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Effects of biomaterials for Lab-on-a-chip production on cell growth and expression of differentiated functions of leukemic cell lines

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Abstract The rapid increase of the applications for Lab-on-a-chip devices has attracted the interest of researchers and engineers on standard process of the electronics industry for low production costs and large scale development, necessary for disposable applications. The printed circuit board technology could be used for this purpose, in particular for the wide range of materials available. In this paper, assays on biocompatibility of materials used for Lab-on-a-chip fabrication has been carried out using two tumor cell lines growing in suspension, the human chronic myelogenous leukemia K562 cell line, able to undergo erythroid differentiation when cultured with chemical inducers, and the lymphoblastoid cell line (LCL), extensively used for screening of cytotoxic T-lymphocytes (CTLs). We have demonstrated that some materials strongly inhibit cell proliferation of both the two cell lines to an extent higher that 70–75%, but only after a prolonged exposure of 3–6 days (Copper, Gold over Nickel, Aramid fiber filled epoxy uncured, b-stage epoxy die attach film, Tesa 4985 adhesive tape, Pyralux uncured, Copper + 1-octodecanethiol). However, when experiments were performed with short incubation time (1 h), only Aramid fiber filled epoxy uncured was cytotoxic. Variation of the results concerning the other materials was appreciable when the experiments performed on two cell lines were compared together. Furthermore, the effects of the materials on erythroid differentiation and CTL-mediated LCL lysis confirmed, in most of the cases, the data obtained in cytotoxic and antiproliferative tests.

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Abbreviations

PCB	Printed circuit board
CTLs	Cytotoxic T-lymphocytes
LCL	Lymphoblastoid cell line
ITRS	International technology roadmap for semiconductors
RCC	Resin coated copper
DAF	Die attach film
PDMS	Poly(dimethylsiloxane)
SAMs	Self-assembled monolayers
ODT	Octadecanethiol
PF before lam	Polyurethane film before lamination
PF after lam	Polyurethane film after lamination
PP	Polyurethane powder
Certonal FC-732T	Certonal FC-732 tempered
EBV	Epstein–Barr Virus
FBS	Fetal bovine serum
EBNA-3	EBV nuclear antigen 3
HLA-A2	Human leukocyte antigen A2
E:T	Effector:target
Cpm	Counts per minute

1 Introduction

The development of biomedical devices, including Laboratory-on-a-chip platforms (LAOC) is based on the employment of biocompatible materials, since most of them, expected to interact with biological samples, must satisfy specific requirements, that combine the need to be non-toxic and biocompatible, with suitable biomechanical properties and physical structure. In agreement with the concept that biocompatibility of a material is a central factor in devices intended for medical or biotechnological applications, the 2005 edition of the International Technology Roadmap for Semiconductors (ITRS) has introduced a section on the requirement for assembly and packaging of medical devices and bio-chips in terms of biocompatibility and packaging reliability [1], which is a clear indication of the growing interest of the electronics industry in this sector.

As far as development of LAOC, several technologies for microfluidic device fabrication and packaging have been proposed to integrate sensing capabilities and electrical interfaces into a single device [2], which require complex steps and expensive facilities, most of them being useful only for prototyping. Nonetheless, the demand for low cost disposable devices has steadily increased, due to the large developments in the area of microsystems. However, the adoption of these technologies for large scale production still remains extremely expensive.

In the last years, printed circuit board (PCB) technology has reached resolution of tens of micrometers which is enough for many microfluidics applications. As an example in [3–5] micro channels and viaducts are used to integrate liquid in quasi-standard FR4-PCB. The main limitation of these approaches in biomedical applications is the biocompatibility of the employed materials. Recently, the range of materials for PCB technologies has been extended by the introduction of new ones, many of these developed for flex circuits and rapidly becoming an industrial standard. Cost effective technologies were also proposed introducing additional steps [6, 7] or novel materials (e.g., aluminum) [8] in a standard PCB process. The use of these "board technologies" is particularly indicated for the development of microtiter plates with embedded sensoring and actuating features allowing fast and parallel analysis of biological samples [9–11]. In particular, a recent work demonstrated the possibility to isolate and manipulate single cells in microwells fabricated on this technology [12]. Moreover the compatibility of these structures with standard PCB process, which is characterized by low production cost and large scale development, allows the development of disposable devices for biological applications.

As a specific example, in standard PCB manufacturing process typically resin coated copper (RCC) and glass fiber filled epoxy films are used. General PCB manufacturing flow is sequential lamination and structuring of dielectric and metal layers. These standard FR4-PCB materials are, in general, not applicable for the realization of microsystems for biological applications. In fact copper, which is the basic metal used in PCB technology, is not biocompatible. Therefore, new dielectric materials had to be evaluated in combination with new biocompatible metal layers, like the aluminum, or with copper metal layers protected with gold metallization and other coatings to guarantee biocompatibility. For realization of LAOC devices some additional materials for hydrophobic/hydrophilic surface modification and adhesive tapes for adding special features to the device, as e.g., a membrane, are needed. In Fig. 1 the schematic cross-section of a PCB proposed for LAOC application is presented which combines metal and dielectrics in a microdevice with fluidics and electronics integrated [12].

Following these considerations it is clear that it is not possible to prepare a list of "universal" biocompatible materials. Therefore, this paper proposes a method to evaluate in vitro the biocompatibility of materials, and applies it to a set of standard and quasi-standard materials in the PCB process in order to create the bases for a biocompatible PCB technology. The employed materials have been grouped in metals, dielectrics, adhesives and, surface treatments (Table 1).

As experimental model systems to determine the possible cytotoxic and antiproliferative effects of the biomaterials, a lymphoblastoid cell line (LCL) and a chronic myelogenous leukemia cell line (K562) were chosen as tumor cells growing in suspension. K562 cells were used for their ability to undergo erythroid differentiation under the stimulation of several inducers [13, 14]. Being LCL cells extensively used for screening of cytotoxic T-lymphocytes (CTLs) [15–17], we employed this cell line to determine the effects of the biomaterials on a specific biological functions, e.g., the CTL-mediated lysis of target cells.

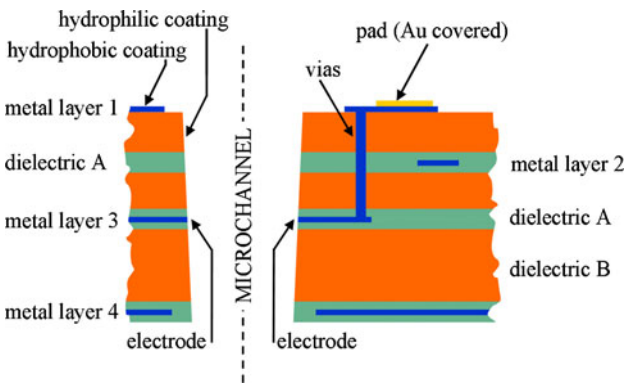

Fig. 1 Schematic cross-section of a PCB-stack with fluidics features developable by a standard PCB process

Table 1 Characteristic of the materials analyzed

Group	Materials	Comments and possible drawbacks	References
Metals	Copper (Cu)	Standard metal in a PCB; due to high chemical instability in wet environment is not used in bio-devices	[3]
	Palladium (Pd), Nickel (Ni), Gold (Au)	Applied with an electroless process to a copper substrate	[8, 12]
	Aluminum (Al)	Widely and cheaply available as a foil; potential substitute of copper in PCB process flows; few nanometers of its oxide self-passivate the aluminum surface making it stable in wet environments. This thin protective layer can be overcome in electrical measurements by increasing the signal frequency	[9, 10, 12]
Dielectrics	Polyimide	Used in the electronics industry for flexible PCBs or as a high-temperature adhesive; used as a substitute of the FR4 substrate material, typically used for rigid PCB devices	[3, 10, 12]
	Pyralux	A B-stage acrylic adhesive, where B-stage means an intermediate stage in the cure reaction of a thermosetting resins. Used as an adhesive to create stacks of multiple dielectric and metal layers. Available in a wide range of thicknesses and in different forms: adhesive sheet itself, or coupled with polyimide	[10, 12]
	Polyurethane	Widely used as flexible and rigid foams, durable elastomers and high performance adhesives and sealants. Available either as powder and as films which can be applied during the lamination process	[18]
	Epoxy films filled with aramid fibers	Applied as an alternative to standard glass filled epoxies used for rigid substrates as hole and microwell drilling by laser ablation is not possible for glass fiber filled materials. Patternable epoxy resins are liquid polymers used as adhesives or encapsulant in microelectronics. They can be applied by screen or stencil printing over large areas and can be also used during the lamination process	[19]
	Die attach film (DAF)	Polymer film consisting of a thermosetting and UV curable resin. This commercially available film could also be used to spaciously laminate different layers together	[20]
	Poly(dimethylsiloxane) (PDMS)	Extensively used for fabrication of microfluidic devices. Low cost. Transparent down to a UV wavelength of 240 nm. Chemically inert towards most reagents and manufactured in multiple viscosities, thus allowing to achieve thicknesses in the range of microns to millimeters. Reversible sealing by electrostatic interaction and irreversible sealing by chemical bond formation is possible. The basic material is hydrophobic, but surface treatments by plasma etching or corona activation are very well known to create irreversible sealing or hydrophilic surfaces. Used as a dielectric or for surface treatments in PCB microfluidics devices	[21, 22]
Adhesive foils	Tesa 4985, Tesa 4983, CMC 15581	Transfer double-sided tapes used for cold bonding. Available with several thicknesses and adhesives	[23]
Surface treatments	Octadecanethiol (ODT)	It is a well known self-assembled monolayer (SAM) allowing to tune the hydrophobicity of Au, Pl and Cu. Moreover it passivates the metal with a protective mono-atomic layer which, in most of the cases, is thin enough for not requiring an increase of the electrical signal frequency. Hydrophilic coating with SAM are also available, but here not tested. The ODT SAM presents a good thermal stability up to 50°C	[10, 24, 25]
	Certonal FC-732	Molding release agent and hydrophobic coating for metals and dielectrics. High performance anti-corrosion surface modifier; used also as an anti-migration barrier or anti-wetting mask. Its low surface energy repels liquids and oils. Widely used in electronics for protection of circuit boards from airborne contamination and humidity, which can lead to corrosion. The dried film is approximately 1micron thick and thermal stable at 175°C	[10, 12]
	Chemlease 41–90 (Chemlease)	Semi-permanent, multiple release system and is also used for molded applications where high chemical aggression is common, providing a chemical stable layer and a hydrophobic coating	[10, 12]

2 Methods

2.1 Materials

The materials under-test (see Table 1 for the complete list and for the major characteristics) were embedded in standard multiwell plates for cell culture (Costar-Corning, USA) in which the biological experiments for biocompatibility were carried out (see Fig. 2a). The materials, mostly foil, were cut by laser machining in round samples of 5.7 mm diameter for 96-well plates and 15 mm for 24-well plates. Blue film was used to cover the sample during the cutting preventing its surface contamination due to the melting materials produced by the laser process. The sample has been embedded in the well with a droplet of PDMS as reported in Fig. 2b. The PDMS was dispensed in each well by an air pressured dispenser (EFD, USA) with a 0.020' tip (internal diameter; EFD Precision Tips, USA) applying a pressure of 1.5 bar for 2.3 s for a 96-well plates and 13.8 s for a 24-well plates. The dispensing parameters were defined by tuning dispensing time, fixed pressure and tip diameter. The covering of the sample edges was optically measured by a Wild H3Z microscope (Heerbrugg, Switzerland) equipped with a MFK II measuring system (Kappa, Germany) at different dispensing time for a 96-well plate (Supplementary Fig. 1S). Figure 1S shows that optimal dispensing time of PDMS for samples embedding was 2.3 s for a 96-well plate. This length of time was chosen in order to obtain the larger space for cell contacts to the materials together with the higher amount of dispensed PDMS for sample embedding. The poly(dimethylsiloxane) (PDMS) was partially dried for 5 min at 45°C after the dispensing. The round samples were deposited by a vacuum tip (EFD, USA) applying a light pressure. The protocol including dispensing, heating and sample deposition was repeated for each row of the multiwell plate. Finally, the PDMS was fully cured at 45°C for 24 h.

Aluminum (18 μm thickness, Al), copper (136 μm thickness, Cu), polyimide (100 μm thickness) were bought in foils and tested without any further process.

In order to understand the possible effect of a non-complete curing of internal layers during lamination process, Pyralux LF0300 adhesive (DuPont, USA) and Aramid fiber filled epoxy F161 (HEXCEL, California) were tested both as uncured (raw material) and cured materials (Pyralux uncured or cured and Aramid fiber uncured or cured). Similarly, polyurethane films (TPU-4201, Epurex Films, Bayer MaterialScience, Germany) were tested either before or after the lamination process (PF before lam and PF after lam), while a second type of polyurethane (94AU925, Merkel Freudenberg Fluidtechnic, Hamburg, Germany) provided as powder (PP) was tested after lamination.

The pressure and temperature profile for Pyralux and polyurethane film are reported in Fig. 3. Aramid fiber filled epoxy was laminated following the curing cycle reported in its datasheet (Hexel, California).

Patternable epoxy (SEMICOAT513E, Shin-Etsu Chemical Co. ltd., Japan) requires up to 150°C to be cured [60 min, 100°C + 90 min, 150°C] (epoxy cured). This temperature is not compatible with the multiwell plate

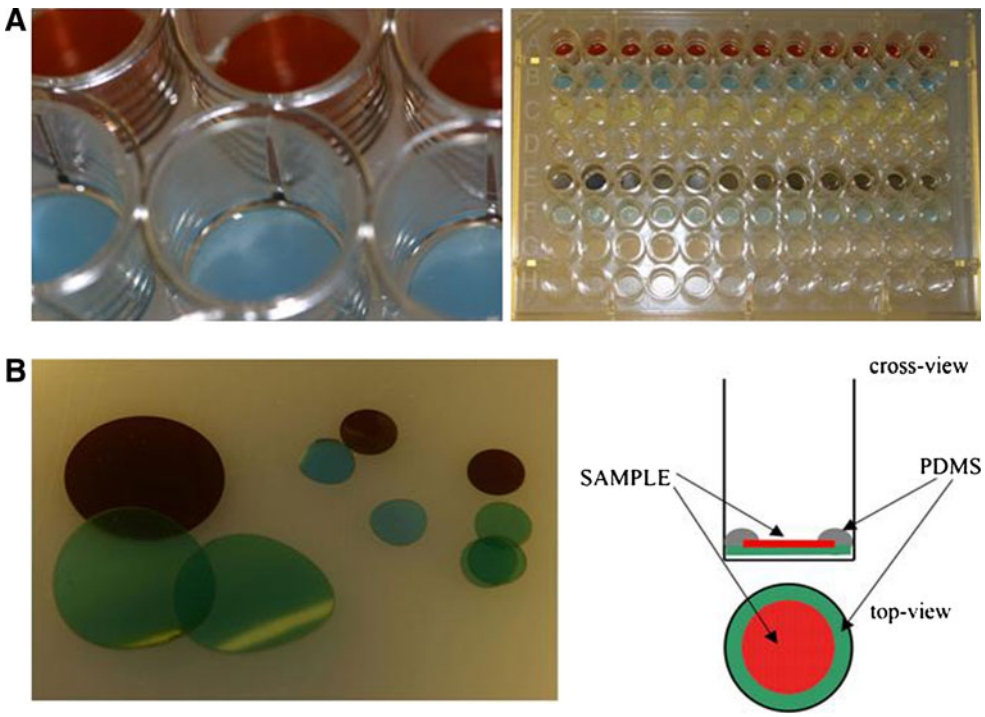

Fig. 2 Samples of under test materials embedded in a standard 96-multiwell. The internal diameter of the well is 5.5 mm (**a**). Materials embedding in standard multiwell plate for biocompatibility tests (**b**). The materials were cut by laser in round samples (*left*) and then embedded in PDMS, used for covering the cut edges to prevent any contamination coming from the support material (*right*)

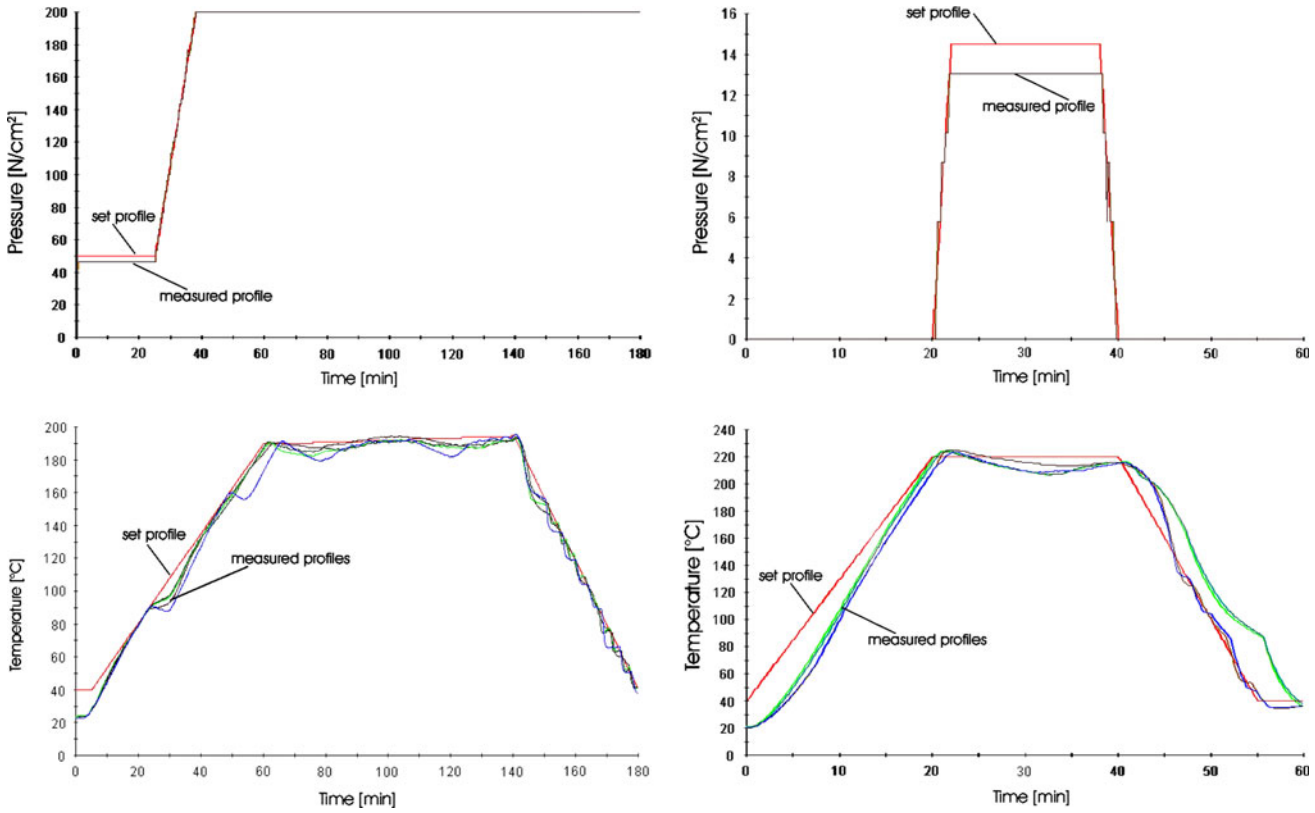

Fig. 3 Profiles of the lamination parameters of Pyralux (*left*) and Polyurethane powder (*right*)

made with polystyrene, which glass transition temperature is 95°C. To overcome this limitation a film of epoxy, 100-μm thick, has been patterned on a support covered with cured Doubling silicone for dental copy (SUPERIUM Dubliersilikon, Weber Dental-Germany), cured, peeled out as a foil and cut by laser. The hydrophobicity of the doubling silicone avoids the sticking of the epoxy film.

The die attach film (DAF) was only tested in the delivered b-stage and from there not fully cured.

Poly(dimethylsiloxane) (PDMS, Sylgard 184, DowCorning) was prepared mixing the two components of Sylgard 184 with a ratio of 1:10 for 5 min and degassed for 30 min at 0.1 bar (4 min were required to reach 0.1 bar) prepared according to [25] and cured in the well at 45°C overnight.

Palladium (Pd) and Gold (Au) have been chemically deposited on copper substrates by electroless process. For Pd deposition, first Nickel (Ni) was deposited as adhesion layer (15 min, 90°C) followed by Pd activation (1 min, 55°C) and Pd deposition (30 min, 60°C). For Au deposition on Ni (Au over Ni), Ni was deposited as adhesion layer (15 min, 90°C) followed by Pd activation (1 min, 55°C) and Au deposition (30 min, 50°C). For Au deposition on Pd (Au over Pd), Ni was deposited as adhesion layer (15 min, 90°C) followed by Pd activation (1 min, 55°C), Pd deposition (30 min, 60°C) and Au deposition (30 min, 50°C). The Cu substrates were cleaned in HCl 0.5% for 30 s before the deposition, rinsing with DI-water and drying with nitrogen.

The acrylic adhesive transfer films Tesa 4985 and 4983 (Tesa tape, USA), with a thickness of 2 and 1.2 mil and thermal stability up to 200°C, and CMC 15581 (CMC technical tapes, Germany), with a thickness of 50 μm and thermal stability up to 130°C, have been prepared in multiwell plates. The tapes, protected on both sides by cover papers, have been cut in samples by laser. All the tapes are transparent and provide a permanent adhesion. The transfer tapes were stuck in the well and PDMS was manually dispensed to cover their edges and cured as for the other multiwell plates.

Certonal FC-732 was deposited filling the well for half of its volume for 5 min, rinsing with water and drying with a nitrogen flux at RT. Certonal was also deposited on an aluminum foil and tempered for 20 min at 150°C on a hotplate (Certonal FC-732T), after which it was cut by laser. A thin layer of Chemlease 41–90 (Chemlease) has been applied on an aluminum foil, tempered for 20 min at 150°C on hotplate and cut in samples.

The Au, Pd, and Cu surfaces have been functionalized with 1-octadecanethiol (ODT, Sigma–Aldrich) making it

hydrophobic. Ethanol was deoxygenated with bubbling nitrogen for 1 h before using it as a solvent for thiols, but not purified further [24]. The metal substrates, embedded in multiwell, have been cleaned with isopropanol for 2 h, rinsing with DI-water and ethanol before the thiol monolayer deposition (24 h at RT). ODT 1 mM has been prepared in ethanol. Any significant variation in the contact angle was recorded using piranha solution [26] or plasma etching [24] for the cleaning of the substrates. After the deposition, the samples were rinsed with ethanol and dried with a jet of high purity nitrogen.

Since all the surface treatments (Certonal, Chemlease and ODT) produce a hydrophobic behavior of the treated surface, the presence of these coatings was verified by measuring contact angle of the processed surfaces, as described in [10].

The surface of some materials is soft or thin layers are applied on, thus hard contact (e.g., with metal tweezers) was avoided. Some metals (e.g., copper) are fast reactive with water therefore water was used only for fast rinsing. The multiwell plates were cleaned with isopropanol (Pyralux cured/uncured, Polyimide, Al, Cu, Pd, Au, PDMS) or ethanol (ODT, Certonal FC-732, Chemlease, Aramid fiber cured/uncured), rinsing with DI-water and drying with nitrogen. The transfer tapes were cleaned with DI-water only rinsing with nitrogen. UV-light was used to sterilize.

2.2 Cell lines and culture conditions

Lymphoblastoid cell lines (LCL) have been obtained after infection of human B-lymphocytes with B95.8 strain of Epstein-Barr virus (EBV) [15]. Both LCL and the human erythroleukemia K562 cells [13] were maintained in RPMI 1640 medium (Sigma–Aldrich, Milwaukee, WI, USA) at 37°C in a humidified 5% CO_2 atmosphere, supplemented with 10% fetal bovine serum (FBS; CELBIO, Milano, Italy), 100 units/ml penicillin and 100 μg/ml streptomycin (Aldrich, St. Louis, MA, USA). For studying the effects on in vitro cell growth, with continuous incubation or after pulse incubation (60 min), cells were seeded at the initial cell concentration of 30,000 cells/ml (K562 cells) or 50,000 cells/ml (LCL cells), cultured in RPMI supplemented with 10% FBS and the cell number/ml determined using a model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL, USA) after different days of cell culture.

2.3 Erythroid differentiation of K562 cells

The experimental protocols for analysis of the effects of the biomaterials on the erythroid differentiation were the following: (a) K562 cells were cultured to within biomaterial-treated 24-well plates for different days and treated with 25 nM mitramycin [27], a powerful inducer of erythroid differentiation; (b) K562 cells were exposed to the different biomaterials for 1 h, then washed, sub-cultured in standard medium conditions and treated with 25 nM mitramycin for the following days (short exposure). The proportion of benzidine-positive cells was determined after 5 and 6 days of cell culture using a solution containing 0.2% benzidine in 5 M glacial acetic acid (10% H_2O_2) as previously described [28].

2.4 CTL-mediated LCL lysis: chromium release assay

Monocyte-depleted PBLs from HLA-A2 positive blood donor were plated at 3.5×10^6 cells per well in 24-well plates in RPMI 1640 containing 10% fetal bovine serum (FBS Hyclone; CELBIO, Milano, Italy) and stimulated with EBV nuclear antigen 3 (EBNA-3) SVR peptide (10 μM). Cultures were restimulated after 7 and 14 days, and the medium was supplemented from day 8 with 10 U/ml rIL-2 (Chiron). On days 14 and 21, T cell cultures were tested for CTL activity using cytotoxicity assay. SVR-specific CTL cultures efficiently lysed SVR-pulsed human leukocyte antigen A2 (HLA-A2) positive LCLs, but did not lyse SVR-pulsed HLA-A2 negative LCLs (data not shown). Cytotoxic activity was tested by a standard 5 h ^{51}Cr-release assay, as previously described [16]. Briefly, target cells were labeled with 0.1 μCi/10^6 cells of $Na_2^{51}CrO_4$ for 90 min at 37°C and pulsed for 45 min with 10^{-5} M of peptide at 37°C. Cells were then washed and used as targets at different effector:target (E:T) ratios. Percentage specific lysis was calculated as 100 × (cpm sample-cpm medium)/(cpm Triton X-100-cpm medium), where cpm is counts per minute.

3 Results

3.1 Effects of biomaterials on cell growth

The experimental protocol for analysis of biomaterials effects was the following: (a) cells (K562 and LCL) were cultured within biomaterial-treated 24-well plates for different days (continuous exposure); (b) cells were exposed to the different biomaterials for 1 h, then washed and sub-cultured in standard medium conditions for different days (short-term exposure). The 1 h short-term exposure was chosen, since this length of time is compatible with most of the protocols available in literature for cellular manipulations using Lab-on-a-chip platforms [10–12, 29–33]. In both protocols, cell concentrations (cell number/ml) were determined after 3, 4, 5, and 6 days of cell growth. In Figs. 4 and 5 only the value determined after three days was reported; at this time, indeed, both control K562 and LCL cells are in the log-phase of cell growth, allowing the

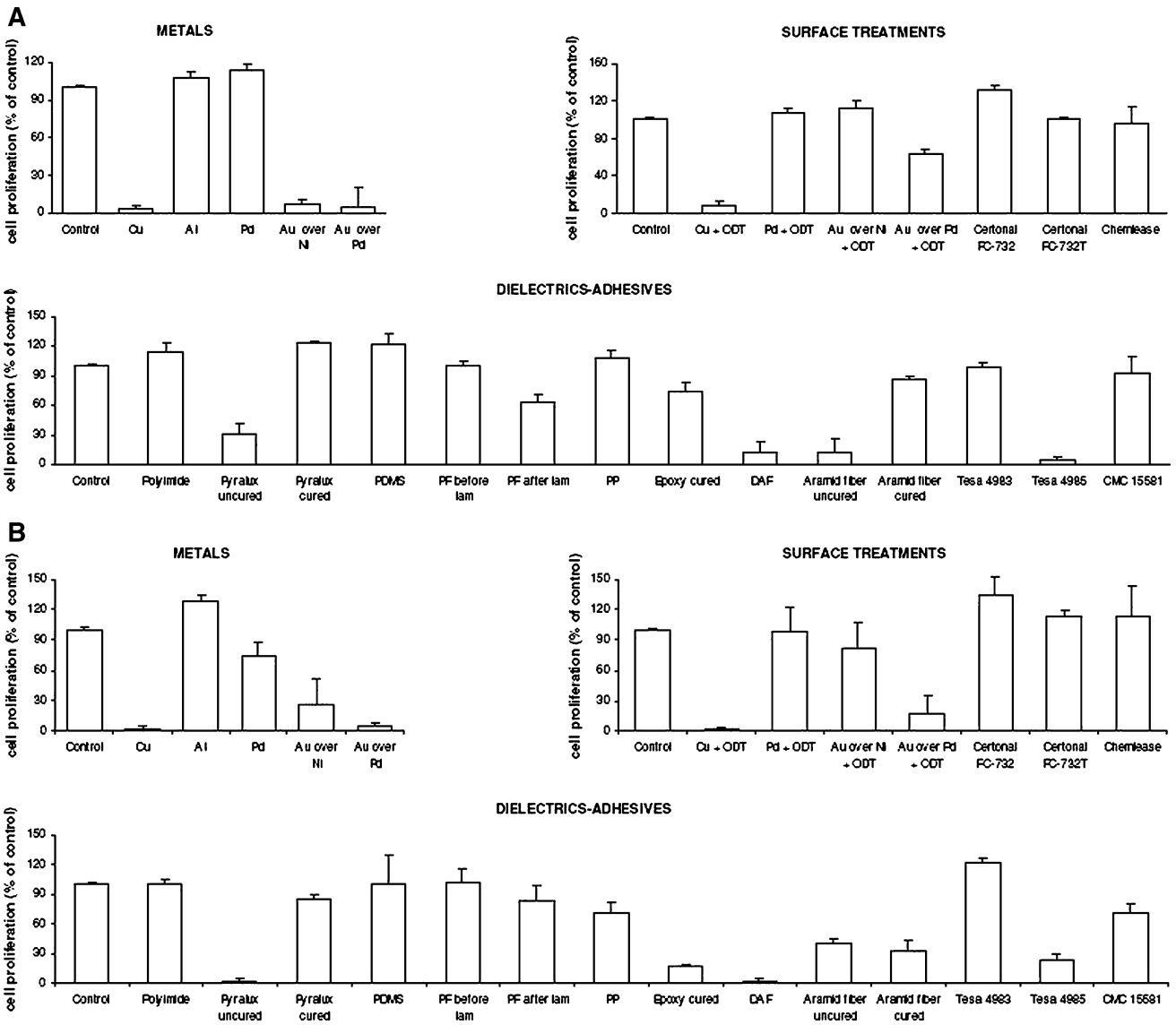

Fig. 4 Effects of the materials on cell proliferation of K562 and LCL cells after continuous exposure. K562 cells (**a**) or LCL cells (**b**) were cultured in RPMI, 10% FBS for different days in 24-well plates containing the indicated materials. At different time points, the cell number/ml was determined and compared with control untreated cells cultured in standard conditions. The effects on cell growth were determined after 3, 4, 5 and 6 days of cell culture. The data reported in the Figure represent cell proliferation in respect to control cells (average ± SD from three different experiments). The cell number/ml was evaluated after 3 days, when both K562 (**a**) and LCL (**b**) control cells are in the logarithmic phase of cell growth

best comparison to detect inhibition of cell growth in experiments aimed at studying possible inhibitors of cellular proliferation [34]. The effects of the employed materials were considered as inhibitory effects in the case of 75% inhibition of cell proliferation, in consideration of the fact that inhibition around 50% of cell growth might be associated in K562 cells, instead to cytotoxic effects, to activation of terminal erythroid differentiation [13].

Results of these experiments are shown in Figs. 4 and 5. In Fig. 4, the data obtained with protocol (a) are shown, allowing to conclude that continuous exposure to materials is not compatible with efficient cell growth, for the following materials: Cu, Au over Ni, Au over Pd (metals), Cu + ODT, Au over Pd + ODT (surface treatments), Pyralux uncured, DAF, Aramid fiber uncured, and Tesa 4985 (dielectrics/adhesives). These materials inhibited cell growth of both K562 and LCL cells. Epoxy cured was inhibitory only on LCL cell growth. Therefore, very consistent effects were obtained on these two cellular systems when continuous exposure to the tested materials was undertaken.

When exposure was limited to 1 h (Fig. 5), most of the materials displayed no inhibitory activity, with the exception of Aramid fiber uncured (which maintained the

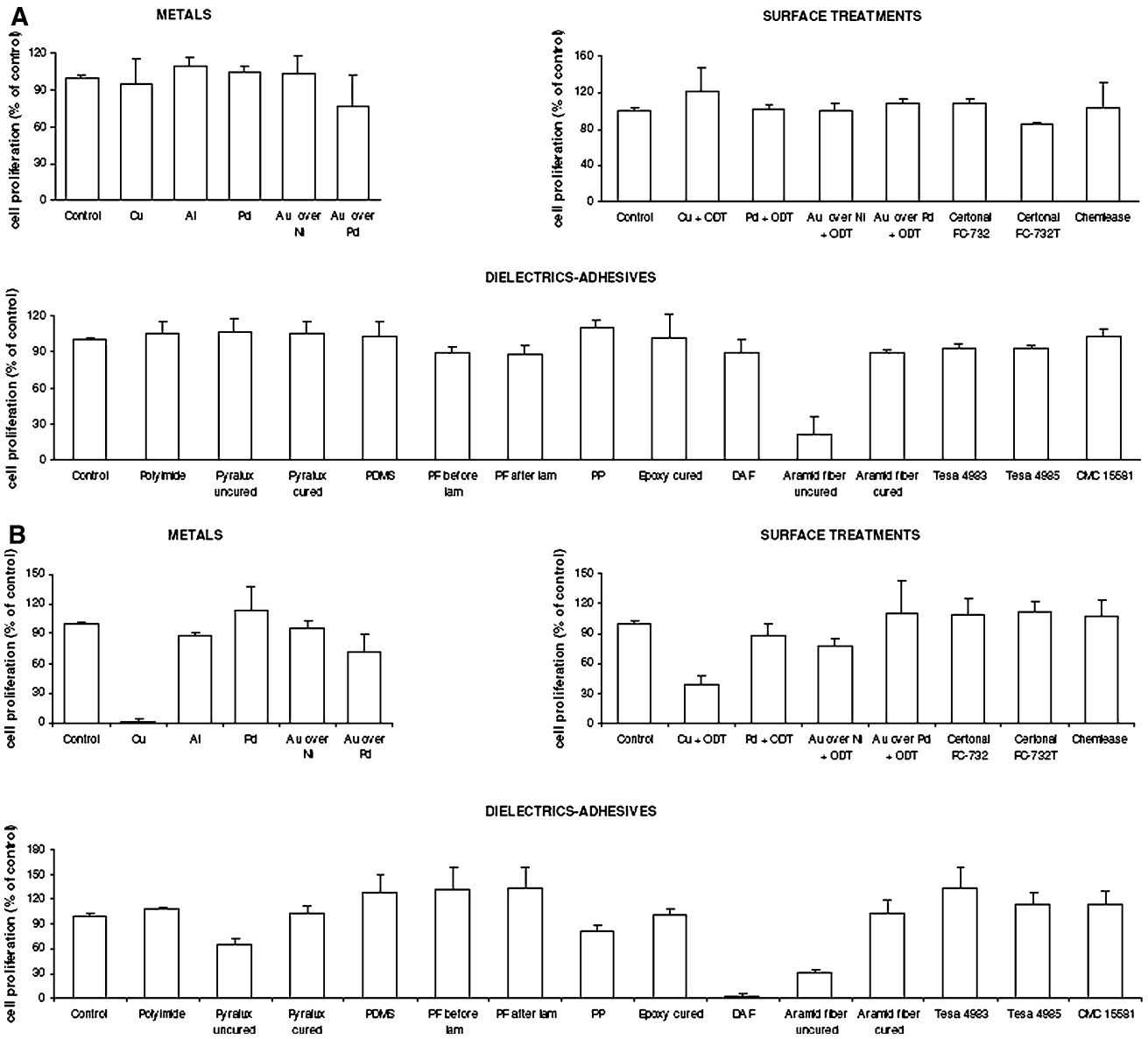

Fig. 5 Effects of the materials on cell proliferation of K562 and LCL cells after short-term (1 h) exposure. K562 cells (**a**) or LCL cells (**b**) were exposed in RPMI, 10% FBS for 1 h in 24-well plates containing the different materials. After two washing steps with RPMI, cells were sub-cultured in standard conditions. The cell number/ml was determined and compared with control cells after three days of cell culture (see legend to Fig. 4). The data represent the average ± SD from three different experiments

inhibitory activity on both cell lines) and DAF, Cu, and Cu + ODT (which maintained the inhibitory activity only on LCL cells). Interestingly, Pyralux cured renders Pyralux compatible with cell growth (see Figs. 4, 5b). In addition, Aramid fiber cured was found to be not active in inhibiting cells growth with the exception of long-time treated LCL cells. These data indicate that caution should be taken when using uncured Pyralux and Aramid fibers for the construction of Lab-on-a-chip platforms.

3.2 Effects of materials on erythroid differentiation

The analysis of the effects of the materials on differentiated functions of K562 cells was performed by determining the proportion of benzidine-positive (hemoglobin containing) K562 cells treated with mitramycin, a powerful inducer of erythroid differentiation. The results obtained are shown in Fig. 6, and clearly indicated a full suppression of the increase of the percentage of benzidine-positive cells with respect to control erythroid induced cells (K562 cells

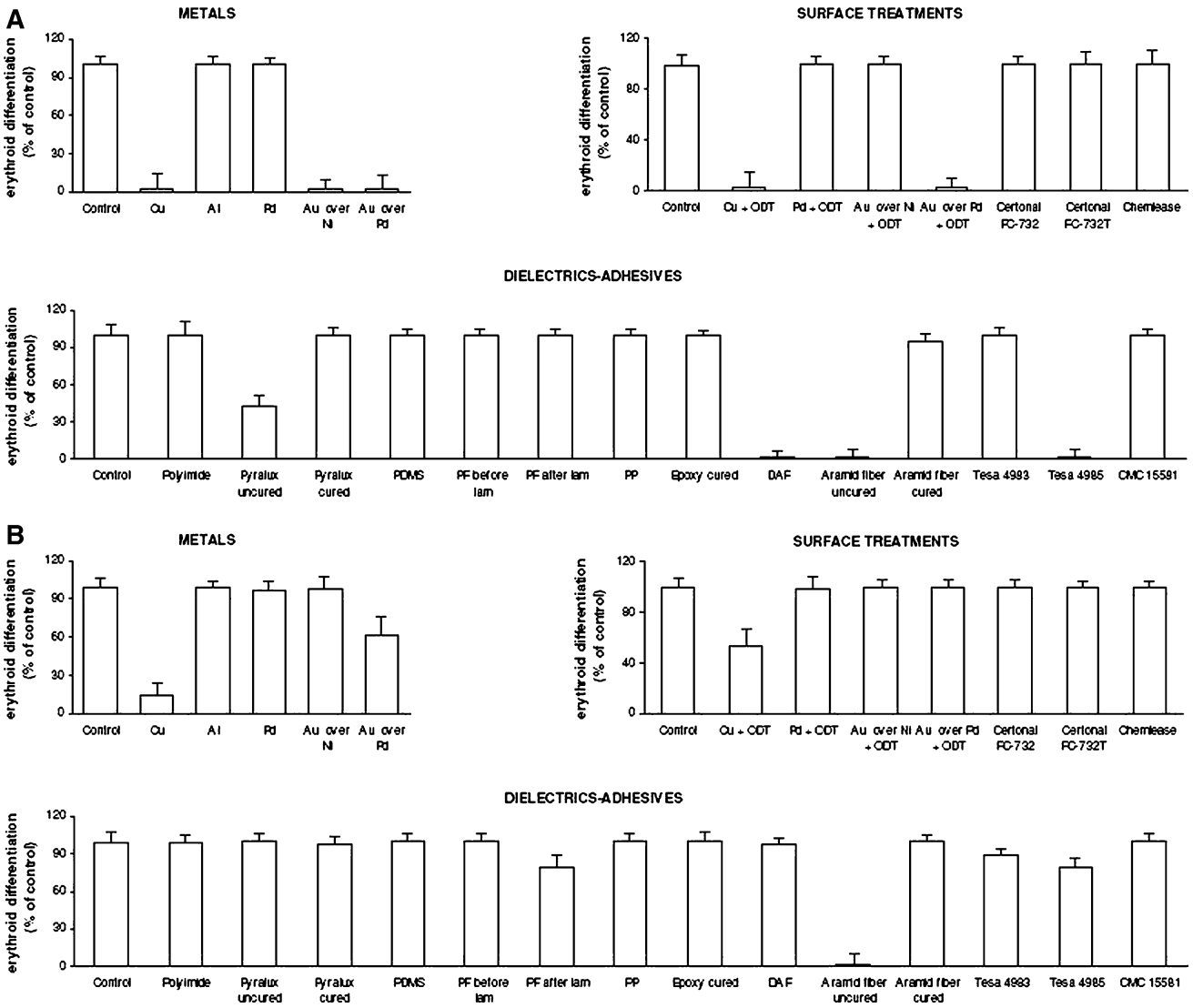

Fig. 6 Effects of the materials on erythroid differentiation of mitramycin-induced K562 cells during continuous exposure (**a**) or after short-term (1 h) exposure (**b**) with the different materials under investigation. The proportion of benzidine-positive cells was determined after 6 days of cell culture. In our experiments, uninduced cells displayed 0.5–3% of benzidine-positive cells. Control mithramycin-treated cells exhibited always very high values of benzidine-positive cells (92 ± 5.5%). The results are presented as % of differentiated cells in respect to control mithramycin-treated cells (considered as 100%). The data represent the average ± SD from three different experiments

treated with 25 nM mitramycin) after a continuous incubation with the following materials: Cu, Au over Pd, Au over Ni (metals), Cu + ODT, Au over PD + ODT (surface treatments), DAF, Aramid fiber uncured, and Tesa 4985 (dielectrics-adhesives). Pyralux uncured induced a significant inhibition of differentiation.

In the case of a short exposure (1 h), Cu and Aramid fiber uncured induced a strong reduction of the percentage of benzidine-positive cells, while also Au over Pd and Cu + ODT exhibited inhibitory activity, although to a lesser extent.

These data are fully in agreement with the results obtained in the cytotoxic and antiproliferative tests (Figs. 4a, 5a).

3.3 Effects of biomaterials on CTL-mediated lysis of LCL cells

The analysis of the effects of the biomaterials on CTL-mediated lysis is shown in Fig. 7, which indicates that CTL-mediated lysis is detectable using all the biomaterials analyzed, with the exception of Tesa 4985. Some of the tested materials caused increased background levels of LCL lysis, including Cu, Cu + ODT, Tesa 4985, and Aramid fiber uncured (see black boxes of Fig. 7). The values of the percentage of lysis were in all cases very similar to those of the control represented by untreated cells. Interestingly, Cu, Cu + ODT, Tesa 4985, and

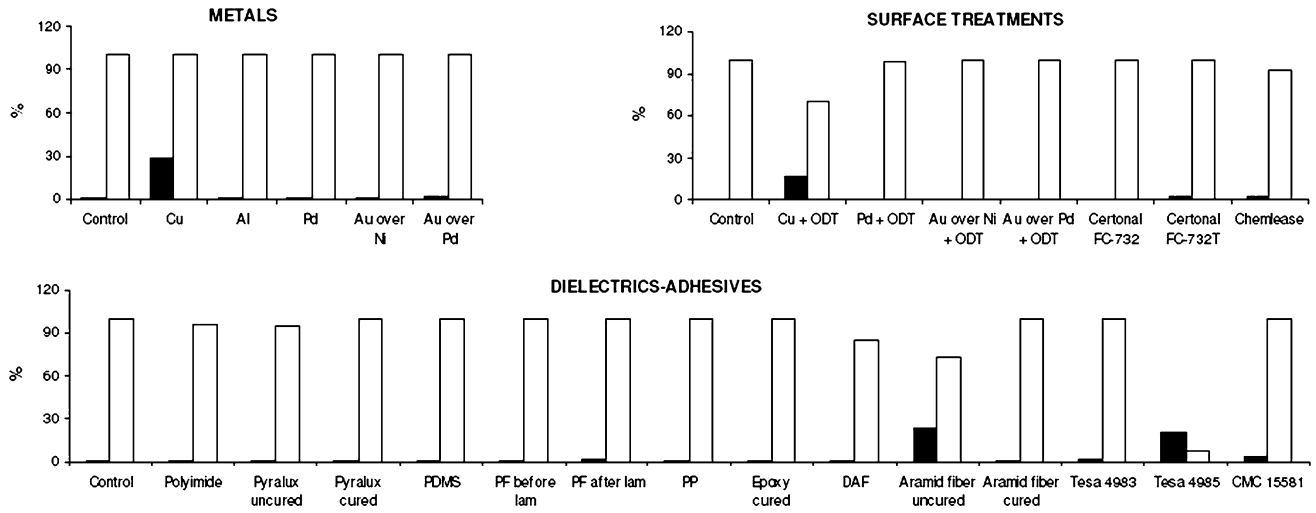

Fig. 7 Effects of biomaterials on CTL-mediated lysis. ^{51}Cr-labelled LCL cells were incubated for 5 h with CTL in a CTL:LCL ratios of 10:1 in 96-well plate with different materials. Incubation for 5 h was performed in order to compare the effects of the employed materials with optimal CTL-mediated lysis of LCL [16, 17]. *Black boxes* % spontaneous release (sample-control), *white boxes* % lysis ratio CTL:LCL 10:1

uncured epoxy filled with Aramid fiber are materials exhibiting antiproliferative activity on LCL cells, as reported in detail in Figs. 4b, 5b.

4 Discussion

The precise analysis of the biological effects of materials employed for the construction of platforms for cell manipulation, including Lab-on-a-chip devices, is a fundamental pre-requisite for the design of the possible applications of devices used in biomedicine and biotechnology. In this paper we have assessed the effects of different potential "biomaterials" on cell growth and expression of differentiated biological functions. In our study we have tested a significant number of materials commonly used for the implementation of Lab-on-a-chip platforms, on two cell lines (LCL and K562) and on several biological functions (cell growth, erythroid differentiation of K562 cells, and CTL-mediated cell lysis of LCL cells).

The results obtained firmly demonstrate that some materials exhibit strong inhibitory effects on both the cell lines and on all the biological functions assessed. When continuous culturing was carried out Pyralux uncured, DAF, Epoxy cured, Au over Ni, Au over Pd, Au over Pd + ODT, Cu, and Cu + ODT inhibited biological functions. Therefore, the use of these materials should be avoided if the device under construction is designed for manipulation of eukaryotic cells under long-term cell culturing experimental conditions. On the other hand, our study clearly indicate that several materials belonging to the groups of metals, surface treatments and dielectric/adhesives are compatible with cell growth and expression of differentiated functions, even if long-term cultures are programmed.

Preparation of the materials appears to be a critical point. In this sense, both uncured preparation of Pyralux and Aramid fiber filled epoxy displayed inhibitory activities in several assays, while cured Pyralux and Aramid fiber filled epoxy did not. Therefore, our data suggest that modifications of biomaterial preparation might overcome in some cases unwanted toxic effects.

Finally when the biological assays performed are considered together, it can be concluded that most of the materials can be used for a short exposure of the cells, with the exception of DAF, Cu, Cu + ODT, and Aramid fiber uncured. Therefore, most of the materials can be employed for the construction of platforms simply dedicated to cell manipulation, isolation and sub-culturing.

As far as the effects on CTL-mediated cytolysis, all the materials are compatible with this CTL biological activity. Only Cu, Cu + ODT, Aramid fiber uncured, and Tesa 4985 should be avoided, since they induce a strong increase of ^{51}Cr release from LCL cells. Therefore, all these materials are compatible with preparation of platforms for analytical purposes. On the contrary, if CTL (or LCL) recovery and sub-culturing are required, DAF, Cu, Cu + ODT, Aramid fiber uncured, and Tesa 4985 should be avoided.

It should be noted that good agreement was found between the effects on cell growth (Figs. 4, 5) and differentiated functions, suggesting that the study of the effects of materials on cell proliferation is predictive of effects on biological functions.

5 Conclusions

The results here presented strongly suggest that analysis of the biological effects of the materials used for production of Lab-on-a-chip preparation should be carefully determined and considered together with the proposed biomedical application of the platforms. It should be underlined that some differences between the cell lines employed exist, when biological effects of the materials are considered together. In addition, we like to emphasize that preparation of the material is critical, as suggested by the comparison of the effects of uncured and cured versions of Aramid fiber and Pyralux. Length of exposure of the cells to the material is also an important parameter, as put in evidence by Figs. 4–7, since differences between 1 h and continuous exposure to the materials are clearly evident. This clearly indicate that for protocols needing exposure of the cells to materials higher than 1 h, careful studies on the effects of all materials employed in the construction of the used Lab-on-a-chip platform should be undertaken. Finally, several materials did not display any inhibitory effects on two cell lines and on all the biological parameters explored and should be considered the first choice to design and produce fully biocompatible Lab-on-a-chip platforms.

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