ON THE DYNAMICAL BEHAVIOUR OF THE CELLS DETACHMENT PROCESS USING ULTRASOUNDS

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Abstract

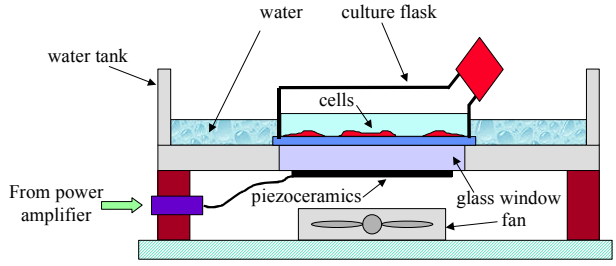
A novel method of cell detachment is reported in this paper. It uses low frequency ultrasounds to stress and detach cells cultured in a flask. An interpretation of the detachment process is basically introduced, based on energy consideration. Results show that cell adhesion can be evaluated by measuring the rate of detachment versus the time of exposure and the influence of some parameters is considered.

Introduction

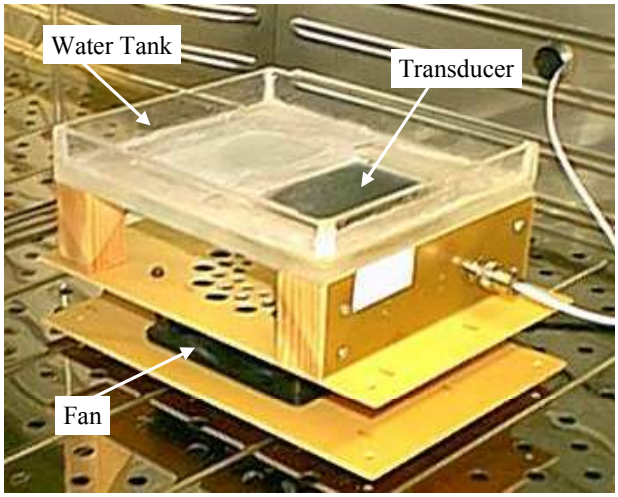
Cell adhesion on a biomaterial is an important phase of a cell-material interactions and the quality of this phase govern the success of the biomaterial integration [1]. The need of quantitative methods for cell adhesion evaluation then become crucial. Cell adhesion on a substrate can be evaluated by the mechanical stress required to detach those cells. Ultrasounds provide an efficient method to stress the cells and detach them. We have conduct a wide experimental study on human osteoblast detachment using low frequency ultrasounds (≅190 kHz). We use piezoceramics transducers mounted on a glass plate that serves as culture flask support. The ceramics are excited with continuous waves and electrical power is recorded during the experiments. The effect of several parameters such as power, thickness of culture medium, cells type, confluence,… are analysed and some of the interesting results on the cell viability after insonification are shown in this paper. Numerical evaluations of stresses at the cell-substrate interface are performed using a simple multilayer model. Preliminary indications on the modelization of the dynamical behaviour of the mechanical detachment process are also given.

Materials and Methods

The ultrasound exposure system includes an insonificator device, a continuous wave power generator and a digital power meter connected to a computer. The insonificator device is shown on Fig. 1a and 1b. It is composed of a piezoceramic transducer (PZT P1-88) bonded to a glass window on which the culture flask is placed. The glass window is insert in tank filled with water. When electrically excited, the transducer, generated plane compressionnal waves through the glass window. After travelling the glass and a thin layer of water, the vibration crosses the bottom of the culture flask on which the cells growth and stress them. The frequency of excitation given by the generator is tuned according resonant frequency of the thickness mode of the piezoceramic (190 kHz). In order to prevent from overheating, the piezoceramic is cooled with a fan. The overall device is placed in a temperature controlled enclosure maintained at 37°C for the cells development.



(a)



(b)

Figure 1: (a) The 190 kHz Insonificator device, (b) photography of the realization.

The output level of the generator is adjusted to settle the absorbed electrical power. Voltage, current and power are recorded with a digital power meter connected to a computer via a GPIB interface.

These measurements are used to estimate the stress T_{cells} applied to the interface between the cells and the flask. Under the assumption that a one dimensional

propagation model in multilayer media can be applied, the stress T_{cells} is evaluated by the expression [2]:

$$(1)\quad \begin{bmatrix} T_{cells}.S \\ j\omega u_{cells} \end{bmatrix} = T_a^{-1} T_e^{-1} \begin{bmatrix} \bar{V} \\ \bar{I} \end{bmatrix}$$

Where S is the surface of the culture subjected to ultrasounds, u_{cells} the mechanical displacement, $\omega = 2\pi f$ is the pulsation of the CW excitation, T_a is the acoustical transfer matrix of the different layers between the transducer and the cells, T_e the electromechanical transfer matrix of the transducer given by the Mason equivalent model [3] and $\left[\bar{V} \quad \bar{I}\right]^T$ is a vector whose components are the complex voltage and current absorbed by the insonificator. They are obtained from the measures of V_{meas}, I_{meas} and P_{meas} by the following formulas:

$$(2)\quad \begin{cases} \bar{V} = V_{meas} \\ \bar{I} = I_{meas} e^{j\phi} \\ \phi = \cos^{-1}\left(\dfrac{P_{meas}}{V_{meas} I_{meas}}\right) \end{cases}$$

Detailed expressions of T_a and T_e are not given here and can be found in reference texts.

The figure 2 shows the linear behavior of the stress/displacement relationship computed from experimental data. This result allows us to consider the cell/substrate interface as collection of mass/spring system.

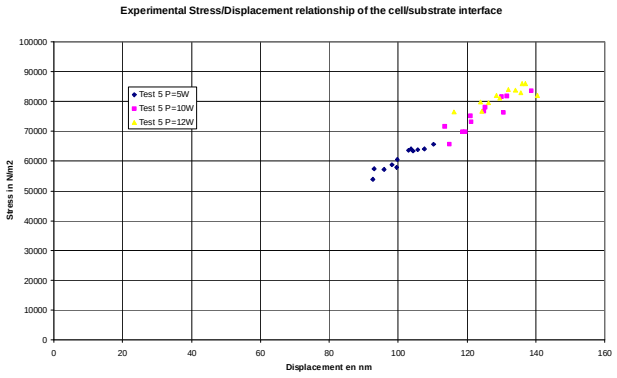


Figure 2: Linear behavior of the cell/substrate interface.

To evaluate the detachment of cells, the number of cells detached after 12 successive periods of 5 minutes of insonification was evaluated using a Coulter Counter Z1 (Beckman Coulter, Roissy, France). The curves of percentage of detached cells versus time of insonification were established. Various parameters supposed to have any influence on the cell detachment with ultrasounds were tested:

- Ultrasound power
- Cell density i.e. incubation time
- Surface roughness on titanium sample

Results

All experiments where done with MG63 cells (human osteoblastic cells). The figure 3 represents one of the typical results obtained on power effects. It shows that the lower the power the more difficult the detachment, i.e. the detachment kinetics for 5W start slowly than for higher powers (10 and 12W).

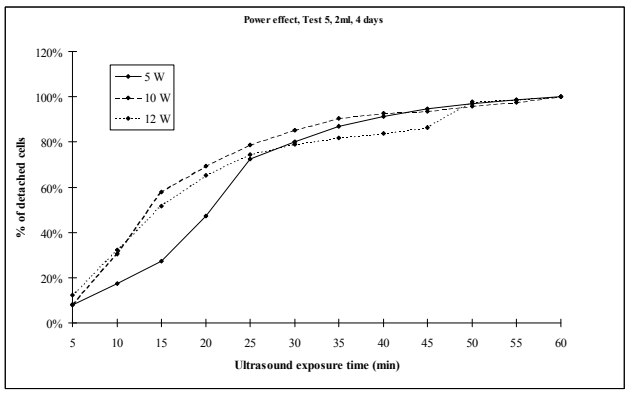


Figure 3: Effect of ultrasound power.

The figure 4 represents the detachment curves for 5 cell densities according to the fact that the cell density increases with the incubation time. We observed that cells detached less rapidly after 4,5 and 6 days than after 2 days of culture. This could be related to the fact that during the first 2 days, the cells are adhering although that later, they are proliferating and consequently less adherent.

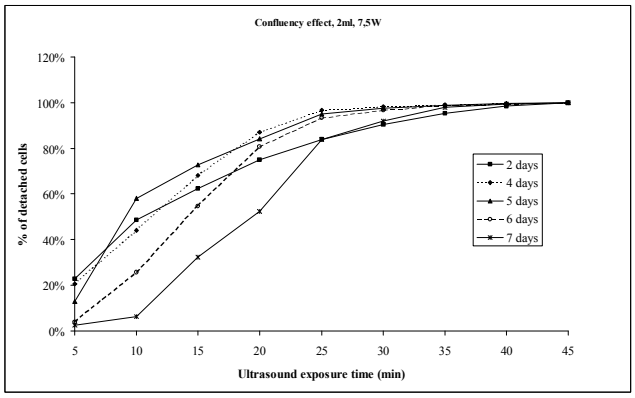


Figure 4: Cell density effect.

The surface roughness effect was studied with cells cultured on titanium samples [4]. Two kinds of surfaces were prepared: optical quality surface and rough surface. The results shown on figure 5 indicate that cell adhesion increases with the roughness of the surface.

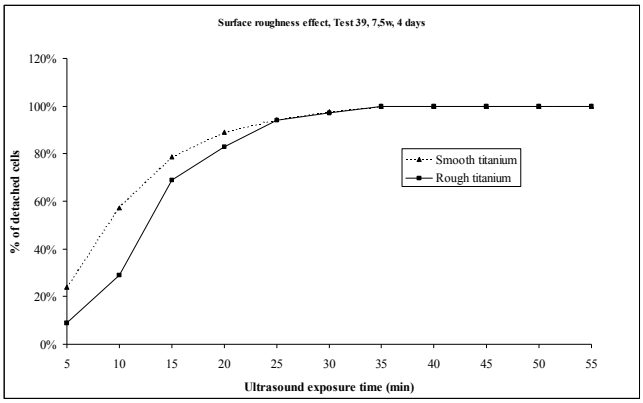


Figure 5: Surface roughness effect.

Finally, figure 6 shows an example of the cell viability obtained after re-inoculation for various power and ultrasounds exposure time. Very good viability are obtained (40% up to 90%).

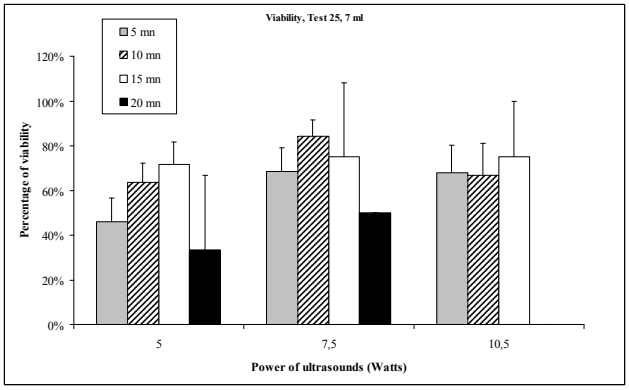


Figure 6: Viability of cells after re-inoculation.

Conclusion

We have developed an US based efficient power controlled detachment methods which can be used to evaluate different degrees of adhesion. The good viability of the re-inoculated cells after ultrasounds exposure show that this method is relatively non-lethal and can be used as an alternative method to classical trypsine enzymatic detachment method. Applying direct mechanical effects on cells by the use of ultrasounds, it provides an efficient tool to study and understand the mechanical behavior of a cell layer for orthopedic applications.

References

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