attachment of cells. In this work, we report on the possible damage to the bare Ge ATR element by the attached living cells. We have assessed the degree of damage by visible microscopy as well as atomic force microscopy after a single and repeated cell culturing on the optical element. This is important because the degree of damage is thought to be directly related to the scattering of light and loss of throughput. We have also measured the protective effect of various extracellular matrix components routinely used in tissue culture such as gelatin, collagen and fibronectin [24,25] as coating on Ge in order to propose a solution to reduce the degree of damage to the Ge surface.

2. Materials and methods

2.1. Optical materials and coatings

Newly polished germanium windows of 1 mm thick and 25 mm of diameter (surface area ~491 mm^2) were used as obtained from Crystan Ltd (UK). The coatings applied to the Ge windows were gelatine type 2 tissue culture grade (2% solution from Sigma, UK), collagen from calf skin type I for tissue culture (Sigma) and fibronectin from bovine plasma (Calbiochem, UK). Gelatine coating was applied at 2 mg/cm^2, or 490 μL of 2% solution per window. Fibronectin was applied at a 5 μg/cm^2, by applying 245.4 μL of a 0.1 mg/mL solution per window. Collagen was dissolved as recommended by the supplier to a working solution of 0.01%, which 490 μL were applied to obtain a coating of 10 μg/cm^2. After application of the coating solutions the windows were left to dry for 2 h at 35 °C.

For FTIR measurements of live cells, a temperature controlled multi-(i.e. 20) bounce ATR accessory trough plate (HATR, Pike technologies) and germanium ATR element (80 mm × 10 mm × 2 mm, Pike technologies) with an angle of incident of 45° were used. The path length in the living cells produced from this accessory in the 2000–1000 cm^{-1} region is approximately 3–6 μm with a depth of penetration of ~0.32–0.64 μm. The trough plate has a measurement area of ~500 mm^2 where the live cells can attach and be measured. The coatings of the ATR plate were applied similarly to the optical windows but volumes adjusted to the growth area of 500 mm^2.

2.2. Live cells preparation

HeLa (ATCC#CCL-2) and PC3 (ATCC#CRL-1435) were maintained in T25 cell culture flasks using RPMI medium supplemented with 10% FBS and 2 mM L-glutamine, in a 5% CO_2 environment at 37 °C. The cells were trypsinised and harvested when they reached ~80% confluence. The cells were then centrifuged into a pellet and re-suspended in L15 medium (Sigma Aldrich) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL

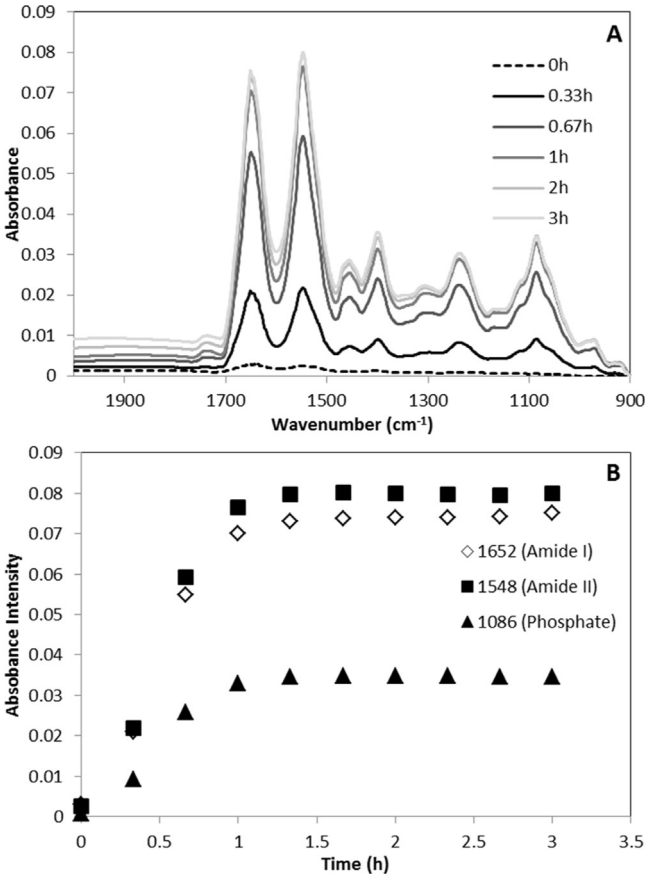

Fig. 1. FT-IR spectra of cells in the first three hours after seeding in the ATR element. (A) absorbance spectra and (B) absorbance intensity of the main peaks in function of time. As background the spectrum of medium was used.

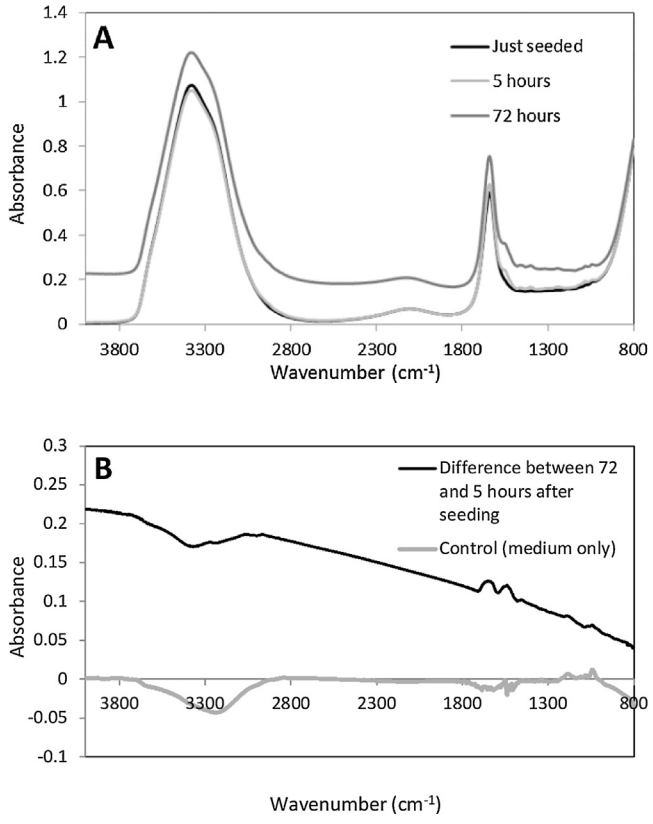

Fig. 2. Baseline shift observed in (A) ATR-FTIR spectra of cells just seeded, 5 h and 72 h after seeding, using as background a spectrum obtained with the empty crystal before seeding, and (B) difference spectrum between 5 h and 72 h of the cell attachment (dark line) and control (medium only, grey line).

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