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Interactions between Biological Media and Piezoelectric Ceramic in Micromixing Applications

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Abstract. We studied the compatibility between a piezoelectric ceramic and a biological suspension: (i) the influence of this substrate on the cell population and (ii) the influence of the cell suspension on the piezoelectric ceramic. We used a 40 mm diameter/0.5 mm thickness niobium substituted PZT wafer. The wafer was put in a Petri dish containing a cell suspension, a second Petri dish containing the same biological medium without ceramic wafer beeing kept as reference. Both Petri dishes were observed for 4 days in order to see the cell population development. The ceramic surface was not affected nor the cells development.

Keywords: Bio-compatibility, Microfluidics, SAW micro-mixer.

1. Introduction

Microfluidic systems miniaturize chemical and biological processes on a sub-millimeter scale. Reducing the dimensions of macroscopic biological or chemical laboratories is advantageous for the following reasons: the small scale allows the integration of various processes on one chip analogous to integrated microelectronic circuitry ([1], [2]). Such integration is the prerequisite for a fully automated data management system covering all steps of a given chemical or biological process. The required reagent volumes are reduced saving both material costs and process time. Finally, the

miniaturization results in enhanced precision by providing more homogenous reaction conditions in shorter times ([3], [4]).

Surface acoustic wave (SAW) oscillators as nano-pumps and micro-mixers in microfluidic bio-medical applications are one of the uprising domain [5].

It is known that biological fluids (cell suspensions, various solutions, some chemicals) may be very aggressive to the materials used in biomedical engineering. It is a less studied aspect of biocompatibility.

In this respect, two aspects were studied: (i) the influence of piezoelectric ceramic substrate on the cell population and (ii) the influence of the cell suspension on this kind of substrate (surface damaging, roughness or porosity increase).

2. Experiments on interaction between piezoelectric ceramic and biological media

The SAW micro-mixer structures tested in our experiments is shown in Fig. 1 (a), where (a) is metallization not removed from the ceramic disk, (b) is interdigital transducers system detailed in Fig. 1 (b) and (c) is the mixing surface. There are two identical structures that will be separate by an adequate diamond cutting operation.

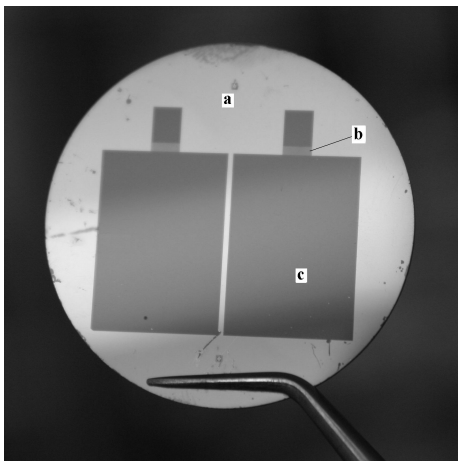


Fig. 1. Two structures of SAW micro-mixer on a piezoceramic wafer.

These structures are made on a piezoelectric ceramic niobium substituted PZT wafer (a) with 40 mm diameter and 0.5 mm thickness. On this wafer an IDT structure (b) was made using a photolitographic process. The metalic line width as well as the interdigital space are 12 μm. Transducers launch surface acoustic waves in the region (c) of the structure that is the active mixing part of our micro-mixer. Subsequently, each of these structures will mount in a suitable mechanical and electrical assembly in order to be used as a mixing device.

This ceramic wafer is the subject of biocompatibility experiences.

First, the ceramic wafer was mirror polished on one face (the active face). Then, the wafer was washed successively with tap water, deionized water and sterile water, in order to remove all the remaining dust from the polishing process. Finally, such prepared mirror polished wafer surface was analyzed by optical microscopy and by atomic force microscopy in order to estimates the surface roughness.

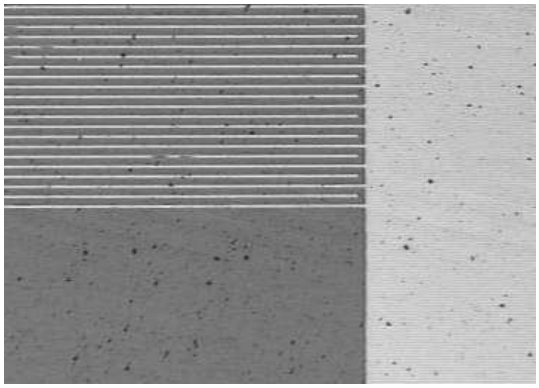


Fig. 2. Structure of Interdigital transducers (IDT).

As biological medium was used a suspension of DC3F cells (pulmonary fibroblasts of Chinese hamsters) in D-MEM buffered with PBS, in a concentration of 5×10^5 cells/50 mm diameter Petri dish.

The ceramic wafer was put in a Petri dish and other two Petri dishes containing the same biological medium but without ceramic wafer was kept as reference. All Petri dishes were maintained at 37°C in 5% CO_2 atmosphere and were observed day by day for 4 days in order to find out the cell population development.

Figure 3 shows the piezoelectric ceramic wafer in the Petri dish containing the biologic medium.

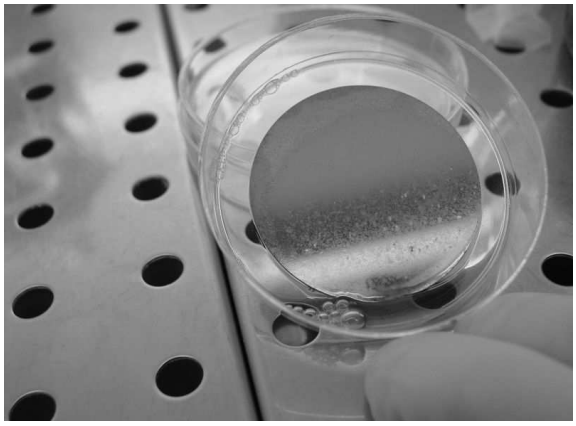
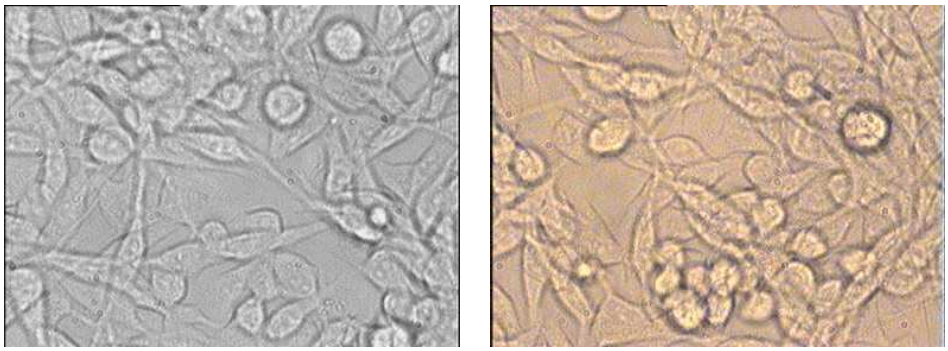


Fig. 3. Petri dish containing the ceramic wafer immersed in the cellular culture.

2.1. Influence of piezoelectric ceramic substrate on the biological fluids

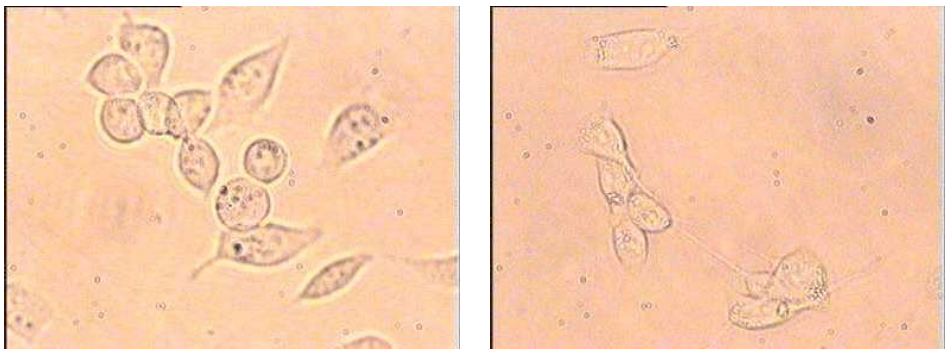
The development of cell cultures in experimental and in reference Petri dishes are shown in Fig. 4 (a) and (b). One may see, at the optical microscope, that in the 3-rd day of experience the cells are attached in two locations in the reference Petri dishes. Also, they form a continuous cellular layer and the color of the culture medium is orange.



(a) (b)

Fig. 4. Continuous cellular layers in two locations of the reference Petri dishes in the 3rd experience day. Phase contrast microscopy 100×.

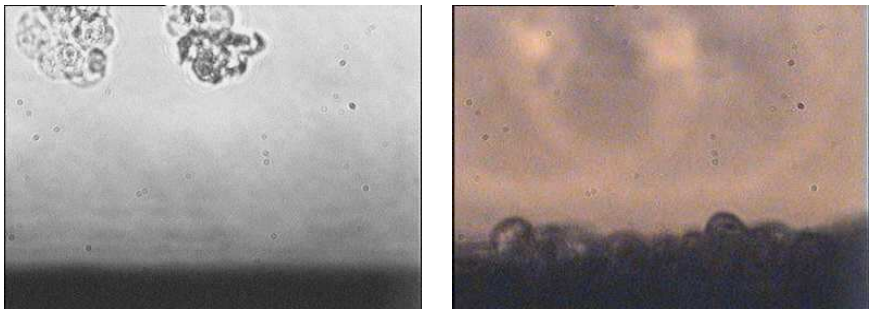
The situation at the same time (the 3rd day) in the Petri dish containing the ceramic wafer is presented in Fig. 5 (a) and (b) and Fig. 6 (a) and (b). One may see, in Fig. 5 (a) and (b) groups of viable cells floating in suspension in the experimental Petri dish.



(a) (b)

Fig. 5. Viable cell configuration in two locations in Petri dish containing piezoelectric ceramic wafer in the 3rd day of experience. Phase contrast microscopy 100×.

Also, groups of dead cells are visible in Fig. 6 (a) and (b) floating in suspension near the ceramic wafer. There are not cells attached on the ceramic wafer and the culture medium is pink.

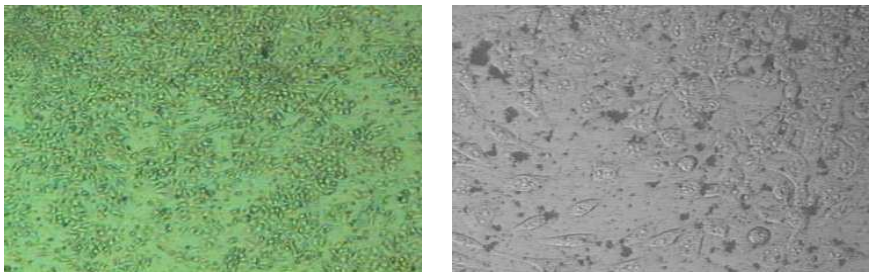


(a) (b)

Fig. 6. Clusters of dead cell in two locations in Petri dish containing piezoelectric ceramic wafer in the 3rd day of experience. Phase contrast microscopy 40×.

In the 4rd day the experience was stopped and the ceramic wafer was washed in HBSS and the fixed in glutaraldehide 25%. The result is visible in Fig. 7 (a) and (b).

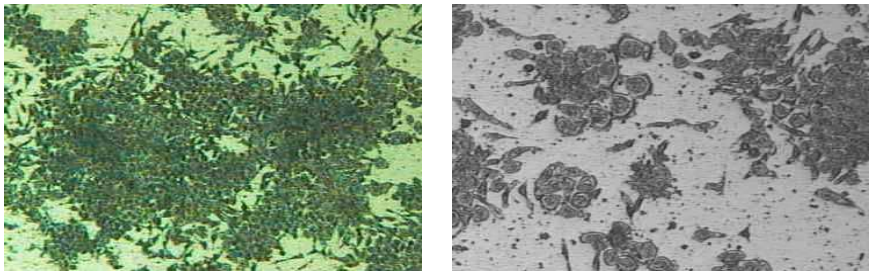
One may see big cellular groups attached to the piezoelectric wafer but without forming a continuous layer. Finally, the surface of ceramic wafer was treated for 4 min with trypsin (5 ml/37°C).



(a) (b)

Fig. 7. Two locations on experimental substrate after washing in HBSS and the fixation in glutaraldehide 25%. Phase contrast microscopy (a) 20×; (b) 40×.

Then, the wafer was washed with HBSS and deionized water in jet. The surface of the ceramic wafer after this operation in shown in Fig. 8 (a) and (b).



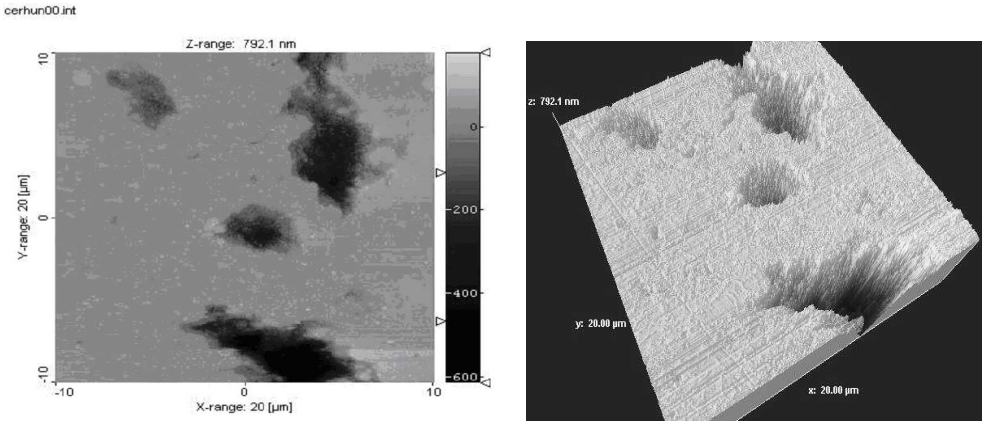
(a) (b)

Fig. 8. Aspect of the ceramic wafer surface after trypsin treatment. Phase contrast microscopy (a) 20× ; (b) 40×.

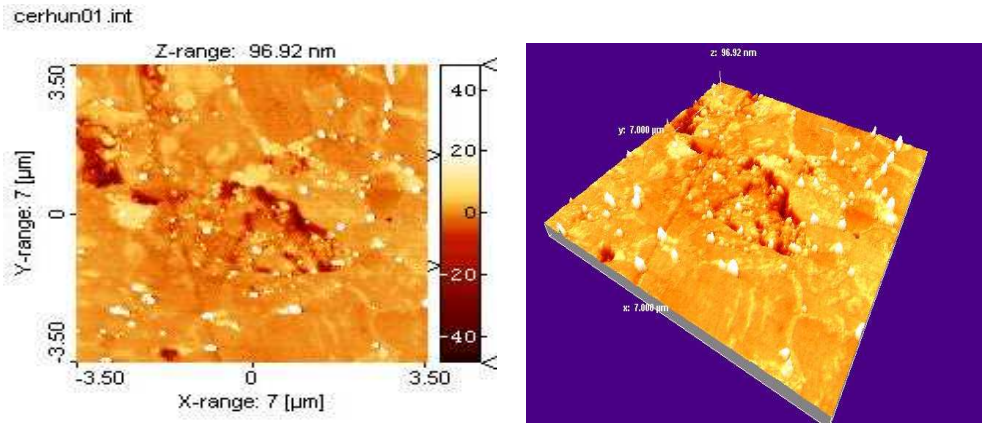
One may see, again, the cellular groups but they don't form a continuous layer. In the same time, the two references Petri dishes shown an abundant cells development.

2.2. Influence of biological media on the piezoelectric ceramic substrate

An atomic forces microscope (AFM) recorded the initial roughness of the mirror polished piezoelectric ceramic. This initial status of the ceramic substrate is presented in Fig. 9 (a)–(d) for four different scanning domains: 20×20 μm^2; 7×7 μm^2; 3×3 μm^2 and 2×2 μm^2 respectively, all in two and three dimensions. One may see in Fig. 9 that the surface is relatively smooth. Some holes of approx. 800 nm depth in the region of the scanning AFM area are observable. In Fig. 9 (d) there are a detailed view. In fact, there were more than four measurements. Because the AFM maximum scanning range is 20 μm, we made a lot of measurements on the wafer surface but the aspect is mainly the same like in Fig. 9.



a



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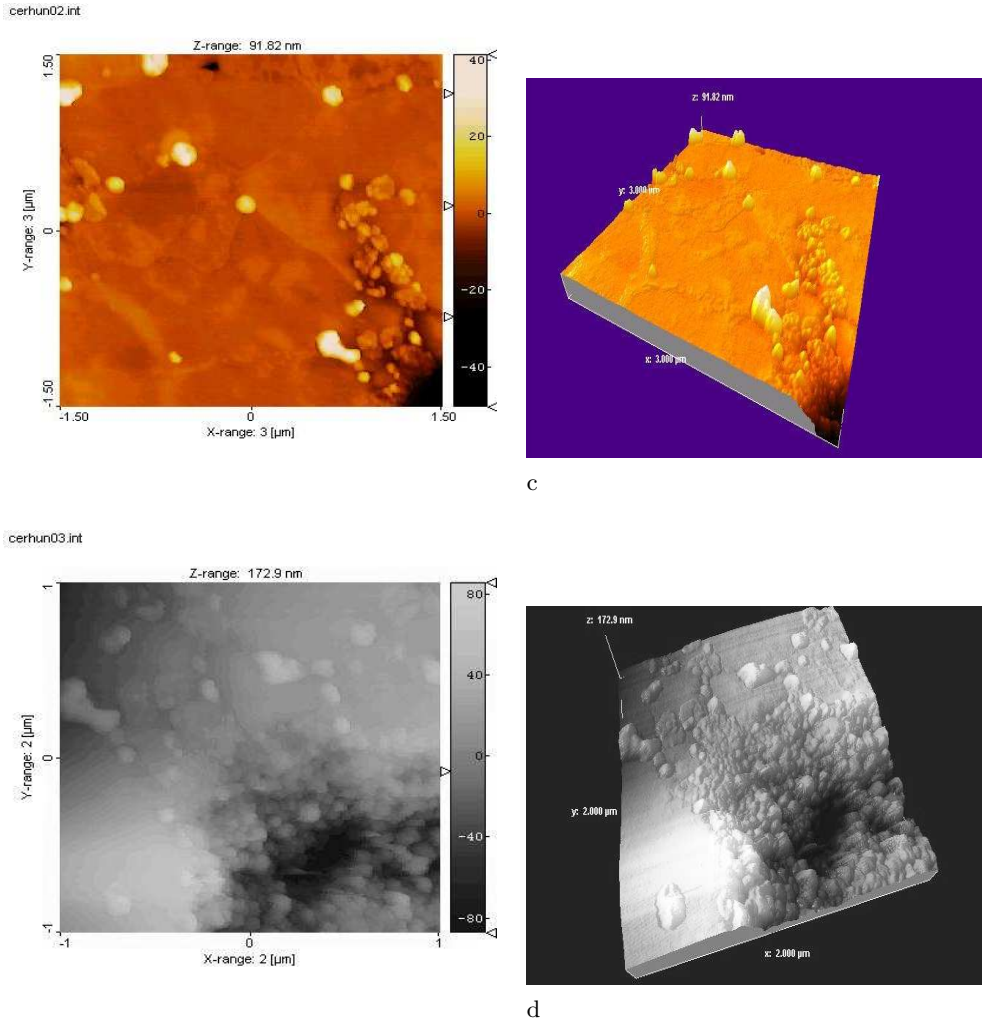
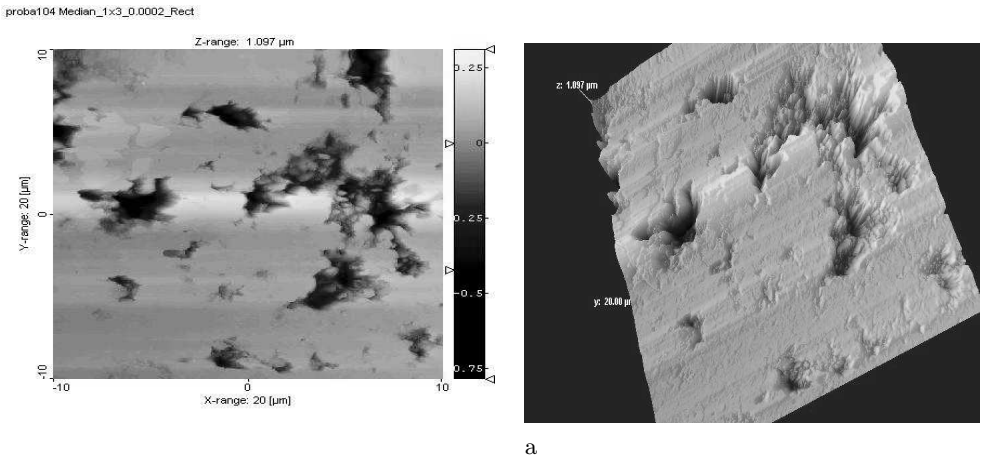


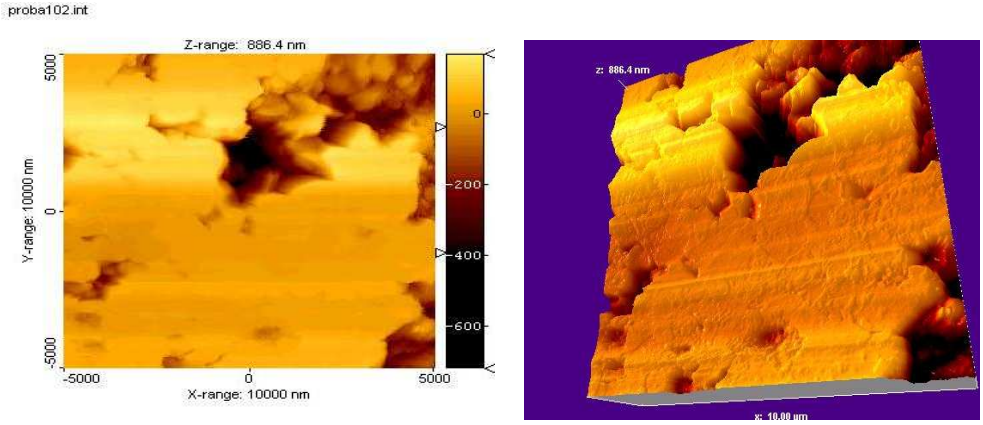
Fig. 9. Surface roughness of the piezoelectric ceramic wafer prior the experiments. Scanning domain: (a) 20×20 µm^2; (b) 7×7 µm^2; (c) 3×3 µm^2; (d) 2×2 µm^2.

After the experiment, the ceramic wafer was cleaned and a new set of AFM measurements were made. The results are shown in Fig. 10 (a)–(d) for the following different scanning domains: 20×20 µm^2; 10×10 µm^2; 5×5 µm^2 and 2×2 µm^2 respectively, all in two and three dimensions. As like in previous measurements we made more than four determinations.

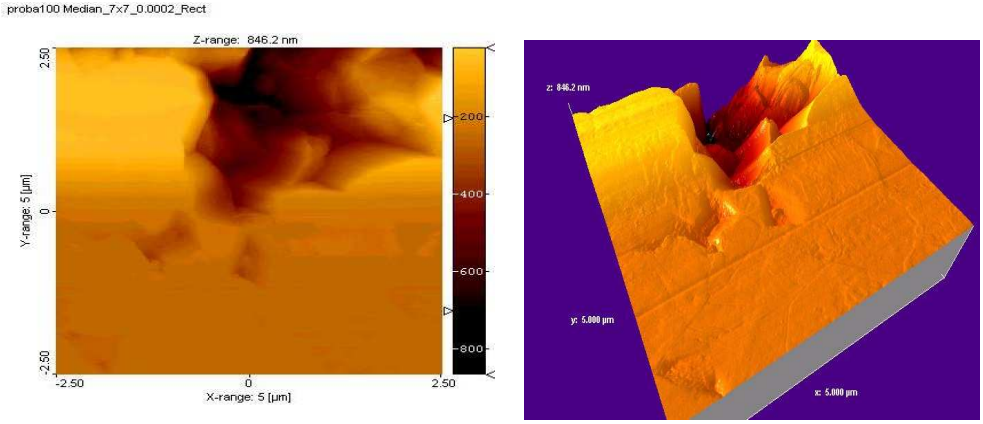
From these recordings it may see that the roughness of the ceramic wafer surface is mainly the same like prior the experiments. A hole of about 750 nm depth may be observed in Fig. 10 (c) at the limit of the scanning range, comparable as dimension and aspect with the hole recorded in Fig. 9 (a).


proba104 Median_1x3_0.0002_Rect
Z-range: 1.097 µm
Y-range: 20 [µm]
X-range: 20 [µm]
z: 1.097 µm
y: 20.00 µm

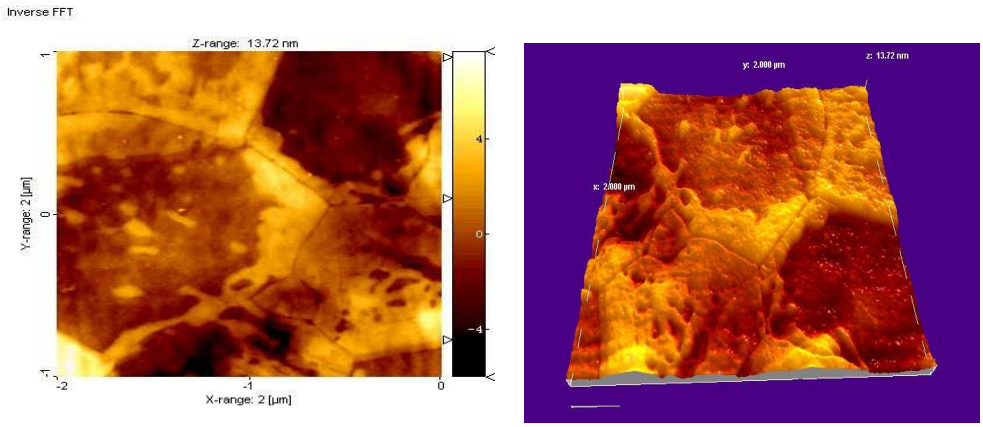
a


proba102.int
Z-range: 886.4 nm
Y-range: 10000 nm
X-range: 10000 nm
z: 886.4 nm
x: 10.00 µm

b


proba100 Median_7x7_0.0002_Rect
Z-range: 846.2 nm
Y-range: 5 [µm]
X-range: 5 [µm]
z: 846.2 nm
y: 5.000 µm
x: 5.000 µm

c



d

Fig. 10. Surface roughness of the piezoelectric ceramic wafer after the experiments. Scanning domain: (a) 20×20 μm^2; (b) 10×10 μm^2; (c) 5×5 μm^2; (d) 2×2 μm^2.

A profile through this hole was traced and presented in Fig. 11 probing that the depth of the irregularities of ceramic surface, about 750 nm in this case, didn't increase following the action of cellular suspension used in experiments.

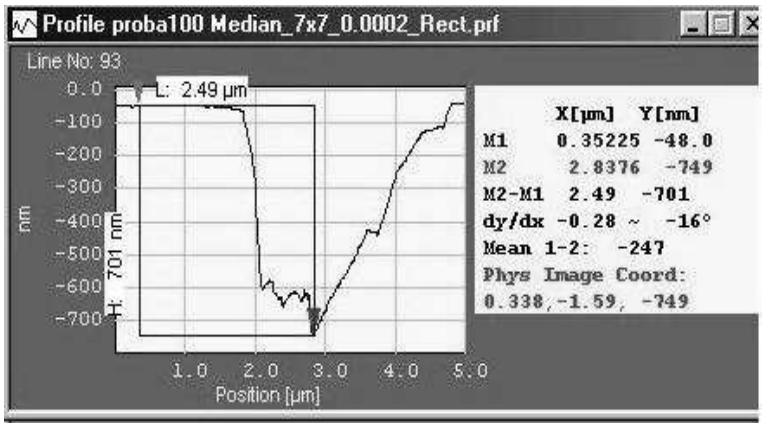


Fig. 11. Depth profile through the hole in Fig. 10 (c).

Concerning cleaning of the ceramic wafer after the experiment, it was a more difficult task than it is believable. In photo in Fig. 12, one may see the experimental ceramic wafer (in the left) compared with a genuine one of the same type and polished in the same manner (in the right).

The photo was made after a ceramic surface washing stage in jet of deionized water. It is remarkable the adherence of the cell remaining on the ceramic surface. Even by using a very strong mixture of oxidants it were some cells remaining on

the ceramic surface. It is possible to totally remove the cells remaining only by re-polishing the ceramic wafer surface, but this operation may drastically damage the SAW interdigital transducers.

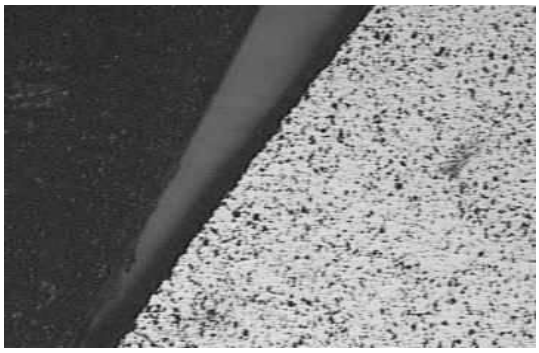


Fig. 12. Comparison between the experimental ceramic wafer and a genuine one.

3. Conclusion

In order to fabricate a microfluidic micro-mixer for applications in medicine and biology, experiments were made on biocompatibility between piezoelectric ceramic and the biological medium consisting in a cellular suspension.

The objectives were to establish if this kind of ceramic substrate has an influence on the development of a cell population and reciprocal, if the action of the biological medium could damage the surface of the ceramic wafer.

For the first objective, the growth of the cellular population was slightly affected by the presence of the ceramic substrate. As one may see in Fig. 5, in the 3^{th} day of experiment the cells had difficulties to fix and to grow on the ceramic substrate. Even in the 4^{th} day, when the experiment finished, the cells failed to form continuous layer on the whole ceramic surface (see Figs. 7 and 8).

Concerning the second objective, the ceramic surface was not affected. The substrate roughness and the surface porosity didn't increase following the contact with the cell suspension. As a negative aspect is the fact that the cell remains (dead cells) on the wafer surface are rather difficult to clean out.

A method to solve this problem may be the coating the entire mixing surface with another material easy to clean (ex. SiO_2) but this solution may reduce the mixing efficiency. Also, the question of the biocompatibility is transferred from the ceramic-biological medium system to the SiO_2-biological medium system.

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