EVALUATION OF THE VIABILITY OF HL60 CELLS IN CONTACT WITH COMMONLY USED MICROCHIP MATERIALS

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ABSTRACT

This paper presents beneficial data when deciding to perform cell experiments in lab-on-a-chip devices. The choice of material can influence the viability of mammalian cells. PDMS, precoated with serum or not, suits well for HL60 cells, demonstrating the best results in the viability experiments, as expressed as an apoptosis/proliferation ratio < 1. We also looked at other materials, such as native silicon-oxide (SiO_2) and borosilicate glass. In the near future other (combinations) of microchip materials will be analyzed for their viability as well as analyzing the effect of mammalian cells on microchip materials in more detail with MALDI-MS.

Keywords: HL60 cells, lab-on-a-chip, viability

1. INTRODUCTION

Today there is a huge interest and effort in analyzing complex biological systems such as living cells using micro- and nanotechnologies. However, transferring classical biological experiments into the chip environment, we have noticed problems concerning cell viability. It is of course crucial that it is verified that cells are in good viable condition before performing any sophisticated cell analyses on lab-on-a-chip devices, because this generated environment can be quite different from the conventional culture flasks. Therefore new viability control experiments were performed to ensure that cells feel comfortable being in contact with these microchip materials.

2. EXPERIMENTAL

Human promyelocytic leukemic HL60 cells (concentration 1.0 x 10^6 cells/ml) were cultured in a 24-wells plate in contact with native silicon-oxide (SiO_2), glass (borosilicate), and polydimethylsiloxane (PDMS), precoated or not with 100% fetal bovine serum (FBS), for 72 hours in an incubator (humidified atmosphere of 37°C and 5% CO_2). Viability was analyzed with real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR). Cyclin D1 is measured as a proliferation marker. Cyclin D1 plays an important role in the transition from the G1 to the S phase in the cell cycle [1]. Tissue transglutaminase (tTG) is measured as an apoptotic marker. tTG prevents the leakage of intracellular compounds from the cell in the apoptotic cascade [2]. Both markers are measured at the mRNA level. Experiments are performed twice in fourfold.

3. RESULTS & DISCUSSION

Figure 1 shows that HL60 cells in contact with native SiO_2 results in a ratio >1, meaning apoptosis, during the first 24 hours of incubation. However, in time this ratio turns towards proliferation after 48 hours, and apoptosis at 72 hours, though both not significant.

0-9743611-1-9/μTAS2005/$20©2005TRF

Precoating of native SiO_2 with 100% FBS benefits the viability. Borosilicate glass (microscope slides) induces the proliferation of HL60 cells, precoated with 100% FBS or not, after 24 hours of incubation. The best proliferative results are obtained when HL60 cells are in contact with PDMS, especially when the PDMS is precoated with 100% FBS. These results are further confirmed by cell counts, showing the highest increase in proliferation over 3 days of incubation (Figure 2). To prevent the cells from diffusing between the SiO_2 and the bottom of the culture plate, which can negatively influence the viability of HL60 cells, PDMS is placed in between. During the 48 hours of incubation, the cells are proliferating, and the obtained apoptosis/proliferation ratio is slightly better compared to the native SiO_2 precoated with 100% FBS alone. Table 1 summarizes the measured Cyclin D1 and tTG expression separately, and the calculated apoptosis/proliferation ratio for all used microchip materials.

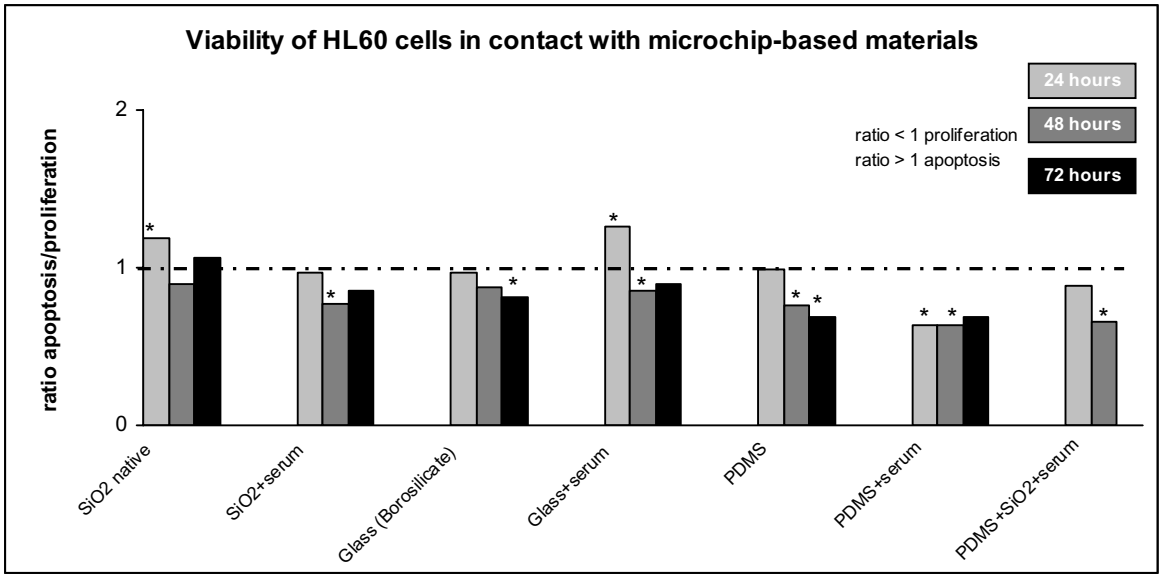

*Figure 1. The apoptosis/proliferation ratio of HL60 cells in contact with microchip materials, measured with real-time RT-PCR. Dashed line refers to the control sample, meaning culture plate only. * = statistical significant, $p \leq 0.05$. The figure represents data from 2 experiments performed in fourfold, with the exception of HL60 cells in contact with PDMS+SiO_2+serum, this experiment is performed once in fourfold.*

*Table 1. Effect of the different microchip materials on the viability of HL60 cells measured with real-time RT-PCR. Cyclin D1 and tTG expression is shown separately, demonstrating cell turnover, as the ratio apoptosis/proliferation. A ratio <1 indicates proliferation and a ratio >1 indicates apoptosis. * = statistical significant, $p \leq 0.05$. The data from HL60 cells in contact with PDMS+SiO_2+serum are from one experiment for 48 hours. All the other experiments are from two experiments for 72 hours.*

	24 hours			48 hours			72 hours		
	Cyclin D1	tTG	ratio	Cyclin D1	tTG	ratio	Cyclin D1	tTG	Ratio
SiO_2	1.00	1.19	1.19	1.02	0.92	0.90	1.31	1.39	1.06
SiO_2+serum	1.11	1.08	0.97	1.05	0.82	0.78	0.91	0.78	0.86
Glass	0.98	0.94	0.96	0.94	0.82	0.87	1.11	0.90	0.81
Glass+serum	0.91	1.15	1.26	0.95	0.81	0.85	1.04	0.93	0.89
PDMS	1.04	1.03	0.99	1.02	0.78	0.76	0.91	0.63	0.69
PDMS+serum	1.36	0.87	0.64	1.29	0.83	0.64	1.25	0.85	0.68
PDMS+SiO_2+serum	0.96	0.85	0.88	1.17	0.77	0.66	-	-	-

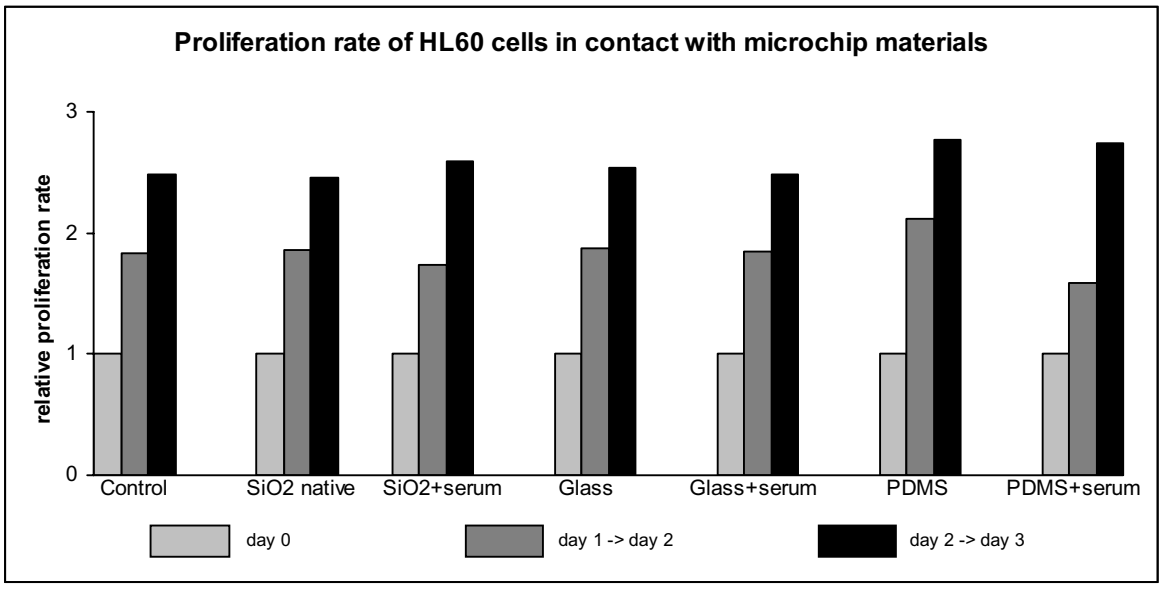

Figure 2. Cell count measurements with HL60 cells in contact with microchip materials performed on a Sysmex cell counter. The relative proliferation rate on day 0 is set at 1 for all materials used. This experiment is performed once.

4. CONCLUSIONS

This paper demonstrates that measuring the viability of HL60 cells in contact with commonly used microchip materials is very advantageous when deciding which material to use for your lab-on-a-chip device. First results show that PDMS, precoated or not with serum, benefits the viability, and can be used in combination with native SiO_2 or borosilicate glass for making fine structures. However, it is not only the material that in the end affects the viability, but the final chip design is also very important. Next steps will concentrate on analyzing other combinations of microchip materials, different modifications and elucidating the mechanism in more detail with MALDI-MS.

ACKNOWLEDGEMENTS

Financial support from the Dutch technology association STW (TMM 6016 'NanoScan' project) is gratefully acknowledged.

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