Epithelial cell interaction with micro-balloons

Introduction

The purpose of the current project was to study the interaction of human lung carcinoma epithelial cells (A549) with different kind of micro-balloons (MB) under dry and liquid phase. The MB's and their interaction with the cells were visualized using transmission and fluorescence microscopy. All experiments were conducted at the group of Prof Kenneth Dawson, UCD.

Microballoons

The description of MBs used in this project is presented in Table 1. MBs were used as dry substrates for cell seeding and also were mixed (while still in liquid phase) with the cell population in different concentrations. Cell attachment and morphology on the MBs substrates and cell mobility were examined.

Table 1: Description of MBs used

MBS	Full name	Charge	Aggregation	Concentration	Toxic
PVA	pH 5 RT	unknown	none	2.4×10^8 MBs/ml	
LBL04	AG-MB/PMAA	-	none	1.47×10^9 MBs/ml	No toxic
LBL07	AG-MB/PSS/PAH	+	none	10^9 MBs/ml	Most toxic

The dried substrate was produced as shown in Figure 1. First, a glass slide was put on the top of a tube, then a drop of 70 µlt of the MBs solution was added on the top of the glass and was covered with a petri dish, so that a flat layer to be formed. The MBs were left to dry overnight. Then the tube together with the glass slide was removed and the dried layer with the MBs was left at the bottom of the petri dish. The same procedure was followed for all three types of MBs until a uniform layer is formed.

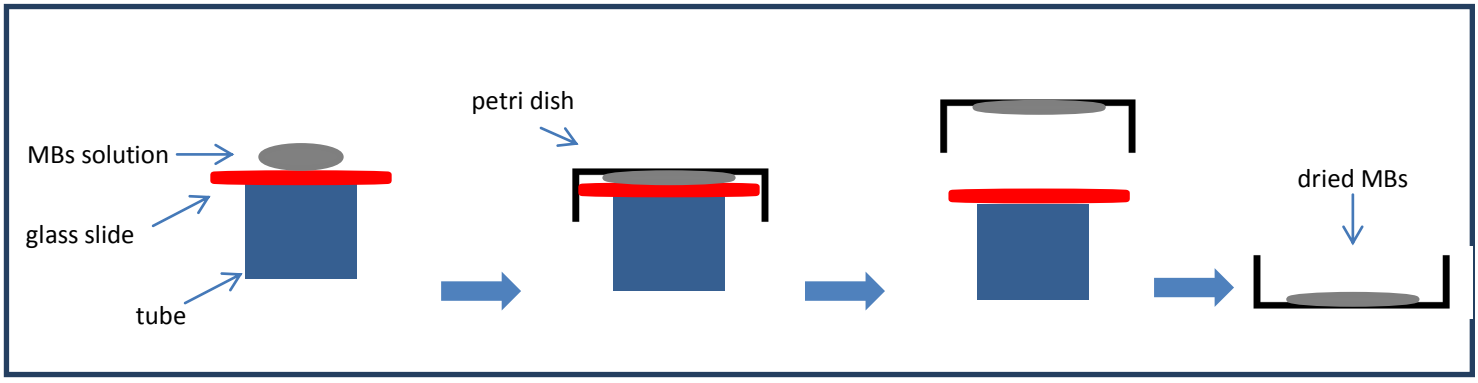

Figure 1: Dried layer of MBs production

Imaging of the MBs layers was conducted using transmission microcopy (Figure 2). Figure 2a shows the PVA, Figure 2b the PHA and Figure 2c the PMAA MBs respectively. The magnification used for all images is x10.

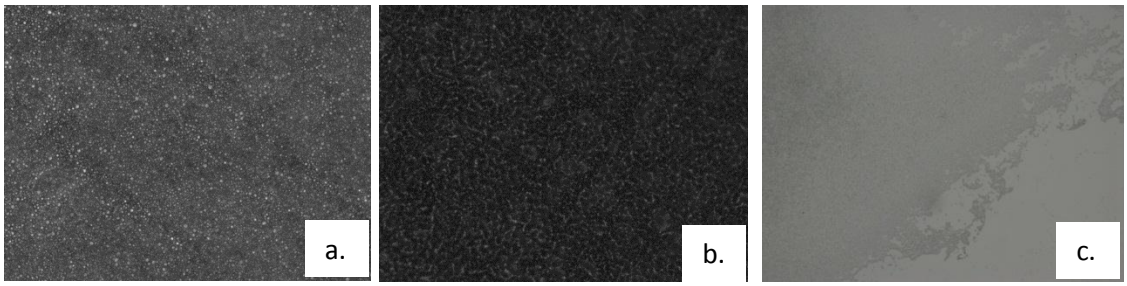

Figure 2: MBs images: a) PVA b) PHA and c) PMAA

As it can be observed from Figure 2, the layer of the MBs formed is compact for the PVA and PHA but not for the PMAA MBs. The PMAA MBs did not attach homogeneously onto the petri dish bottom, showing detachment in some areas and peeled off of the upper layer, the reason for the detachment is not known. Thus, the PMAA layer was not considered appropriate substrate for cell seeding; the cell culture experiments that follow were conducted for the PVA and PHA MBs.

Cell seeding experiments

A549 cells were cultured according to the standard protocol (fed every 2nd day and trypsinised when reached 80% confluency), and were seeded onto the MBs layers. On each substrate 160.000 cells were seeded and then 2ml of medium was added. Then the seeded substrates were incubated at 37^{o}C for 24hrs to let cells attach on to the MBs layers. 24 hrs post seeding the medium was removed and 2 ml of transparent imaging solution was added. The composition of the imaging solution was: 750mM NaCl, 100mM HEPES, 5mM $CaCl_2$, 25mM KCl and 5mM $MgCl_2$, the pH was adjusted to 7.4. The imaging of the cell seeded substrates was conducted at 37 ^{o}C in live cell chamber. Figure 3 shows the imaging of the MBs cell seeded layers. 3a shows the cells attached on the PVA MBs and 3b the cells attached on the PHA MBs. Cells preference to attach on the PAH MBs is obvious from the images below, since the cell population seems to be much higher compared to the PVA layer.

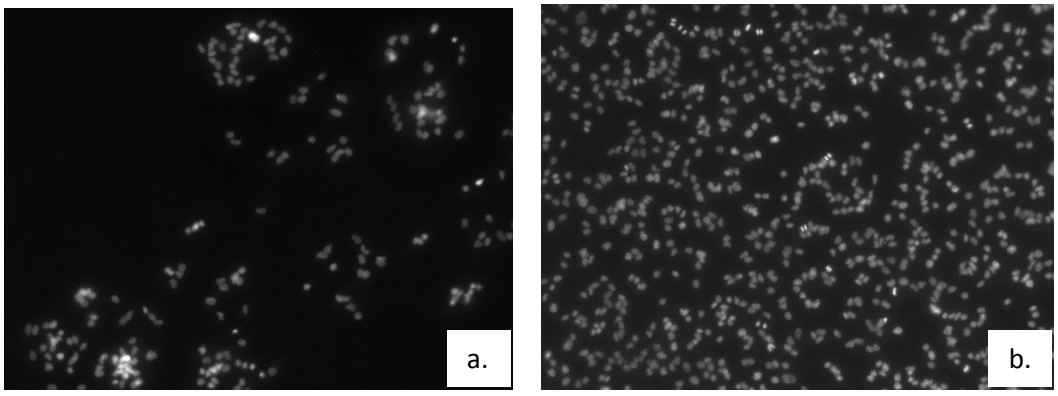

Figure 3: Cells attached on the a) PVA MBs and b) PHA MBs

More detailed images of PHA MBs together with cells are presented in Figures 4a and 4b with magnifications x10 and x20 respectively. The cells can be easily distinguished on the MBs layer.

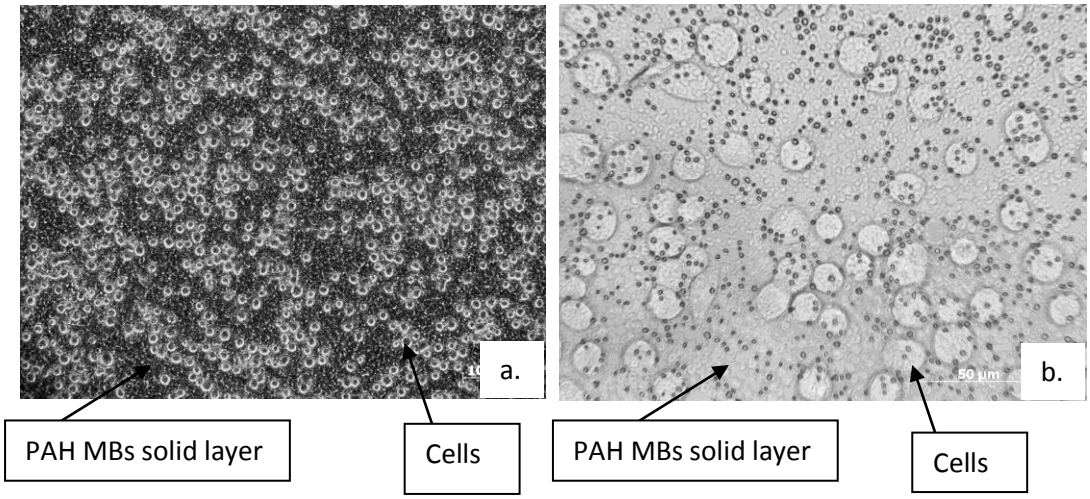

Figure 4: Cells attached on the PHA MBs with magnifications x10 and b) x20

Following to the transmission microscopy, fluorescence microscopy was conducted for both PHA and PVA cell seeded layers to examine the morphology of the cytoskeleton of the cells attached on the MBs layers. For the fixation of the cells 4% formalin was used. All samples were fluorescently stained with phalloidin (Texas Red) for the actinskeleton staining and with Dapi for the nucleus staining. Figures 5 and 6 show the staining results for the PAH and PVA MBs respectively. In all figures the actin is labeled red and the nucleus bleu.

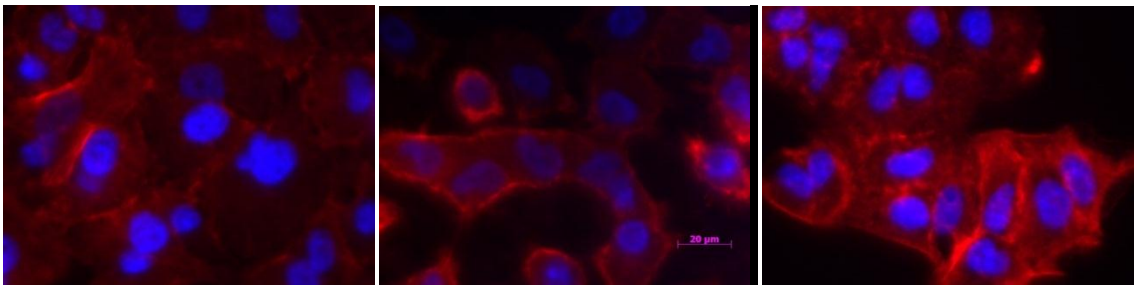

Figure 5: Cell staining with Texas red and Dapi for cells attached on the PHA MBs layer

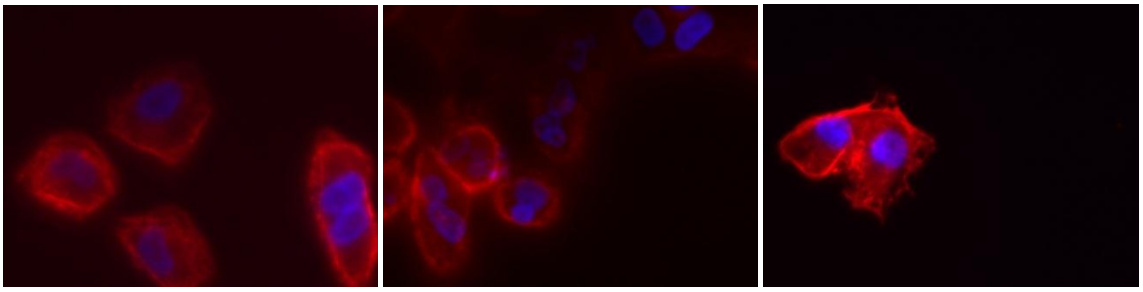

Figure 6: Cell staining with Texas red and Dapi for cells attached on the PVA MBs layer

As it can be observed from Figures 5&6 cells show very similar morphology, typical for healthy attached epithelial cells. On the PHA substrate though cells are more confluent and very close to each other showing a triangle morphology of the actinskeleton.

Cell mobility

Cell mobility was first tested on the dried PAH and PVA MBs substrates. The cells were seeded onto the MBs layers as described previously and the cell culture medium was replaced by the transparent imaging solution. All samples were placed at 37 ^{o}C in live cell chamber and pictures were taken every 5 minutes for 12 hrs. Figure 7 shows the cells behavior on the PAH and Figure 8 on the PVA MB layer for the first 5 hours of imaging. For the PAH substrate very low mobility of the cells can be observed, cells move mainly around their axis and interact with the neighbor cells. On the PVA layer (Figure 8) cell mobility is higher but mainly over the area without MBs. Also a few cells within the 5 hrs, leave the MBs layer and go to the petri dish glass surface, verifying that A549 are not attracted by this layer.

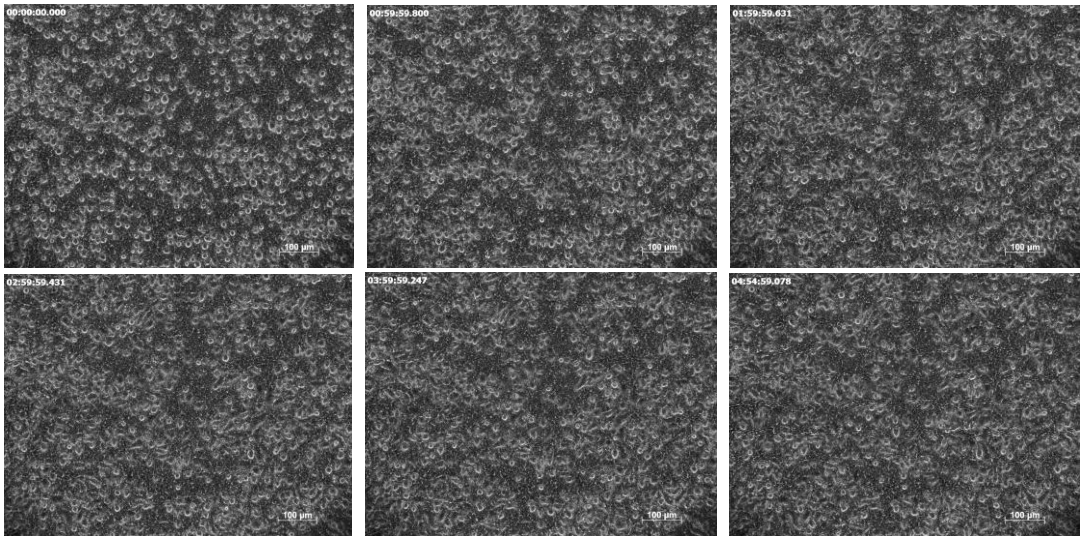

Figure 7: Cell mobility onto the PAH substrate over 5 hours.

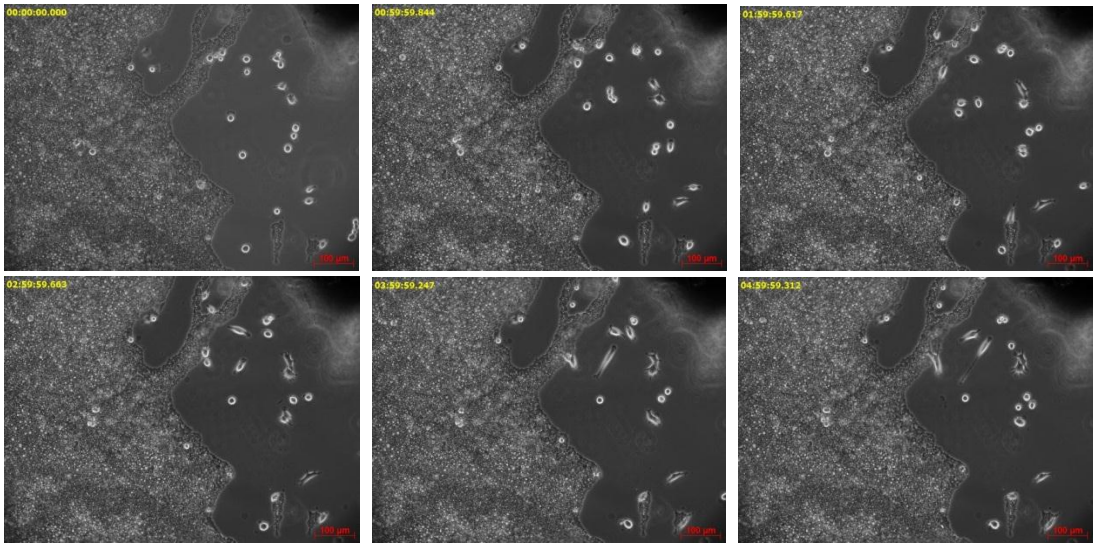

Figure 8: Cell mobility onto the PVA substrate over 5 hours.

Following to the mobility on the dry PAH and PVA layers cell interaction with MBs was also conducted for mixture of cells with the MBs while MBs are still wet. Here only the PHA MBs were used. Three different MBs-cells mixtures were prepared. 160.000 cells were mixed with 10, 5 and 1 µlt of MBs. The mixtures of MBs-cells were visualized overnight. Figures 9, 10 and 11 show the cell mobility on the 10, 5 and 1 µlt of MBs mixtures with cells respectively.

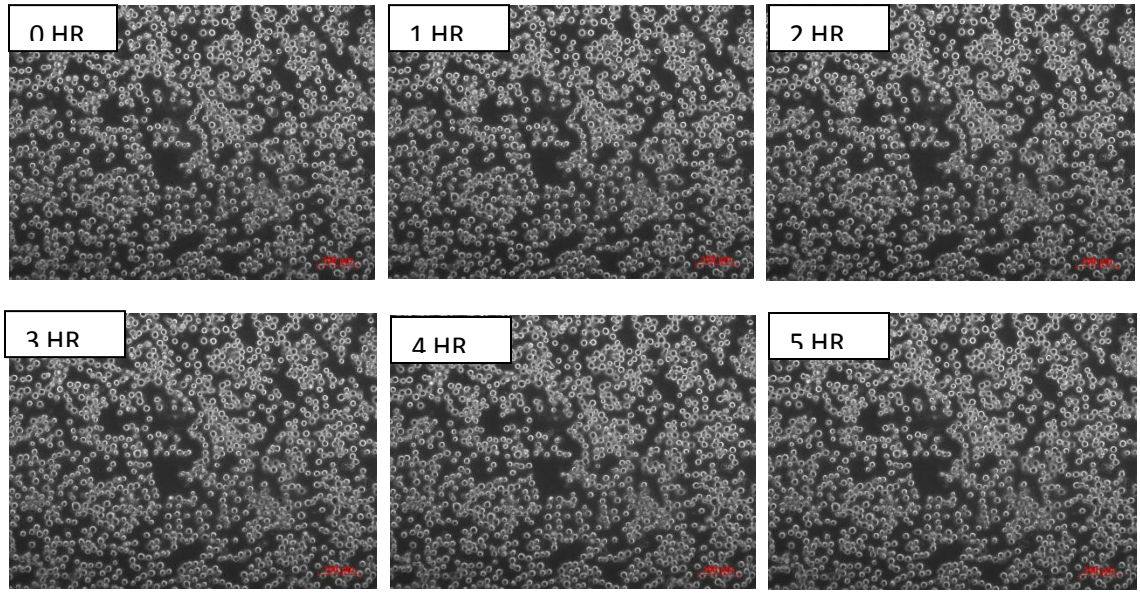

Figure 9: Cell mobility in the cell-PAH MBs mixture with 160.000 cells and 10µlt MBs.

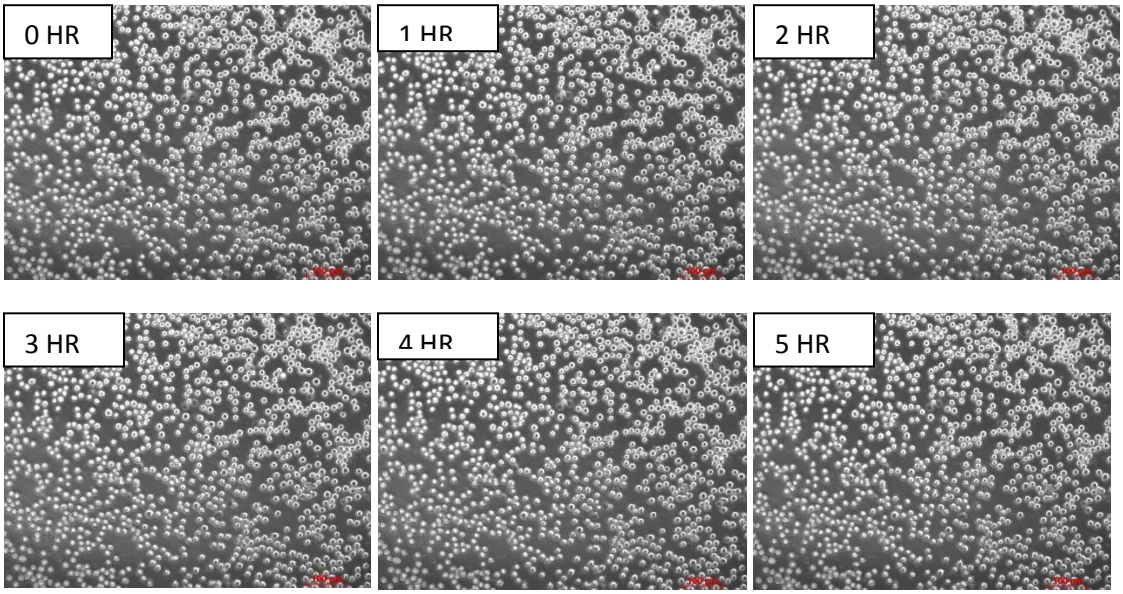

Figure 10: Cell mobility in the cell-PAH MBs mixture with 160.000 cells and 5µlt MBs.

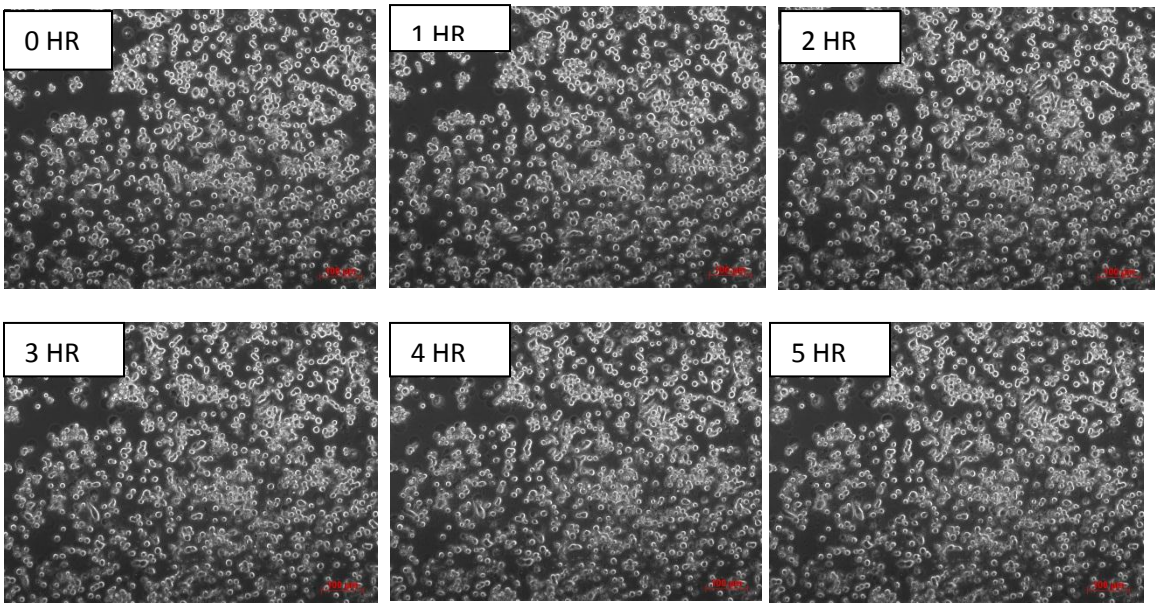

Figure 10: Cell mobility in the cell-PAH MBs mixture with 160.000 cells and 1µlt MBs.

Figures 9, 10 and 11 show the MBs dispersed together with the cells in the imaging solution. As it was expected the MBs float in the liquid, cells are assumed to be below the MBs. The number of MBs that float seems to be similar independently to the amount that was mixed with the cells. From the overnight videos, it could be seen that the MBs shown in Figure 10 were moving faster compared to the other two mixtures but apparently it is not possible to present here, also from Figures 9 and 10 it can be seen that the MBs are very close to each other and only small movements around their axis are allowed. We assume that the movement of the MBs is because of the cells that move underneath them; however since we could not take images of the cells we cannot be sure that this is the reason of the cells movement.

Conclusions

In this project, layers of three different types of microballons (PVA, PHA and PMAA).were produced. Uniform layers could only be achieved with PAH and PVA MBs. For this reason PAH and PVA were used for cell culture studies. 24 hrs post seeding, PAH was proved to be a better substrate for the A549 cells. Fluorescence staining with phaloidin and Dapi, showed that the cells morphology was very similar on the two

substrates. Continuing, cells mobility on PHA and PVA dried MBs surface was conducted. On PAH, cells had very low mobility, however they stayed on the MBs and were slightly moving around themselves. On the contrary, PVA did not attract the cells, since they moved relatively fast out of the MBs layer and migrated to the uncovered glass surface. Very slow mobility was also observed with the different MBs that were mixed with cells.

Future work

For the future experimental work it is suggested:

1. The MBs-cell ratio in the mixture would be optimized.
2. Examine in details the mobility of the MB due to the cell interaction with them.
3. Test the proteins involved in the cell-MBs interaction.
4. Find the appropriate balance for the MBs (before they solidify) so that they form an appropriate layer for the cells to attach, proliferate and create an appropriate environment for new cells to differentiate on it