Polylactic acid, a sustainable, biocompatible, transparent substrate material for Organ-On-Chip and Microfluidic applications

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Abstract

Organ-on-chips are miniaturised devices aiming at replacing animal models for drug and toxicity studies and studies of complex biological phenomena. The field of Organ-On-Chip has grown exponentially, and has led to the formation of companies providing commercial Organ-On-Chip devices. Yet, it may be surprising to learn that the majority of these commercial devices are made from PDMS, a material widely used in microfluidic prototyping, but which has proven difficult to use in industrial settings and poses a number of challenges to experimentalists, including loss of small compounds. To alleviate these problems, we propose a new substrate for organ-on-chip devices: Polylactic Acid (PLA). PLA is a material derived from renewable resources, and compatible with high volume production such as microinjection moulding. The polymer can be formed into sheets and prototyped into the desired devices in the research lab. The aim of this paper is to assess and prove the suitability of Polylactic acid as substrate material for Organ-on-a-chip applications. Surface properties, biocompatibility, small molecules adsorption and optical properties of PLA are investigated and compared with PDMS and other reference polymers.

Keywords: Organ-On-Chip, PLA, transparency, biocompatibility, rapid prototyping, cell tracking

Significance

Organ-On-Chip (OOC) technologies are a powerful and emerging tool that allow the culture of cells constituting an organ and enables scientists, researchers and clinicians to conduct more physiologically relevant experiments without using expensive, and complex animal models. Since the emergence of the first OOC devices, the translation from research to market has happened relatively fast. To date, at least 28 companies are proposing body and tissue on-a chip devices.

The material of choice in most commercial organ-on-chip platforms is an elastomer, Polydymethyloxane (PDMS) commonly used in microfluidic R&D. which is subject to poor reproducibility. In this study we showed that Polylactic Acid (PLA), is a suitable material for the production of microfluidic devices. We show that PLA overcome all the drawbacks related to PDMS. PLA can be prototyped in less than 45 minutes from design to test a new transparent no autofluorescent, biocompatible and bio-inert OOC device and is also suitable for mass-manufacturing. PLA-based OOC platform have the potential to transform the OOC industry and provide a sustainable alternative for Lab-On-Chip and point-of-care devices.

Introduction

Organ-On-Chip (OOC) is the convergence of two important and emerging research areas, microfluidics and tissue engineering, meeting the requirements for more physiologically accurate human tissue. OOC devices provide advanced models for cell and tissue culture, accelerating and facilitating the understanding of human biology, toxicology, disease progression and prognosis while; overcoming important ethical, financial and interspecies variances related to animal experimentations [1].

Since the birth of the first OOC device in 2007[2] , complex functionalities have been added to a simple structure, leading to the so-called body-on-a-chip or human–on-a-chip fields. The translation from the research to the market was relatively fast. To date, 28 companies are proposing body and tissue on-a chip devices.[3] The material of choice in most organ-on-chip platforms has been poly (dimethylsiloxane) (PDMS) a thermo-curable elastomer. Of the companies present in the OOC market, the majority (57%) sell PDMS products (Suppl. info 1). Although PDMS is biocompatible, transparent, gas permeable, flexible, and relatively easy to manufacture at small scale, a variety of issues have been encountered by consumers while using PDMS as substrate material. These problems include channel deformation, high evaporation, leaching of uncured oligomers, absorption of hydrophobic compounds and unstable surface treatment which can lead to inconsistent and unpredictable results with respect to biological outcomes [4]. While some of these issues have been overcome with time-consuming protocols (e.g. soxhlet extraction in ethanol, or other organic solvent, to remove uncrosslinked oligomers [5]), PDMS moulding still remains a difficult process to fully automate [6] significantly slowing down the translation from the research to the mass market production.

To alleviate the drawbacks of PDMS, recent studies have focused on the use of thermoplastic as well as 3D printable materials. Poly methyl methacrylate (PMMA), Polycarbonate (PC), Cyclic olefins (COP and or COC) and Polystyrene (PS) are some common materials proposed from different suppliers as scalable alternatives. These thermoplastic materials have the advantage of being relatively cheap, and they translate easily to the mass market, however, they are fossil-based, therefore unsustainable, and can leach compounds under certain conditions.

In a recent study, we proposed Polylactic acid (PLA), a biocompatible thermoplastic material from renewable resources and widely applied to tissue engineering, as new substrate material for the production of environmentally sustainable single-use microfluidic devices. We optimised PLA workability in conjunction with a layer-by-layer laser-based prototyping technique and demonstrated basic microfluidic functions [7]. Now, motivated by a number of studies have explored the existing relation between the scaffold morphology and surface chemistry of PLA with different cells [8], we provide evidence that PLA is a suitable replacement to other polymers in Organ-on-a-chip applications. We investigate surface properties, biocompatibility, adsorption of small compounds, and optical properties of PLA in comparison to PDMS and with other reference polymers such as polystyrene. Finally, we provide an example of cell-tracking through a PLA-based organ-on-chip device.

Results

An OOC device provides support for tissue attachment and organization to simulate tissue and organ-level physiology. The first interaction between the cells and the device happens at the material surface. The requirements of a suitable substrate material for healthy cell environment and tissue adhesion, are wettability, surface roughness and chemical composition[9].

PLA functionalisation

PLA has a naturally hydrophobic of a surfaces, which is limiting cell attachment. To address this, surface treatments are required to increase the substrate wettability [10]. The most widely utilised and preferred surface modification methods rely on oxygen plasma and UV-ozone treatments [11]. However, several studies have reported the non-permanent nature of those functionalization methods, hence a hydrophobic recovery effect [12]. Such a phenomenon is particularly exhibited by PDMS, with a drastic increase of the contact angle 1 week after the treatment [12]. To overcome the hydrophobic recovery issue, PDMS device needs to be used just straight after the functionalisation process, but it limits the practicality, accessibility and the mass production of the devices. The hydrophilic nature of a surface can be assessed by measuring indirectly the surface energy via contact angle analysis. The contact angle value gives a quantitatively measure of the hydrophilic or hydrophobic nature of a surface. In particular, a material is hydrophilic if it has a contact angle below 90˚, while hydrophobic if above 90˚. PLA has a contact angle about 77-80˚, while the one of a typical treated PS well-plate used for cell culture applications is 45˚±4˚.[12] This value of 45˚ is the appropriate contact angle to avoid electrostatic interactions with hydrophilic molecules. In order to permanently modify the surface properties of PLA, a wet-chemistry approach was adopted. (Fig. 1) A Sodium hydroxide (NaOH) solution was employed to achieve an alkaline surface hydrolysis allowing of the ester linkages of the PLA chains at the surface to produce carboxylic (-COOH) and hydroxyl (-OH) functional groups (Fig.1 A, B and C). Using this functionalisation process, the final contact angle can be tuned by changing the functionalisation time and the solution concentration (Fig. 1. D,E). This functionalisation protocol is stable over time, with no evidence of a hydrophobic recovery effect (fig. 1.F). In accordance with previous results reported by Tham et al. [13], this method acts on hydrolysing and eroding the material surface, inducing a visible change in the surface roughness (fig. 1G). Interestingly, a pillar formation structure is noticed with an increase of the pillar density with respect to the solution concentration. While increase of surface roughness is visible on SEM imaging, the pillar formation is partly damaged by the conductive gold layer. In conclusion, the proposed functionalisation protocol allows to tune the surface wettability of PLA to obtain the desired contact angle. The resulting formation of the -COOH and of -OH groups at the surface can readily be used for further conjugation of surface modifying species [13], in order to provide the best environment for the specific cell culture application.

PLA biocompatibility

Biocompatibility in OOC devices can be defined as the absence of toxic effects to the cells or tissue interacting with the material, as well evidence of optimal growth/survival conditions to the cultured cells and tissues. PLA has been widely used in medicine as material for resorbable sutures, orthopaedic implants, scaffold for tissue engineering and drug delivery systems[8].Therefore the biocompatibility of PLA has been well established by a number of studies, typically demonstrating lack or low inflammation after implantation, compatibility with surrounding tissue[14], [15]. In this study, we specifically aimed at comparing the biocompatibility of pristine PLA versus functionalised PLA, as well as against other common organ-on-chip and microfluidic materials: PS (control material), PDMS, PMMA, and PC. Cell proliferation and cell death with two cancer cell lines adenocarcinomic human alveolar basal epithelial cells (A549) and

human hepatocellular carcinoma cells C3A). At 48 hr of cell culture almost all the substrates performed comparably well to PS control. The culture of A549 cells on both *f*PDMS and *f*PMMA demonstrated a statistically significant increase in the cell proliferation of ~11% and 18% respectively (Fig 2A). With regards to cell death (as measured by cell membrane integrity) for C3A at 48 hr only the substrates made of *p*PLA, *f*PLA and *f*PDMS performed comparably with the control. For the A549 at 48 hr of culture no cell death was noted (Fig 2B), while at 7 days with the exception of PLA all polymers caused the same percentage of cell death as compared to control (Fig 2). For the C3A cells, *p*PLA and *f*PLA showed a decrease in cell death of ~6%and 10% respectively. The data demonstrated a slight benefit of utilization of *f*PLA with as compared to PS plate. In the case of the A549 cells culture on both *p*PLA and *f*PLA showed a decrease in cell death of ~12%.
If cancer cells can be used to model cancer microenvironment in microfluidic human OOC, one of the most interesting tool that an OOC can provide is to study the interactions between different kind of cells such as normal and cancer cells. Therefore, we also independently investigated the PLA biocompatibility with primary cells, that are extremely sensitive cells with a finite life span and a limited expansion capacity. We cultured Human umbilical vein endothelial cells (HUVECs) primary cells for 7 days on the same substrates with the addition of COC. Both pPLA and fPLA performed as the control PS (no statistical difference). No cell attachment was noticed on the pristine PDMS (cell death shown as nil on Fig2C), while PC and COC showed the higher cell death percentage (figure 2C). No cell attachment was noticed on the pristine PDMS. Overall, the toxicity data indicates that PLA is a suitable material for cell culture. Translating from a polystyrene substrate to a PLA substrate was found to be marginally better for A549 and C3A cells, and similar from HUVEC cells.

Absorption and Adsorption of small molecules on PLA substrates

Several reports have shown that, unless treated, PDMS can absorb small hydrophobic compounds such as steroids and/or hormones.[16]–[20] This behaviour can be explained by the porous and hydrophobic nature of its polymeric network. This phenomenon can be a major drawback in bioassays, in particular drug testing assays where drugs engineered for rapid delivery are typically smaller than 500 Da and diffuse uncontrollably inside the microfluidic device [18].[22] Several methodologies have been proposed to overcome this issue; however, by and large they result in delays in prototyping or manufacturing time. On the other hand, PLA, as a thermoplastic material should not show any absorption behaviour, but it can be subject to surface adsorption. We have previously shown, that laser-cut PLA microstructures do not adsorb small hydrophilic compounds [7]. In this study, the absorption of both hydrophilic and hydrophobic compounds were assessed in laser-cut *p*PLA and *f*PLA and PDMS channels. To test the absorption of hydrophobic compounds Nile Red, a small hydrophobic fluorophore (318.37 Da), was selected as representative molecule, while fluorescein salt (376.27 Da) was selected to test the absorption of a typical hydrophilic compound. The devices have been designed and manufactured with a channel cross-section of about 0.8 mm^2 (Fig. 3A). A fluorescence image of each empty channel was taken with a microscope in order to create the null absorption reference point (Fig3.B). To create the maximum absorption reference point, the channel was filled with the fluorescent solution (Fig3.B). The solution was incubated inside the channel for 60s and then aspirated out of the channel from the outlet. Each channel was then cleaned with deionised water if loaded with Nile Red or with ethanol if loaded with fluorescein salt solution. A picture was taken after 60s of the cleaning channel cycle and the fluorescence intensity measured with the imaging software ImageJ and normalised with respect to the reference points. The rinsing-cleaning-imaging cycle was repeated 20 times (Fig3.B)

and the normalised fluorescence intensity was plotted against the number of cycles (Fig.3C). This experiment shows PLA does not absorb hydrophobic or hydrophilic compounds, even when functionalised, while PDMS predictably absorbs small hydrophobic compounds (reaching 50% of absorption after 20 cycles), making PLA a superior material in this respect.

Optical properties of PLA: Transparency and autofluorescence

In order to study the organ functionality and biological responses at the cellular and molecular level, the optical properties of an organ-on-chip system substrate material, are of fundamental importance and relevance.[21]. It is well established that PDMS, PMMA, PC, COP and COC have good or excellent optical properties and transparency. Furthermore, they have a low fluorescence background or autofluorescence, when irradiated near UV wavelength, a desirable feature for fluorescent imaging [22]. The optical properties of PLA have been investigated and reported previously[23],however, very little is known about the polymer's autofluorescence properties. The transparency and autofluorescence of PLA substrate was investigated, analysing possible changes due to functionalisation in comparison with PDMS, PMMA, PC and COC substrates. We observed no difference nobetween *p*PLA and *f*PLA with regards to transparency as transmittance of the light in the visible region (Fig. 4A). *f*PLA shown 92% of transparency, higher than PDMS, but lower with respect to the other materials tested. While, PMMA showed the highest transparency (96 %), followed by COC, PC, PLA and PDMS (Fig.4B). In OOC applications fluorescence imaging is often required over a period of time rather than instantly.[24] Hence the analysis of the autofluorescence over time under illumination is highly beneficial. In this study, the autofluorescence was measured over a period of 600s (10min).(Fig.4B). *p*PLA and *f*PLA were analysed and compared to glass, with the data showing a gradual fluorescence decay for both, with higher intensity than glass. Contrary to the transparency behaviour, a notable difference was noted between the pristine and the functionalised PLA - *f*PLA showing a higher autofluorescence due to the increased presence of –OH groups in the functionalised sample. Furthermore, the autofluorescence of *p*PLA and *f*PLA were measured and compared to COC, PC, PDMS and PMMA with reference to glass. In these experiments, different wavelengths were tested, covering the range used in fluorescence microscopy. The fluorescence background was taken after 60s illumination [22]. As expected, for all the materials tested, the autofluorescence decreased with increasing wavelengths. An exception to this was PC which demonstrated a lower fluorescence background at 532 nm. PLA showed autofluorescence levels no higher than ~ 1 times the one of glass and comparable with the other tested materials. (Fig. 4 C,D,E). Although the numerical fluorescence background measured for both pristine and functionalised PLA were higher than other materials, these results are consistent with previous autofluorescence studies on other thermoplastic materials used for OOC applications and are not hindering PLA suitability for fluorescence imaging [22], [24]

Cell tracking in a PLA Organ-On-Chip device

The role of an Organ-on-a-chip device is not only to culture different cell types successfully but also to provide a micro-environment for cells which would be superior to typical 2D cell culture. To this end, the device can be engineered in order to guide and spatially confine the cells. Recently, Biselli et al.[21] used an OOC device as powerful tool to study and quantitatively analyse the cancer-immune cells interactions via imaging analysis. The velocities, turning angle and path of the moving cells were used as indicator to check anticancer immune responses. Having demonstrated that the transparency of *f*PLA substrates is comparable to that of other common polymers used in microfluidic, we designed and manufactured an Organ-On-Chip device for cell tracking in a confined channel. A device composed of three layers was manufactured using a combination of hot embossing and Sacrificial layer laser Assisted Method, SLAM approaches [7] (Fig.5.A). In this experiment, PC-3 (prostate adenocarcinoma) cells were cultured on the *f*PLA device and a time lap

video was recorded using a Juli Smart microscope (Digital Bio) in a CO_2 incubator at 37˚C. A microphotograph of the region of interest was taken at 2 minutes intervals using a 4x magnification objective and videos analysed with a custom written software as per material and method session. Kinematic descriptors such as speed, mean curvature, and angular speed exhibit were extracted and compared for a PS Petri dish, PLA disc or inside a device. The kinematic descriptors between the PLA substrate and the PS were very similar despite slight difference on the surface topology [25]. However, the diffusion coefficient that is typical of a kind of motion and not strictly related to the kinematics of individual cells is equal for the two videos, thus demonstrating that cells under the microscope have the same behaviour (Fig 5B.iv). On the other hand, the kinematic descriptors extracted from the cell culture in the PLA OOC device showed cell motility differences (Fig5 C). This might be due to the difference in the oxygen permeability of the material. For instance, PLA has an oxygen diffusion coefficient of 2.5e-12 m2/s [26], while the air of 2.14e-5 m2/s. Up to now, one of the main driving reason in choosing PDMS for OOC application was its good oxygen permeability, with a diffusion coefficient of 3.5e-9 m2/s, while COC, another emerging material for OOC application, has an oxygen diffusion coefficient close to nil [27]. The differences observed in cell motility can be overcome by granting a better surface topology or providing a protein coating in culture channels to improve cell adhesion and applying a medium flow for gas exchange to feed the cells with the required amount of oxygen [28]. In conclusion the transparency of *f*PLA allows cell tracking in the device and a random cell confined walk can be observed (Fig.5D). In conclusion, the transparency of *f*PLA allowed comparable cell tracking in the device and in a control PS Petri-dish (Fig.5.A.).

Discussion

We introduced PLA as a novel material for Organ-On-Chip devices. PLA can be machined via laser-cutting as shown in this article, or by 3D printing as shown elsewhere. In this paper, we showed that PLA offer many distinct advantages as a substrate for Organ-On-Chip applications. First, PLA can be functionalised via a rapid (60s) functionalisation process using Sodium Hydroxide, reducing its native contact angle of about 70 to 40. Secondly, cells can be as easily cultured on PLA surfaces, as on conventional polystyrene (PS) petri-dishes or on conventional microfluidic PDMS material. PLA also performs better with regards to Cell proliferation and cell toxicity compared to the majority of uncoated polymeric material such as PMMA, PC, COC. Thirdly, we demonstrate that PLA performs largely better than PDMS with regards to the adsorption and absorption of small molecules. Taking a hydrophilic representative molecule, Nile Red, of same size as oestrogen, a classic target compound in cell studies, we showed that a PDMS adsorbed 50% of the Nile Red after 20 load and wash cycles, while a similar PLA channel showed no adsorption, whether or not it had been functionalised. Fourthly, PLA substrates are highly transparent and present very little auto-fluorescence, enabling high quality imaging, which is demonstrated through a cell-tracking experiment in an Organ-On-Chip device. In this experiment, a random cell confined walk was observed both in the PLA Organ-On-Chip device and in a control on polystyrene (PS).

PLA is compatible with a range of complex integration, such as conducting electrodes, which we demonstrated in a previous publication [7]. While here we use a layer-by-layer approach to prototype Organ-On-Chip devices, PLA can be also formed, molded, and printed. PLA is a popular material for filament deposition modelling, or 3D-printing. However, while 3D printed microfluidic devices have demonstrated elsewhere [ref] they lack good transparency to enable high-resolution imaging. On the other hand, we showed that high transparency can be achieved using molding and laser-cutting techniques, which applies to both prototyping and high-volume manufacturing.

In summary, we have demonstrated that PLA is a new suitable material for Organ-On-Chip devices. Our approach is designed to address many disadvantages inherent to conventional PDMS microfabrication technique. The lack of adsorption and absorption of small compounds, without the need for functionalisation, ensure a faster fabrication. Furthermore, in conjunction with

its biocompatibility, inertness to small molecules, optical qualities, PLA microfluidics will open opportunities to develop more sustainable solutions for microfluidic laboratories, in industry or in research. PLA represents a more environmentally sustainable solution than other thermoplastic fossil-based materials proposed in the market as alternative to PDMS. Ultimately, we envision that microfluidic device engineers will embrace a Design for Sustainability approach, and look into biopolymers, such as PLA, to develop single-use devices, for a wide range of applications, from Organ-On-Chip to Point-Of-Care devices.

Materials and method

Materials

Polylactic acid (Natureworks® 2003D) was purchased from Naturework in pellet format, PMMA Clarex® was purchased from Weatherall Ltd., in a 1 mm sheet form, PDMS Sylgard 184 was purchased from Dow Corning, while COC (Topas), PC (RS components) were acquired in 1 mm sheet. All the reagents used were purchased from Sigma Aldrich, unless differently specified in the text.

PLA Functionalization

The functionalisation process was an alkaline surface hydrolysis. NaOH (sigma Aldrich) was dissolved in DI water to prepare 0.01, 0.1, 0.5, and 1 M solution concentrations. The Pristine PLA sample is dipped inside the solution and kept in for specified amount of time (0, 10, 30, 60, 120 sec). A contact angle of about 40˚, recommended for cell culture application [9], [29], was achieved by dipping the substrate for 60 seconds. Then the sample was washed with water and dried with compressed air. To measure the effect of the functionalization time, and solution concentration, the contact angle of the samples was measured using a custom-made static contact angle apparatus [30] The images acquired via a Dinolite microscope (model) were analysed with ImageJ plugin contactj. Each measurement was carried out 6 times. To assess the chemical changes at the surface an attenuated total reflection, ATR-FTIR, analysis was carried out collecting 200 scans in the range of 4000-400 cm^{-1}, with a resolution of 4 cm^{-1} using a Nicolet Is5 spectrophotometer. The effect of the surface functionalization on the surface were studied using a white light interferometer (Zygo) with 1 nm resolution in vertical direction. The Roughness (which one, Ra) quantitative determination was carried out using Metro.Pro 8.2. For morphology analysis SEM (Quanta FEG 650 SEM) images were acquired in high vacuum mode.

PLA Biocompatibility

The human hepatoblastoma C3A cell line was obtained from the American Type Culture Collection (ATCC, USA). The cells were maintained in Minimum Essential Medium Eagle (Gibco, UK) with 10% foetal bovine serum (FBS) (Gibco, UK), 2 mM L-glutamine, 100 U/ml penicillin/ streptomycin, 1 mM sodium pyruvate and 1% nonessential amino acids (all Sigma, UK) (termed complete medium), at 37°C and 5% CO_2. The A549 cells (XX) were purchased from ATCC and maintained in RPMI (GIbco, uk) + 10% FBS + 100 IU/ml non-essential AA + 2 mM L glutamine + 100 U/ml Penicillin/Streptomycin. For the biocompatibility experiments, both cell types were seeded in 1 24 well plate at a cell density of 50000 cells per well in 1 ml of complete medium. The medium was changed every 48 hours up to a period of 7 days. Subsequent to appropriate cell culture period (48 hr or 7 days) the cell supernatants were collected and frozen at -80°C and later used for the adenylate kinase assay. In the alamar blue assay (proliferation), the supernatant from all well was removed, and stored in a freezer -80ºC as described above. 1 ml of diluted alamar Blue reagent (Sigma, UK) (0.1 mg/ml) reagent was added to all (Sigma, UK). The plates were incubated at 37ºC for 90 minutes and fluorescence was measured at 544/590nm. The loss of cell membrane integrity was evaluated utilising a ToxiLight™ bioassay kit (Lonza, USA). Briefly, 20 µl of cell supernatant was transferred to a luminescence compatible plate before the addition of 80 µl of adenylate kinase (AK) detection buffer. The plates were incubated for 5 min at room temperature and luminescence quantified. These

experiments included a negative control – polystyrene cell culture treated plastic and a positive control - 0.1% Triton X-100 (Sigma, UK).

Organ-On-Chip device preparation

To manufacture the PLA Organ-On-Chip devices, firstly PLA sheets were manufactured using a manual hydraulic heated press as per [7] . A prototyping approach described in [ref] was adopted Briefly, each layer was cut using a CO2 laser cutter (Epilog Mini Elix 30W) using SLAM approach previously developed, which consist of applying a thin tape (100 µm) on the material prior to laser-cutting [7]. Then the different layers are cleaned by sonicating in pure Ethanol. To manufacture the functionalized device, the cleaned layers are dipped in a 1M NaOH solution for 60 s, then cleaned with water, 2-Propanol (Sigma Aldrich) and compressed air. The surfaces are then chemically activated under UV exposure (254nm) for 45 s. The different layers are then bonded together placing them in a sandwich composed of two glass slides, and kept them in contact at 50 C for 10 minutes. Once the assembly has cooled down to room temperature, it is disassembled and the device is ready to be tested.

Small molecules absorption and adsorption

To assess the bio-inertness of the material, pristine PLA was compared with functionalised PLA and with PDMS. To manufacture the device, specifically 6 channels of 1 mm width and 0.8 mm depth were cut through the PLA layers. The remaining cut pieces were used as negative mould to manufacture the PDMS device. The PLA device were then assembled as per chip preparation section. To manufacture the PDMS device, PDMS was mixed with the curing agent with a weight ratio of 1:10 and poured into a PS petri dish where the negative mould had been previously attached using a double sided adhesive tape (3M tape 469 MP). The PDMS is then left for 1h30 at room temperature prior to being placed for 1h at 80°C on a heated plate. In the same way a PDMS layer with input and output access holes were prepared. The two layers were then bonded together using an oxygen plasma treatment for 2 minutes (Diener electronic plasma surface technology). To test the absorption of hydrophobic compound Nile Red (sigma Aldrich) was dissolved in EtOH 99% to obtain a solution concentration of 1 µM. To test the absorption of hydrophilic compounds fluorescein salt was dissolved in DI water (1uM). A fluorescent microscope (AM4115T-GFBW) was used to take the fluorescent images and the image analysis freeware Imagej was used to analyse the fluorescence intensity. An advantage of this absorption experimental set-up is that it is fast and it does not require specific apparatus.

PLA Optical properties

The transparency of the materials was tested analysing the transmittance of the light in the visible region using a UV-VIS spectrophotometer (Shimadzu UV-2550). The autofluorescence of the plastic materials of interest was measured using a Leica SP8 3X STED laser scanning microscope equipped with two Internal Spectral Detector Channels (PMT). Current from the PMT was amplified and data was acquired using the software LAS X from Leica. Laser Kit WLL2 (supercontinuum white light laser [pulsed]), 200 excitation lines from 470-670 nm was used as light source. The fluorescence was filtered by suitable laser filters for 405 nm (emission filter 460/510 nm), 532 nm (emission filter 580/640 nm) and 633 nm (emission filter 650/720 nm). Materials were wiped with ethanol preceding the imaging and were attached to a microscope slide before placing them on the microscope stage. The microscope objective (20x) was focused at the bottom surface of the samples and the objective was moved towards them. Auto-fluorescence imaging was done after 60 seconds illumination of each sample at each laser wavelength of interest (405, 532 and 633 nm excitation wavelengths). For each sample three unbleached sample spots were identified and selected for illumination. The analysis of the material auto-fluorescence long term recovery was done by illuminating and recording the samples for 10 min at both 405 nm (emission filter 460/510 nm) and 633 nm (emission filter 650/720 nm) wavelengths

Cell imaging and tracking on a PLA device

Cell Culture. PC-3 prostate cancer cells were cultured at 37°C in a 5% CO_2 humidified atmosphere in RMPI (10% fetal bovine serum, 100,000 units/liter penicillin, 50 mg/L streptomycin and 200mM glutamine). Cell seeding in PLA support (1st method). PC-3 cells were seeded on PLA lab-on-chip at a final concentration of 20.000 cells/80uL. Every hour new RPMI fresh medium was slowly added to prevent the channels from drying out completely. Images were taken every 1 hour. Cell seeding in PLA support (2nd method). PC-3 cells were seeded on PLA lab-on-chip at a final concentration of 20.000 cells/80uL. Tips containing 20ul of RMPI were positioned at the end of each microfluidic channel to allow replacing the medium every 24 hours (instead of 1 hour). Images were taken every 24 hours.

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Author contributions

Author contributions: A.O. V.L.C, N.H., V.P. and M.K-K, designed research; A.O., D.V., A.K. F., A. V.P performed research; R.R., E, V.S. contributed to reagents and analytic tools; A.O. A, D, E, N.H, V.S, M.K-K analyzed data; and A.O and M.K-K wrote the paper.

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References

[1] S. N. Bhatia and D. E. Ingber, “Microfluidic organs-on-chips,” *Nat. Biotechnol.*, vol. 32, no. 8, pp. 760–772, 2014.

[2] B. Zhang, A. Korolj, B. Fook, L. Lai, and M. Radisic, “Advances in organ-on-a-chip engineering,” *Nat. Rev. Mater.*, vol. 3, pp. 257–278, 2018.

[3] B. Zhang and M. Radisic, “Organ-on-a-chip devices advance to market,” *Lab Chip*, vol. 17, pp. 2395–2420, 2017.

[4] T. P. B. Yihua Wang, “Uncured PDMS inhibits Myosin In Vitro Motility in a Microfluidic Flow Cell,” *bioRxiv*, pp. 1–23, 2018.

[5] S. Li *et al.*, “preparation of highly reliable PDMS double emulsion micro fl uidic devices †,” *RSC Adv. Rapid*, pp. 25927–25933, 2016.

[6] M. Iqbal, S. Haswell, and I. Gibson, “Lab-on-a-chip or Chip-in-a-lab : Challenges of commercialization lost in translation,” *Procedia Technol.*, vol. 20, no. July, pp. 54–59, 2015.

[7] A. E. Ongaro *et al.*, “Laser ablation of polylactic acid sheets for the rapid prototyping of sustainable , single-use , disposable medical micro-components.”

[8] T. E. Grigoriev, T. B. Bukharova, A. V Vasilyev, G. E. Leonov, Y. D. Zagoskin, and V. S. Kuznetsova, “Effect of Molecular Characteristics and Morphology on Mechanical Performance and Biocompatibility of PLA-Based Spongious Scaffolds,” pp. 977–983, 2018.

[9] S. Engineering and B. Science, “Effect of Surface Wettability of Osteosarcoma Cells on Plasma-,” vol. 26, no. September 2011.

[10] R. Civitarese *et al.*, “Organ-On-A-Chip Platforms : A Convergence of Advanced Materials , Cells , and Microscale Technologies Organ-On-A-Chip Platforms : A Convergence of Advanced Materials , Cells , and Microscale Technologies,” no. October, 2017.

[11] P. K. Chu, J. Y. Chen, L. P. Wang, and N. Huang, “Plasma-surface modification of biomaterials,” vol. 36, pp. 143–206, 2002.

[12] P. M. Van Midwoud, A. Janse, M. T. Merema, G. M. M. Groothuis, and E. Verpoorte, “Comparison of Biocompatibility and Adsorption Properties of Di ff erent Plastics for Advanced Micro fl uidic Cell and Tissue Culture Models,” 2012.

[13] C. Y. Tham, Z. A. A. Hamid, Z. Ahmad, and H. Ismail, “Surface Modification of Poly (lactic acid) (PLA) via Alkaline Hydrolysis Degradation,” no. May, 2015.

[14] M. Kaduri, M. Poley, O. Adir, and N. Krinsky, “Biocompatibility , biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems,” *Chem. Eng. J.*, vol. 340, no. January, pp. 9–14, 2018.

[15] Y. Ramot, M. Haim-zada, A. J. Domb, and A. Nyska, “Biocompatibility and safety of PLA and its copolymers ☆” *Adv. Drug Deliv. Rev.*, vol. 107, pp. 153–162, 2016.

[16] B. J. Van Meer *et al.*, “Small molecule absorption by PDMS in the context of drug response bioassays,” *Biochem. Biophys. Res. Commun.*, vol. 482, no. 2, pp. 323–328, 2017.

[17] K. J. Regehr *et al.*, “Biological implications of polydimethylsiloxane-based microfluidic cell culture †,” pp. 2132–2139, 2009.

[18] R. Gomez-sjoberg, A. A. Leyrat, B. T. Houseman, K. Shokat, and S. R. Quake, “Biocompatibility and Reduced Drug Absorption of Sol - Gel-Treated Poly (dimethyl siloxane) for Microfluidic Cell Culture Applications,” vol. 82, no. 21, pp. 8954–8960, 2010.

[19] J. A. C. K. D. W. Ang, N. I. J. D. Ouville, S. H. T. Akayama, and M. O. E. L. S. Ayed, “Quantitative Analysis of Molecular Absorption into PDMS Microfluidic Channels,” vol. 40, no. 9, pp. 1862–1873, 2012.

[20] M. W. Toepke and D. J. Beebe, “PDMS absorption of small molecules and consequences in microfluidic applications,” no. c, pp. 1484–1486, 2006.

[21] E. Biselli *et al.*, “Organs on chip approach : a tool to evaluate cancer -immune cells interactions,” *Sci. Rep.*, pp. 1–12, 2017.

[22] A. Piruska *et al.*, “The autofluorescence of plastic materials and chips measured under laser irradiation,” pp. 1348–1354, 2005.

[23] A. You, M. A. Y. Be, and I. In, “Structural and optical properties of poly lactic acids,” *J. Appl. Phys.*, vol. 77, no. August 1998, pp. 2957–2973, 2016.

[24] B. Lu, S. Zheng, Q. Quach, and Y. Tai, “A study of the autofluorescence of parylene materials for m TAS applications,” *Lab Chip*, vol. 10, pp. 1826–1834, 2010.

[25] D. Baptista, L. Teixeira, C. Van Blitterswijk, S. Giselbrecht, and R. Truckenmüller, “Overlooked ? Underestimated ? Effects of Substrate Curvature on Cell Behavior,” *Trends Biotechnol.*, vol. xx, pp. 1–17, 2019.

[26] H. J. Lehermeier, J. R. Dorgan, and J. D. Way, “Gas permeation properties of poly(lactic acid),” *J. Memb. Sci.*, vol. 190, no. 2, pp. 243–251, 2001.

[27] R. D. K. Christopher J. Ochs, Junichi Kasuya, Andrea Pavesi, “Oxygen Levels in Thermoplastic Microfluidic Devices during Cell Culture,” *Lab Chip*, vol. 14, no. 3, pp. 459–462, 2015.

[28] A. Al-ani, D. Toms, D. Kondro, J. Thundathil, Y. Y. Id, and M. U. Id, “Oxygenation in cell culture : Critical parameters for reproducibility are routinely not reported,” *PLoS One*, pp. 1–13, 2018.

[29] J. P. F. Max J. Lerman, Lembong Josephine, Shin Muramoto, Greg Gillen, “The Evolution of Polystyrene as a Cell Culture Material,” *Tissue Eng. Part B*, vol. 24, no. 5, pp. 359–372, 2018.

[30] A. H. Guillaume Lamour, “Contact Angle Measurements Using a Simplified Experimental Setup,” *J. Chem. Educ.*, vol. 87, no. 12, pp. 1403–1407, 2010.

Figures

Figure 1

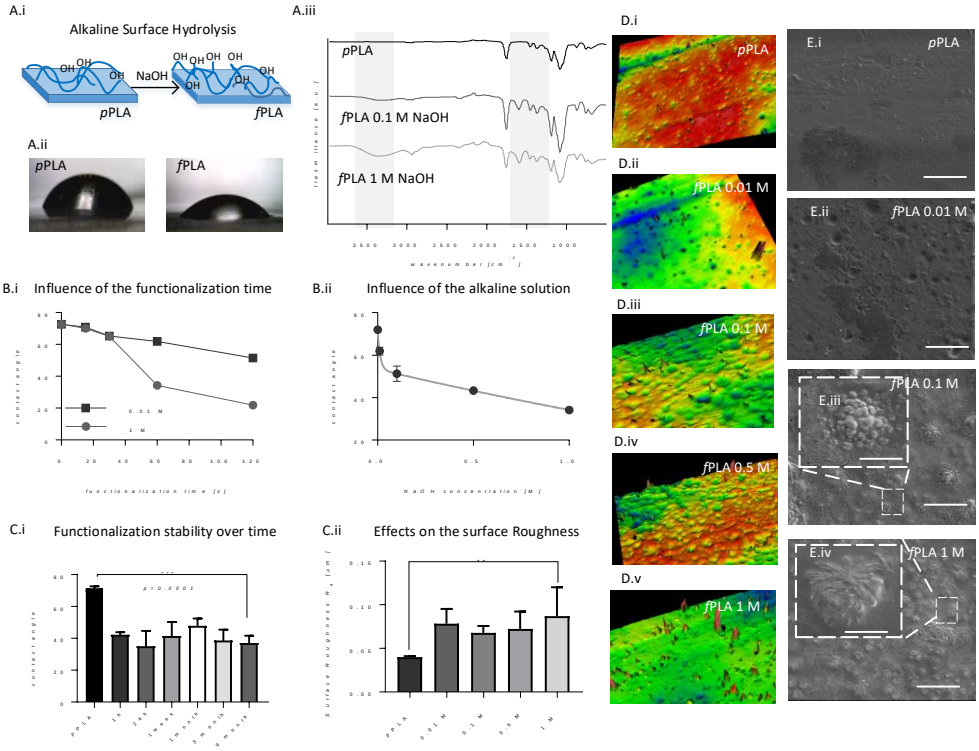

Figure 2

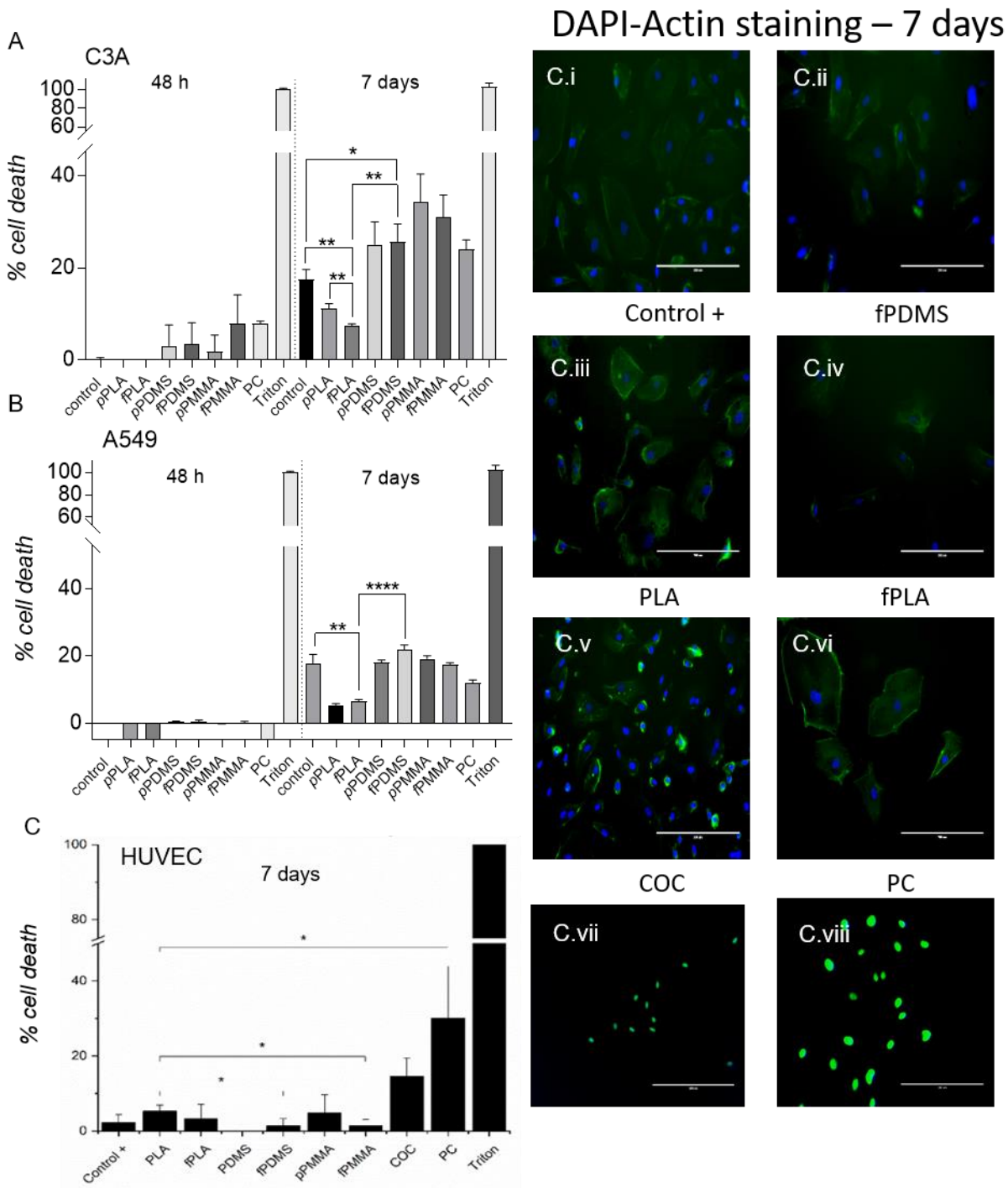
A
C3A
48 h
7 days
% cell death
control
pPLA
fPLA
pPDMS
fPDMS
pPMMA
fPMMA
PC
Triton
B
A549
C
HUVEC
Control +
PDMS
COC
DAPI-Actin staining – 7 days
C.i
C.ii
C.iii
C.iv
C.v
C.vi
C.vii
C.viii
Control +
fPDMS
PLA
fPLA
COC
PC

Figure 3

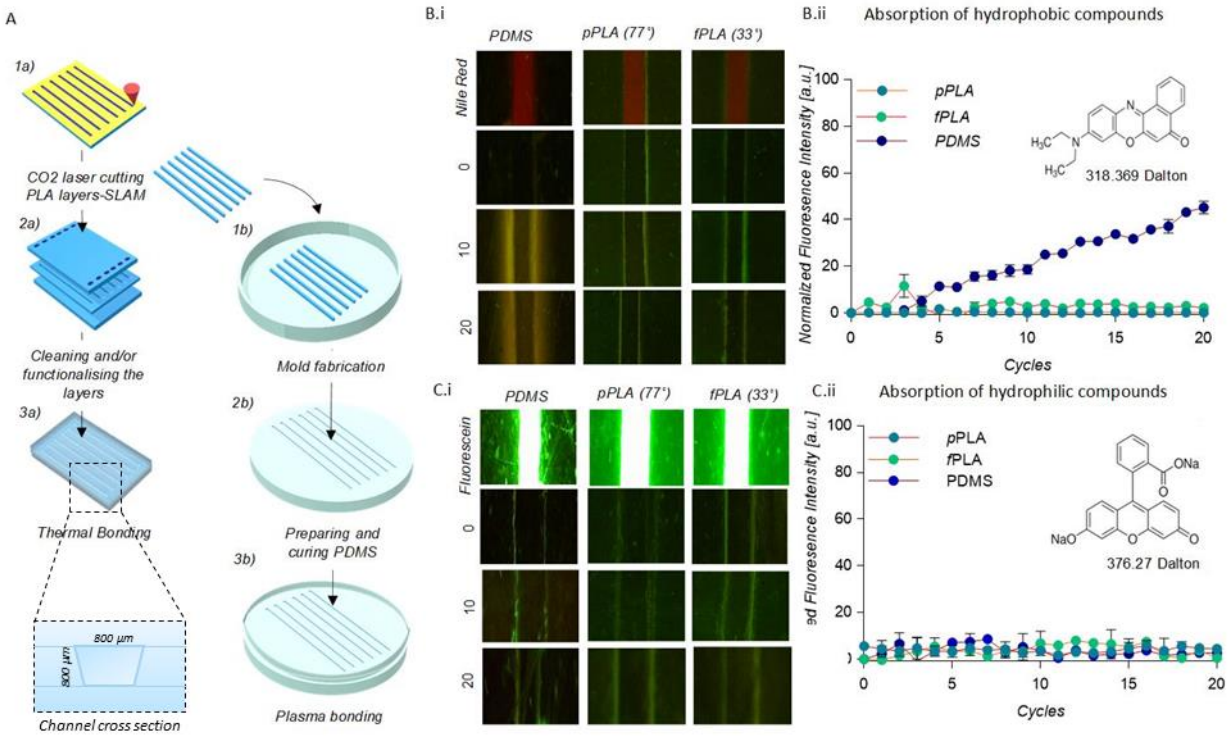
A
1a)
CO2 laser cutting PLA layers-SLAM
2a)
Cleaning and/or functionalising the layers
3a)
Thermal Bonding
800 µm
800 µm
Channel cross section
1b)
Mold fabrication
2b)
Preparing and curing PDMS
3b)
Plasma bonding
B.i
PDMS
pPLA (77°)
fPLA (33°)
Nile Red
0
10
20
B.ii
Absorption of hydrophobic compounds
Normalized Fluoresence Intensity [a.u.]
pPLA
fPLA
PDMS
318.369 Dalton
Cycles
C.i
PDMS
pPLA (77°)
fPLA (33°)
Fluorescein
0
10
20
C.ii
Absorption of hydrophilic compounds
pPLA
fPLA
PDMS
376.27 Dalton
Cycles

Figure 4

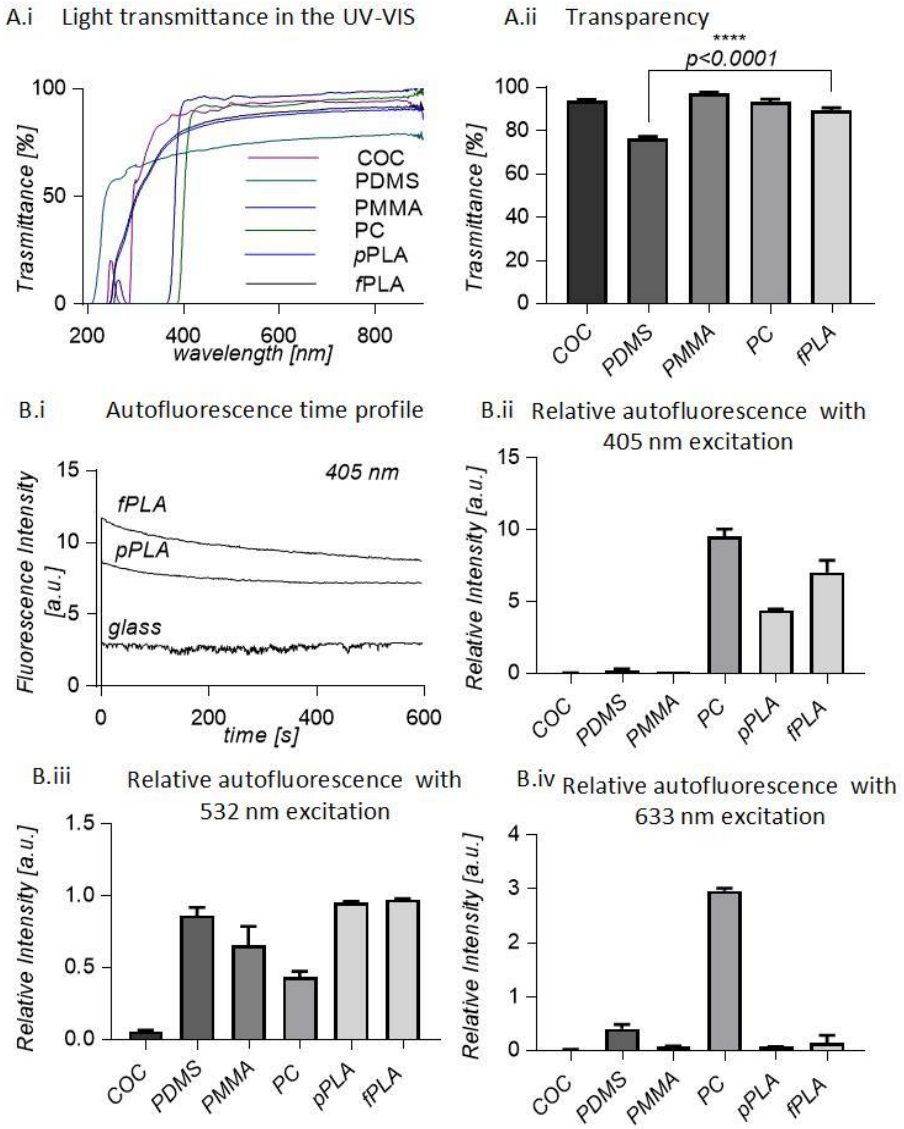
A.i Light transmittance in the UV-VIS
Trasmittance [%]
wavelength [nm]
COC
PDMS
PMMA
PC
pPLA
fPLA
A.ii Transparency
****
p<0.0001
Trasmittance [%]
COC
PDMS
PMMA
PC
fPLA
B.i Autofluorescence time profile
405 nm
fPLA
pPLA
glass
Fluorescence Intensity [a.u.]
time [s]
B.ii Relative autofluorescence with 405 nm excitation
Relative Intensity [a.u.]
COC
PDMS
PMMA
PC
pPLA
fPLA
B.iii Relative autofluorescence with 532 nm excitation
Relative Intensity [a.u.]
COC
PDMS
PMMA
PC
pPLA
fPLA
B.iv Relative autofluorescence with 633 nm excitation
Relative Intensity [a.u.]
COC
PDMS
PMMA
PC
pPLA
fPLA

Figure 5

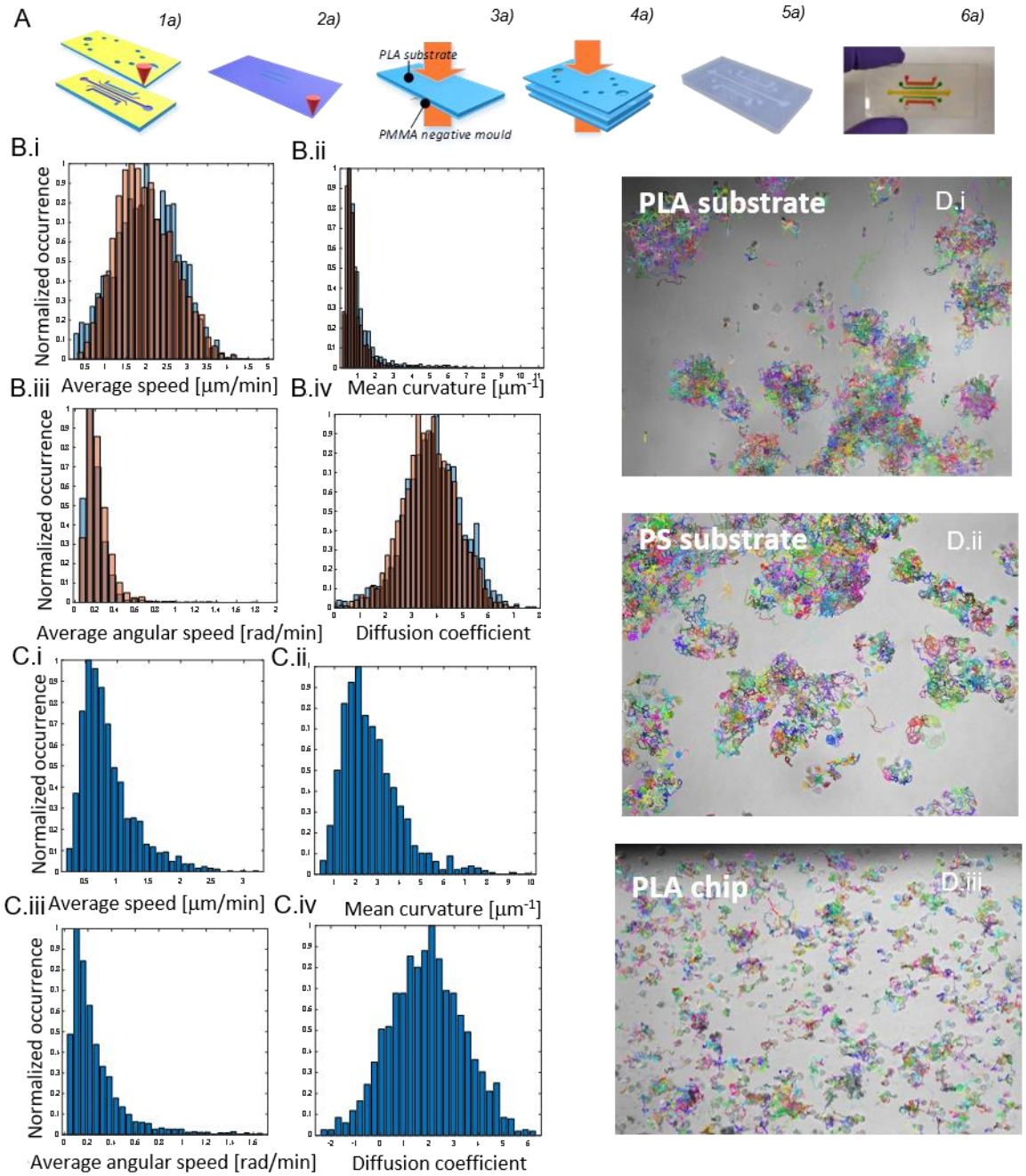
A
1a)
2a)
3a)
4a)
5a)
6a)
PLA substrate
PMMA negative mould
B.i
B.ii
Normalized occurrence
Average speed [µm/min]
Mean curvature [µm-1]
B.iii
B.iv
Average angular speed [rad/min]
Diffusion coefficient
C.i
C.ii
C.iii
C.iv
PLA substrate
D.i
PS substrate
D.ii
PLA chip
D.iii

Figure Legends

Figure 1 *Tuning the surface properties of PLA. A.i) The functionalization mechanism via Alkaline Surface Hydrolysis leads to the formation of hydroxyl groups; A.ii) Photographs of a DI water drop on the pristine PLA substrate and on the functionalized PLA substrate shows the decrease of the contact angle after functionalisation (A.iii); A.iv) FTIR spectrum of pristine PLA (dark blue), functionalized PLA using a concentration of 0.1 M of NaOH solution (blue line), and a concentration of 1 M of NaOH (light blue). The grey areas underline the change in the spectrum due to the functionalization. The first one (from left to right) at 3200 cm-1, indicative of the formation of hydroxyl groups, the second one highlights a stretching of the C-C groups indicative of a partial hydrolysis of the treated surface. B.i) Influence of the functionalization time on the final contact angle for two different solution concentration, 0.01 M (green box) and 1 M (blue circle). The two connecting lines are just to guide the eye. For all the points the error bars are shorter than the high of the symbol. B.ii) influence of the NaOH concentration on the final contact angle after 60 s of functionalization. The experimental results are fitted with a two-phase exponential decay function (orange line). For some points the error bar is shorter than the high of the symbol. C.i) Stability of the functionalization method adopted over three month. C.ii) Effect of the solution concentration on the final surface roughness of the functionalized substrate after 60 s functionalization. D) Interferometer pictures of the unfunctionalized and funcionalized PLA surfaces with different solution concentration after 60 s functionalization time. E) SEM pictures of the unfunctionalized and funcionalized PLA surfaces with different solution concentration after 60s functionalization time.*

Figure 2*. Assessing the material biocompatibility. A) percentage of cell death of human hepatocellular carcinoma cells (C3A) on the different substrates at 48h and 7 days time points; B) percentage of cell death of adenocarcinomic human alveolar basal epithelial cells (A549) on the different substrates at 48h and 7 days time points. C) percentage of cell death of HUVECs cells on the different substrates at 7 days time point. The cells did not adhere on the pristine PDMS. C.i,- viii DAPI-actin staining of Huvecs cells at 7 days time point on the different substrates.*

Figure 3 *. Assessing bio-inertness of the substrate materials. A) Protocol to manufacture PLA and PDMS microdevices. 1a) Sacrificial layer assisted method to cut through channel of 1 mm width from a PLA substrate. 2a) the different layers are cleaned and/or functionalised for 60 s with 1M NaOH solution. Then the unfunctionalized and functionalized layers are thermal bonded together at 70 C for 1h using binder clips To increase the contact area 3a). The cut PLA parallelepipeds are then used negative mould to fabricate the PDMS device to have the same channel cross section for the different devices. In insert the channel cross section. 1b) the parallelepipeds are then attached to a PS dish. The PDMS is mixed and placed in the mould and cured 2b). 3b) Plasma bonding of the PDMS device. B) Absorption of hydrophobic compounds. B.i) top view of the channel for (from left to right) PDMS, pristine PLA and functionalized PLA. From the top to the bottom picture of the channel filled with a solution of Nile Red in ethanol (1µM), representing one of the reference point; then picture of the empty channel, representing the 0 reference point, and picture of the channel at the 10th and 20th cycle. B.ii) Normalized fluorescence intensity, measured at the centre of the channel, plotted in function of the cycle number. In insert molecular structure and molecular weight of the Nile Red. C) Absorption of hydrophilic compounds. C.i) Top view of the channel for (from left to right) PDMS, pristine PLA and functionalized PLA. From the top to the bottom picture of the channel filled with a solution of fluorescein salt in DI water (1µM), representing one of the reference point; then picture of the empty channel, representing the reference point, and picture of the channel at the 10th and 20th cycle. B.ii) Normalized fluorescence intensity, measured at the centre of the channel, plotted in function of the cycle number. In insert molecular structure and molecular weight of the fluorescein salt*.

Figure 4. *Optical Properties: A) Transparency of the materials. A.i) UV-VIS spectrum of the different substrate materials; A.ii) Comparison of the transmittance of the light in the visible region of the different substrate materials. B) Autofluorescence of the materials, B.i) Fluorescence decay profile under 10 minutes continuum illumination of functionalised PLA, pristine PLA and glass; B.ii) Autofluorescence intensity relative to glass of the different substrate materials after 60 s continuum illumination with 405 nm excitation, 532 nm excitation B.iii) and 633 nm excitation B.iv..*

***Figure 5**. Assessing PLA suitability for microfluidic cell culture and Organ on a chip applications with respect to cell tracking. A) schematization of the protocol to manufacture PLA microfluidic devices. 1a) SLAM approach to create inlet and outlet holes in the top layer and to microstructure the channel on the middle layer. 2a) rastering a PMMA substrate to create a mould for the bottom layer with the diffusion channels. 3a) hot embossing the structure from the PMMA mould to PLA, using a temperature of 70 C and a pressure of 0.5 MPa for 15 minutes. 4a) NaOH functionalization and UV activation of the surfaces with thermal bonding of the different layers. T=50 C for 5 minutes. 5a) then assembled device is cooled down maintaining the layers in strictly contact. 6a) picture of the final device filled with food dyeB) Statistics of the Kinematic descriptors of the PC-3 prostate cancer cells on a disc of PLA (blue bars) and on a PS well plate (orange bars) B.i) speed, B.ii) mean curvature B.iii) angular speed and B.iv) diffusion coefficient. C) Statistics of the Kinematic descriptors of the PC-3 prostate cancer cells on a PLA device C.i) speed, C.ii) mean curvature C.iii) angular speed and C.iv) diffusion coefficient. D) Extracted PC-3 prostate cancer cells trajectories through the Cell-hunter software on a disc of PLA D.i), on a PS substrate D.ii) and on a PLA device.*