Frequency effect on the cell detachment by ultrasound.

L. Peyre[1], D. Callens[1], A. Lefebvre[2], E. Radziszewski[1], H. Hildebrand[2] J.-J. Fabre[1]

[1] *Institut d'Electronique de Microélectronique et de Nanotechnologie/Département d'Opto-Acousto-Electronique, Université de Valenciennes Le Mont Houy, 59313 Valenciennes Cedex 9, France, Email: Ludovic.Peyre@univ-valenciennes.fr*

[2] *Groupe de Recherche sur les Biomatériaux, Faculté de Médecine H. Warembourg Place de Verdun, 59405 Lille Cedex, France*

Introduction

Intensive use of biomaterials in medicine and biology requires the understanding of many physiological phenomena. One of the most important for mechanical integration and biocompatibility is cell adhesion [1]. Theory as well as cells adhesion evaluation techniques have been developed [2-3]. These techniques are based on the application of mechanical stress on cells. All of them are interested in shear stress influence; none to our knowledge gives information about longitudinal adhesion strength.

As mechanical waves, ultrasounds seemed to be an efficient tool to evaluate cell adhesion [4]. Different techniques have been considered to use the potentialities of ultrasounds to evaluate cell adhesion.

In this study, we employed a technique allowing the application of various normal forces on cells cultured on various substrates. The insonification system consists in ceramics emitting bulk longitudinal waves through the flasket used for cell culture. Our interest went on adherent MC3T3-E1 cells. The substrate used in this study was glass.

Firstly, we observed surface vibrations thanks to a laser interferometer. This allowed us to obtain spatial distribution of normal force applied on cell culture. We particularly observed frequency influence on this distribution.

Secondly, we submitted cell cultures to ultrasounds of various power and frequencies and measured detachment rate.

Detachment rate curves obtained can be explained thanks to interferometer observations. We then succeeded in deducing a value for the adhesion force of MC3T3-E1 cells in our experimental conditions.

Materials and methods

Cell culture

We used MC3T3-E1 embryonic mouse osteoblasts. We cultured them in Dubelco's Modified Eagle Medium supplemented with 10% decomplemented newborn calf serum. The cells cultures were incubated during 3 days at 37°C in an atmosphere of 5% CO_2 at 95% humidity. Medium was changed just before insonification to avoid presence of dead cells. Incubation time has been chosen so that the experimentation is done with non-confluent cells. After the application of ultrasounds, detached cells were counted using electronic counter (Coulter Z1), the counting of non-detached cells allowed us to obtain detachment rate.

Insonification

We made an apparatus specifically designed to generate ultrasounds of various frequencies and power through the whole surface of cell substrate. It consists of two piezoelectric ceramics stuck to a glass slide. One side of the slide has been metallized to facilitate interferometer observations. The cell substrate was constituted of glass plate allowing good acoustic transmission because of its flat shape. Contact between cells culture flaskets and ultrasonic generator was made using a thin petroleum layer. A computerized system made it possible to measure electrical power used during experiments; frequencies were fixed by ceramics thickness and electrical adaptation.

We made four of these apparatus to obtain a frequency range from 148 kHz to 2 MHz (148 kHz, 592 kHz, 1 MHz, 2 MHz). The electrical power delivered to ceramics was varying from 100 mW to about 2500 mW. We used ten minutes insonifications before the counting of detached cells.

We undertook a study to avoid measurements errors due to a temperature growing during insonification.

Laser interferometry

We used interferometric probe SH-130 (B.M. Industry, Lisses, France) allowing to observe longitudinal displacements of some Angstrom at the glass/ceramic interface. Spatial resolution used for interferometric cartography was 100µm. The size of the cartography was reduced to cover the whole cell culture surface.

Results

Interferometry

We give four examples of cartography on figure 1. These images represent normalized displacement amplitude. We can observe that no symmetry appear on these images, but we can also observe that we are not in piston mode. Thus all cells are not submitted to the same stress. Some of them are highly stressed while others are not stressed. To consider this phenomenon, we decided to work using surface point of view. If we define vibration amplitude threshold, a part of investigated surface as higher vibration amplitude than the threshold. We calculated surface rate versus threshold and obtained curves shown on figure 2.

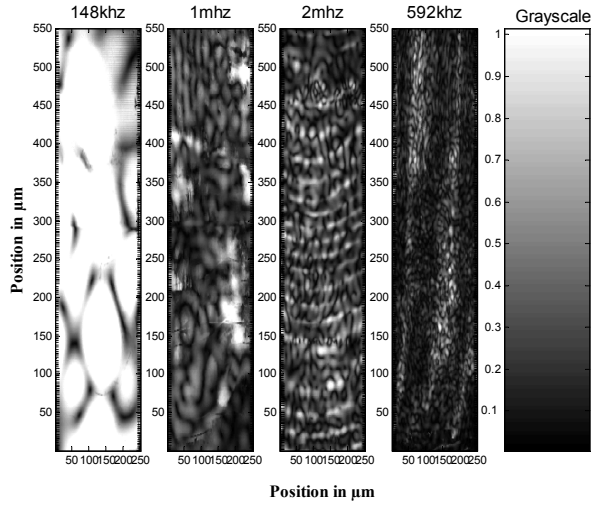

Figure 1: Normalized displacement cartography of glass/ceramic interface. Frequencies used were 148kHz, 592kHz, 1MHz, 2MHz. Power adjustment was 2 Watts.

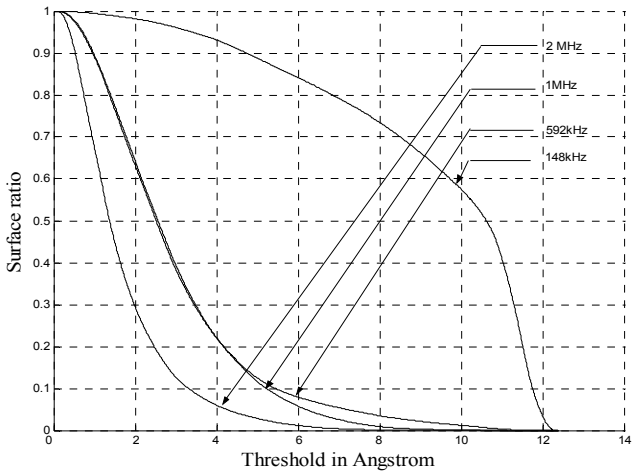

Figure 2: Curves of cell culture surface ratio versus vibration amplitude threshold. Power adjustment was 2 Watts.

Cell counting

We performed measurements described earlier for two flaskets for each power value at each frequency. Results for two series of manipulation are shown on Figure 3. As expected, for a 148 kHz frequency, detachment rate grow with electrical power applied to ceramics. For higher frequencies, cell detachment rate remains overall lower than 10%. It means that only low frequencies are able to detach cells.

At 148 kHz we reach a detachment rate value of about 50%. As cells grown roughly uniformly we can consider that in this case, about 50% of cell surface has been detached.

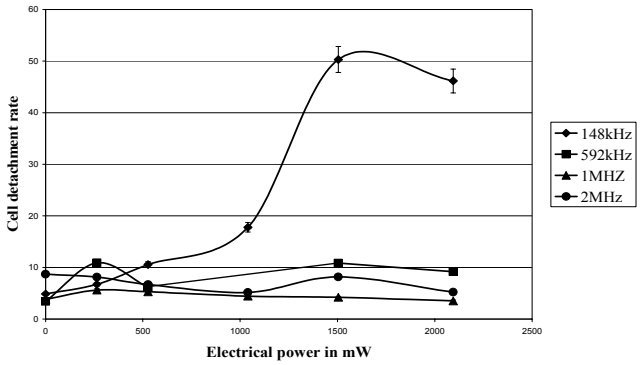

Figure 3: Cell detachment rate versus electrical power applied to ceramics for four frequencies (148kHz 592kHz 1MHz 2MHz).

Interpretation

We can see that we have got strong detachment for low frequencies (148 kHz), but weak detachment for higher frequencies. It can be explained by interferometer observations. Indeed, low frequencies detach less than 10% of cells what corresponds to 10% of the cellular culture surface. This give a value of threshold needed to detach cells higher than 6 Å. With a frequency of 148 kHz cell detachment rate can be as high as 80%. Observation of cell detachment curves gives us a rate of 50% for 148 kHz. This corresponds to a vibration amplitude of 10,5 Å. From this value, it is possible to obtain longitudinal stress needed to detach cells with acoustic propagation theory. We found a value of 164nN for longitudinal detachment force. We are in the order of magnitude of values found by other techniques [3].

Conclusion and prospects

By the use of ultrasound, we performed a technique allowing to detach cells from substrate. Frequency effect study permitted us to obtain a value for the longitudinal adhesion force of cells on glass. Under our experimental conditions, we found a value for this force of 164nN. This is a new information upon cell adhesion phenomenon because all existent techniques give information on shear adhesion force [3].

We are now performing studies on different substrates, at different frequencies and power.

References

[1] Orsello, E.C. *et al*, Molecular properties in cell adhesion: a physical and engineering perspective. Trends in Biotechnology **19** (2001), 310-316

[2] Bell, G.I. Models for the specific adhesion of cells to cells. Science **200** (1978), 618-627

[3] Missirlis, Y.F. *and* Spiliotis, A.D. Assessment of techniques used in calculating cell-material interactions. Biomolecular Engineering **19** (2002)

[4] Myrdycz, A. *et al*, Potentialities of ultrasounds for the non destructive evaluation of cell adhesion. Bone **25** (1999), 75s-79s