Directed Cell Growth on Laser-Transferred 2D Biomaterial Matrices

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Abstract: Patterned arrangement of living cells to form tissues on a given surface is the prerequisite of successful bioengineering, tissue building and biosensor technology. The aim of the present study was to deposit various biomaterials onto a given substrate to establish a patterned cellular matrix using laser-based technologies. We used the Pulsed Laser Deposition and the Absorbing Film-Assisted Laser-Induced Forward Transfer methods to deposit various biomaterials, such as fibronectin, collagen and endothelial cell growth supplement. Subpicosecond KrF excimer laser was used for irradiation of the target materials. When cultured neuroectodermal stem cells, astrocytes, endothelial and neuroblastoma cells were layered on the deposited biomaterial patterns a guided growth of these cells was induced along the patterned thin film. Some cell types showed various interactions when approached each other. The above methods are suitable to build an architecture of substrates which supports and guides the growth of cells and may enable the cells to induce directed and rapid repair of injured tissues.

INTRODUCTION

Controlled positioning of living cells onto a given surface is a fundamental problem from the point of view of bioengineering, tissue building and biosensor technology. A possible way to realize the above goal is the preparation of cell growth-supporting layer onto the substrate in the required pattern. It is thought that living cells attach only to the structured layer of the growth-promoting molecules along a formed pattern. Such applications have been reported using self-assembled monolayers [1], microcontact printing [2] and soft lithography [3,4]. Based of the well-known properties of the laser-based techniques we assume that the pulsed laser deposition (PLD) and the absorbing film assisted lased induced forward transfer (AFA-LIFT) techniques may also be suitable for preparation of the above-mentioned structured thin layer of "cell friendly" biomaterials.

In the last decade PLD of organic and biological thin films has been extensively studied due to the increased interest in various biomedical applications. A number of experiments have shown that the nanosecond PLD technique is suitable for deposition of different types of biocompatible polymers [5-11] and for materials found in the mammalian organism, such as hydroxyapatite, dentine and pepsin [12-14]. The main drawback of the nanosecond PLD is the possible thermal or photochemical damage of the sensitive biological samples. A potential solution of this problem can be the use of femtosecond lasers during the deposition procedure since the ultrashort laser pulses reduce the thermal effects, thus allowing the effective and harmless deposition of sensitive biomaterials. Accordingly, in the last years the aim of new bio-PLD experiments was the widening of the range of target biological/biocompatible materials and the application possibilities of pulsed laser deposition technologies using femtosecond lasers [15,16]. These works proved that femtosecond lasers are also suitable for thin film deposition of organic materials under the optimal experimental conditions.

MAPLE DW (Matrix-Assisted Pulsed Laser Evaporation Direct Write) technology was developed for transfer of biological/biocompatible materials from a "ribbon" to a substrate. Using this method Ringeisen *et al*. produced mesoscopic patterns of viable *Escherichia coli* on Si(1 1 1) glass, and nutrient agar plates [17], while Barron and co-workers deposited two types of mammalian cells, human osteosarcoma and rat cardiac cells into a biopolymer matrix *via* MAPLE DW [18].

In our earlier experiments we successfully developed and applied a modified laser induced forward transfer arrangement (AFA-LIFT) for the controlled transfer of *Trichoderma longibrachiatum* conidia and different types of living cells [19-21]. The biological samples were transferred from absorbing silver thin layer coated fused silica substrates (donors) to gelatin- or matrigel-coated glass plates (acceptors) using a nanosecond KrF excimer laser. The metal film promoted the movement of cells and conidia and prevented the photonic and thermal damages. Our results proved that this direct-write technique can also be efficiently applied for the controlled transfer of biological targets. Fernández-Pradas *et al.* used similar arrangement for the production of DNA microarrays applying Nd:YAG laser and titanium film as absorbing layer [22, 23].

Earlier investigations proved that the nanosecond lasers are suitable for transfer of biomaterials. However, it is likely that the efficiency of the direct-write methods could be in-

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creased by decreasing the thermal damaging effects of the nanosecond pulses similarly to the case of nanosecond PLD [24].

The aim of the present study was to deposit structured cell growth-promoting thin layers with PLD and AFA-LIFT techniques using femtosecond excimer laser and demonstrate that cells from the cell-culture solution preferably attach to and grow on these surfaces and the end result of this procedure will be precisely controlled positioning of cells or tissues.

MATERIALS AND METHODOLOGY

PLD Technique

First thin films of cell growth-promoting substances were deposited using a standard PLD apparatus with a chamber-pressure of 1.3×10^{-3} Pa. The deposited materials were Na-alginate, fibronectin, endothelial cell growth supplement and collagen embedded in starch matrix. All these molecules are known to promote cell growth in culture in low (<1 %) concentration. The samples were pellets pressed from these powdery mixtures at 4.4×10^{4} N/cm^2 pressure. Femtosecond laser pulses were generated using a XeCl excimer-pumped dye laser system producing pulses at 496 nm, which, after frequency doubling were amplified in a KrF excimer laser cavity [25]. This system was capable of producing pulses of 450 fs duration at 248 nm. The laser beam was focused by a spherical lens to yield different fluences of 3.46, 2.75, 2.19, 1.55, 0.9 J/cm^2, the repetition rate was 10 Hz. For the spectroscopic investigations several layers were deposited onto KBr tablets by 15000-35000 pulses depending on the applied fluences. The chemical composition of the films was measured using a FTIR Bruker spectrometer. The recorded spectra demonstrated that 2.19 J/cm^2 is the optimal fluence to reach the best agreement between characteristic peaks of the deposited films and the starch. As the concentration of the target materials was very low, we suggested that they should be deposited together with starch as vehicle. Consequently, the result of the PLD procedure only from the behaviour of cells during the cell-growing experiments could be deduced. To investigate the effect of starch only on cell growth, we performed control experiments in which only a starch thin layer pattern was used. For this purpose we deposited thin layers onto silicon plates by 25.000 laser pulses at 2.19 J/cm^2 fluence from pure starch and starch mixed with Na-alginate (0,025% w/w), fibronectin (0,0125% w/w), endothelial cell growth supplement (0,025% w/w) and collagen from calf skin(0,025% w/w), respectively. All the chemicals were obtained from Sigma, Budapest, Hungary. A metal mask was used for the patterning of the prepared layers (Fig. **1**).

AFA-LIFT Technique

In contrast to previous work of Zergioti *et al.* [24], we had to apply absorbing film in the direct-write transfer procedure since the absorption of the investigated solutions was too low to absorb the pulse energy. Therefore in the second case the putative growth-promoting materials were transferred with absorbing film assisted laser induced forward transfer technique. The target materials were Na-alginate (20 mg/ml in DW), endothelial cell growth supplement (15 mg/ml in PBS), rat serum (undiluted) and collagen from calf skin (50 mg/ml in 0.1M acetic acid). Solutions of these materials were spread on the 100 nm thick silver film coated fused silica plates (donor). The metal layer was irradiated through the quartz plate by single pulses of the femtosecond KrF excimer laser. The UV beam was focused by a fused silica lens having 4 cm focal length. The applied fluence on the silver-quartz interface was 390±35 mJ/cm^2, the illuminated area was 0.04 mm^2. At this fluence the silver layer was removed from the irradiated spots together with the contacting target material. The latter one was collected on sterile glass substrates (acceptor) placed parallel to the donor at a distance of 600 μm. Donor and acceptor plate pair was connected to an X-Y-Z translator and it was translated after each laser pulse (Fig. **2**). During this procedure matrices of transferred spots of the target materials could be produced. The spots were allowed to dry and then were stored at -20 ºC until further use.

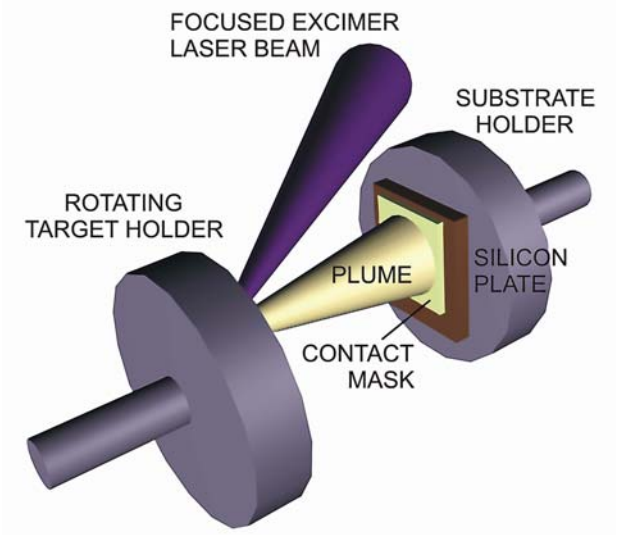

Fig. (1). The target-substrate arrangement with a metal contact mask in the PLD chamber. The target materials were Na-alginate, fibronectin, endothelial cell growth supplement and collagen embedded in starch matrix. The samples were pellets pressed from these powdery mixtures at 4.4×10^{4} N/cm^2 pressure. Femtosecond KrF excimer laser beam was focused by a spherical lens to yield different fluences of 3.46, 2.75, 2.19, 1.55, 0.9 J/cm^2, the repetition rate was 10 Hz.

Cell Types, Purification, Cell Culture

Purified and cultured rat astroglial cells, neuroblastoma cells, bovine endothelial cells and mouse clonal neuroectodermal stem cells were used for the studies. All chemicals were obtained from Sigma and Invitrogen, Budapest, Hungary. Astroglial cells were obtained from newborn rat brain hemispheres by homogenizing the hemispheres with culturing medium. The supernatant was carefully removed, the remaining pellet was plated onto Petri dishes and cultured in HDMEM containing 10% fetal calf serum. Endothelial cells were obtained from fresh pig aorta by rinsing the aortic lumen with 0.2% trypsin in HDMEM. After trypsin digestion the cells were centrifuged, plated onto Petri dishes and cultured in HDMEM containing 10% fetal calf serum. Human neuroblastoma (clone SH-SY5Y, Sigma, Hungary, Budapest) and mouse neuroectodermal stem cells (clone NE-4C/1, kindly provided by dr. Emilia Madarász) are immortalized cells which can be kept in culture for long periods of time without loss of phenotype. These cells were passed 3-4 times

and then frozen and kept in a –86 °C freezer. After plating all the cells were left to grow for 10-14 days or until they formed a monolayer then they were harvested and their concentration was adjusted to $5x10^5$ cell/ml. Culture medium containing the harvested cells in the above concentration was plated onto the surface of PLD-treated silica plates in form of spots (3-4 mm in diameter). The spots were confined to various patterned areas of the PLD-treated surface (Fig. **3A**) but the different spots were not allowed to mix with each other in order to follow the spontaneous migratory potential of plated cells. Glass plates with transferred spots using the AFA-LIFT technique were treated similarly. Briefly, clonal NE-4C/1, endothelial and astroglial cells were plated onto the treated glass surface to cover the spots, too. In case of serum spots both clonal neuroectodermal cells and astroglial cells were plated onto the surface without allowing mixing of the two cell populations. On both PLD- and AFA-LIFT-treated surfaces the cells were first allowed to attach for 1 h, then the floating cells were carefully washed off and the surface was covered by new culture medium. The cells were allowed to grow for further 24 h, then the medium was washed off and the cells were fixed with 4% phosphate-buffered paraformaldehyde (0.1 M, pH 7.4). All the plates used were gamma-sterilized before use in cell culture or the AFA-LIFT procedure.

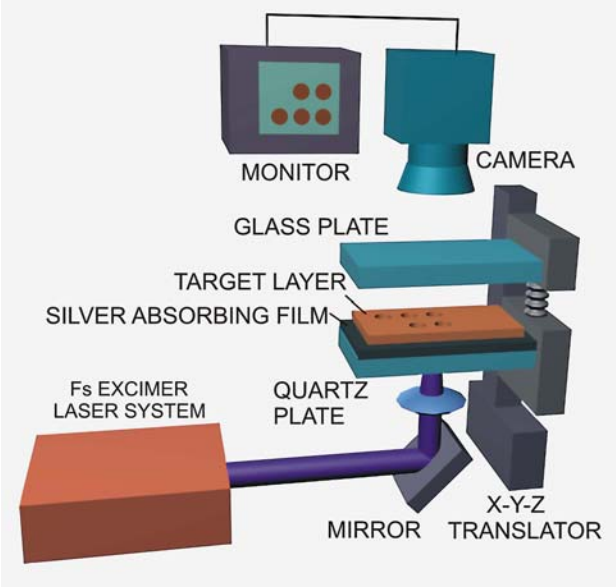

Fig. (2). AFA-LIFT arrangement for guided transfer of biological samples. The target materials were Na-alginate (20 mg/ml), endothelial cell growth supplement (15 mg/ml), rat serum (undiluted) and collagen from calf skin (50 mg/ml). Solutions of these materials were spread on the 100 nm thick silver film coated fused silica plates (donor). The metal layer was irradiated through the quartz plate by single pulses of the femtosecond KrF excimer laser. The UV beam was focused by a fused silica lens having 4 cm focal length. The applied fluence on the silver-quartz interface was 390±35 mJ/cm^2, the illuminated area was 0.04 mm^2.

Immunocytochemistry

To visualize the various cell types on the plates the following antibodies were used: rabbit anti-PGP9.5 (Protein Gene Product 9.5, LabVision, 1:50) to detect neuroblastoma cells, rabbit anti-GFAP (Boehringer Mannheim, 1:1000) for astrocytes and chicken anti-Green Fluorescent Protein (GFP, Chemicon, 1:4000) for the NE-4C/1 stem cells. Although the GFP expressed by the stem cells can be detected without immunohistochemistry, their native fluorescence is not equally strong in all the cells, therefore we used anti-GFP immunohistochemistry to detect them. Specimens processed for immunohistochemistry were preincubated in 5% milk (prepared from fat-free milk powder) for 1 h, then incubated with one or more of the above antibodies overnight at 4°C. The immune reaction was completed by using the avidin-biotin technique (reagents were purchased from Vector Labs, Burlingame, CA, USA). The fluorescent chromophores used were avidin-FITC or avidin-Texas Red (Vector Labs). In cases where no immunocytochemistry was used, reflected light illumination (Leica halogen cold light source, Leica CLS100X) of the specimens was applied to investigate and visualize the cells. Flurorescent or reflected images were photographed using an Olympus DP70 digital camera mounted on an Olympus BX50 microscope. Digital images were resized and their contrast and brightness adjusted.

RESULTS

Guided Cell Growth by PLD-Treated Surface Patterns

The growth of various cell lines were tested either on the special growth-promoting surface, being most specific for them (endothelial cell growth supplement, ECGS) or on surfaces known to support growth of several cell types (collagen, alginate and fibronectin). Pig endothelial cells have shown a specific and guided growth on endothelial cell growth supplement. Cells plated on the pattern (Fig. **3A**) first grew along the PLD-treated surface and left the untreated areas free (Figs. **3B,C** and **E,G**). When the expanding cells reached the borders of the patterned areas they grew onto the uncoated areas, too (Fig. **3D,E,F**). The guided growth was best seen in case of linear surface patterns, where cells first grew along the long ECGS-treated lines and then cells spread from these lines onto the ECGS-free areas (Fig. **3G**).

Collagen from calf skin and Na-alginate also appeared to promote specific guided growth of astrocytes, neuroectodermal stem cells and SH-SY5Y neuroblastoma cells. Astroglial cells showed a less vigorous growth than human neuroblastoma cells. The neuroectodermal cells were least actively growing in the investigated period of time. Astroglial cells were strictly confined to the patterned surface provided large enough collagen- or alginate-treated areas were available for the cells (Figs. **4A-D, G,H**). If astroglial cells were plated onto a collagen-treated surface where the area was relatively small, they quickly overgrew the coated surface (Figs. **4C,D**). However, in case of alginate surface patterns the astroglial cells rather migrated along the coated lines and showed less extensive growth and migration characteristics (Figs. **4G,H**). Neuroblastoma cells grew so quickly that larger collagen- or alginate-treated areas were completely populated within 24 hours (Fig. **4E**). Guided growth of cells could also be seen in this case, as growth from the edges of the plated cells was always growth-directed on both substances (Fig. **4F**).

In cases, when neuroblastoma cells and astrocytes were plated to neighbouring pattern fields interesting signs of competition for space between the two cell lines were observed. Neuroblastoma cells showed a nearly uncontrolled growth on collagen or alginate surfaces until they reached

the areas already populated by astroglial cells. Neuroblastoma cells were not able to invade the territories occupied by astroglial cells and tended to grow on the free glass surface between the coated stripes (Fig. **5A**). In other cases the rapidly proliferating neuroblastoma cells approached the astroglia cell lines adhered to the the edges of coated surface and their further growth appeared to be prevented by a glial barrier-like structure (Fig. **5B**). In this case the glial cells took up a palisade-like arrangement thus preventing the growth of neuroblastoma cells.

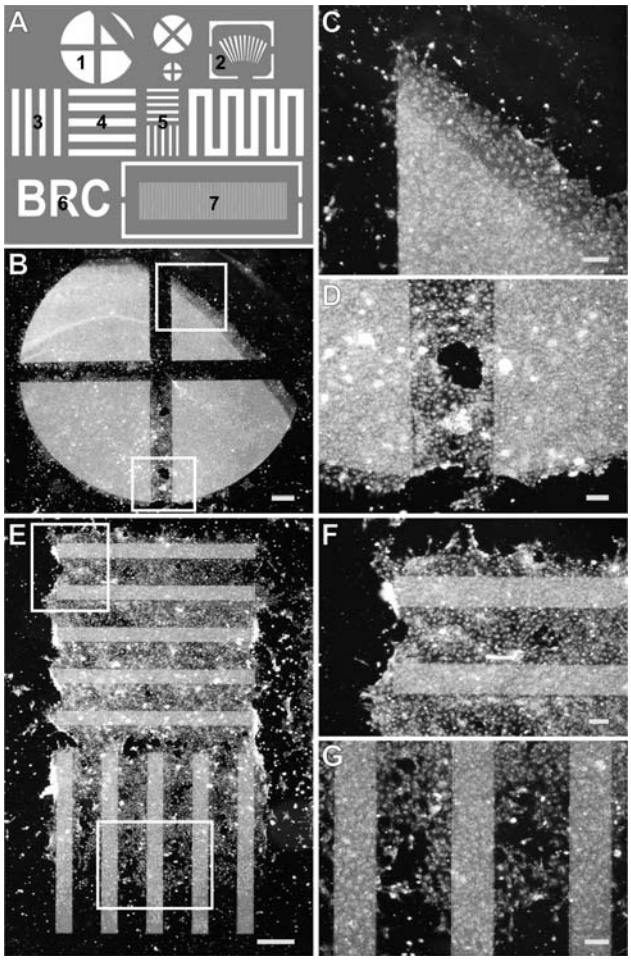

Fig. (3). Growth of pig endothelial cells on endothelial cell growth supplement thin layer pattern induced by the PLD technique. **A** shows the native pattern before loading the cultured cells onto the treated surface. The various pattern areas are numbered in order to identify them in the figures where the growing cells are shown. In **B-D** the area No.1 is shown with attached and intensively growing endothelial cells (reflected illumination microscopy). Boxed areas in **B** show the areas displayed at higher magnification in **C** and **D**. Note that cell growth respected the edges of the patterned areas except where density of the cells was so high that the cells populated the free silicon surface (**D**). Similar growth of cells was observed on linear parallel patterns (**E-G**). The boxed areas in **E** (area No. 5) show two fields populated by higher and lower density of cells (**F** and **G**, respectively). It can be assumed that the cells first attach to the patterned surfaces and then form bridges on the untreated surface between the lines (**G**). The cells do not tend to grow outside the patterned areas. Scale bar in B and E = 200 μm, in C,D,F,G = 50 μm.

On fibronectin-coated surfaces no directed growth of neuroblastoma and glial cells was observed, as the vigorously growing cells did not adhere to the fibronectin-coated surfaces only, however, a similar palisade-like arrangement of the astroglial cells was seen (Fig. **5C**). At the neuroblastoma-astroglia interface the cultured astrocytes formed a dense line facing the edge of very densely growing neuroblastoma cells (Fig. **5C**).

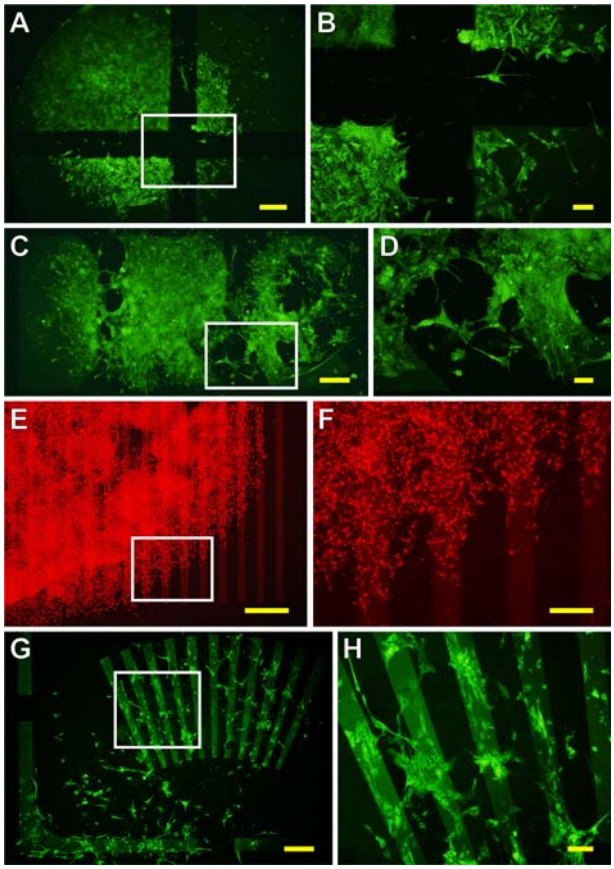

Fig. (4). Attachment and guided growth of astroglial and neuroblastoma cells on collagen and alginate patterns. In **A-D** cultured astroglial cells are shown attached to the collagen thin layer deposited by the PLD method. The growing astrocytes strictly follow the borders when density of cells is not too high (area No.1; **A-B**, boxed area is shown in **B**), while overgrowing astrocytes rapidly form dense cellular bridges between the deposited structures (area No. 6; **C-D**, boxed area is shown in **D**). Similar directed growth of astrocytes was observed on Na-alginate-coated surface (area No. 2; **G-H**, boxed area is shown in **H**). Compare the irregular attachment of astrocytes in **G** (uncoated surface) with the guided growth seen on the coated surface. In contrast, the very rapid proliferation and growth of neuroblastoma cells can be seen only at the free borders of the cells where they tend to follow the alginate stripes (area No. 7; **E-F**, boxed area is shown in **F**). Scale bars: In **A,C,E** and **G** = 200 μm, in **B,D,F** and **H** = 50 μm).

No regular or directed growth was observed when the above cell types were plated onto starch-coated surface patterns (Fig. **5D**).

Guided Cell Growth on AFA-LIFT Treated Glass Surfaces

Glass surfaces which received collagen, serum, Na-alginate and ECGS spots transferred by the AFA-LIFT technique induced different behaviour of the cells than PLD-treated surfaces. Collagen and alginate spots did not induce

directed growth of astroglial or neuroectodermal stem cells *i.e*. the cells grew to an equal extent on uncoated and coated surfaces (Figs. **6A-C**; not shown for alginate). In contrast, these cells grew around the serum spots but did not enter the spots (Figs. **6C** and **D**; not shown for astroglial cells). Only ECGS spots induced guided growth of endothelial cells, the rapidly proliferating cells started to differentiate when they reached the edge of the spot and entered it in a pattern where most of the invading cells grew towards the centre of the spot along their longitudinal axis (Fig. **6E**).

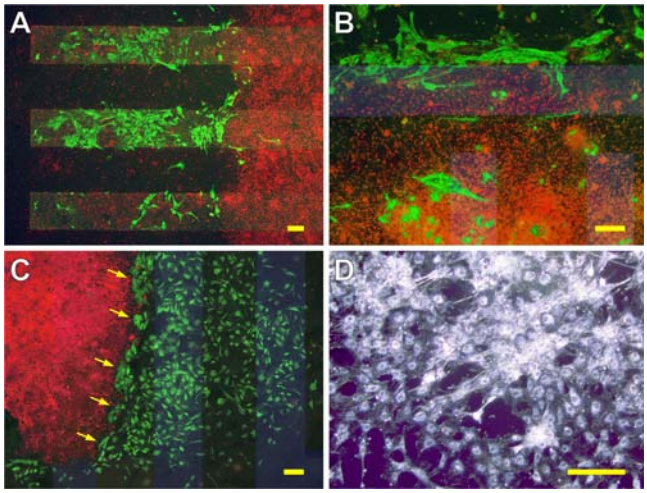

Fig. (5). Interaction between plated neuroblastoma and glial cells. In **A** and **B** regularly growing astrocytes (green, area No. 4) and neuroblastoma cells (red) are guided by a Na-alginate thin layer pattern. Expansively growing neuroblastoma cells approached the astroglial cells and their growth appeared to be blocked by the glial cells. This effect is well seen in **B** where the astroglial cells seem to be attached to the edge of the Na-alginate thin layer where they prevent further expansion of neuroblastoma cells. In **C** the interaction of the above cells can be seen on fibronectin-coated surface. Note that the fibronectin coating did not induce specific guidance. The glial cells appear to form a glial barrier around the neuroblastoma cells (arrows). **D** shows the irregular growth of astroglial cells on starch-coated surface. Scale bars = 100 μm.

DISCUSSION

In the present study we have shown that various biomaterials transferred by PLD or AFA-LIFT methods onto silicon plates successfully guide or rarely prevent the growth of different cell types. Moreover, various forms of interaction between the competing cell types and between the substrate and the cells were observed in some cases. These findings suggest that the above methods are suitable to build an architecture of substrates which support and guide the growth of cells. The guided growth may enable the cells to induce directed and rapid repair of injured tissues.

At present there are two basic streams of laser-induced tissue repair strategies. One way is to alter the morphological or chemical surface characteristics of a given substrate [26-32] and then to apply living cells onto this surface (cell patterning), the other way is the controlled transfer of living cells onto the surface in a given pattern [21, 33-35]. Restructuring the chemical and topographic features of the surface enables selective placing, growth, localization, phenotypic and genotypic control of living cells. In the present study we used the above mentioned laser-based methods to transfer growth promoting biomaterials onto biologically inert surfaces. Although both PLD and AFA-LIFT can be used for the transfer of biomaterials the effects achieved by the two techniques cannot be directly compared because of their different mechanisms of action.

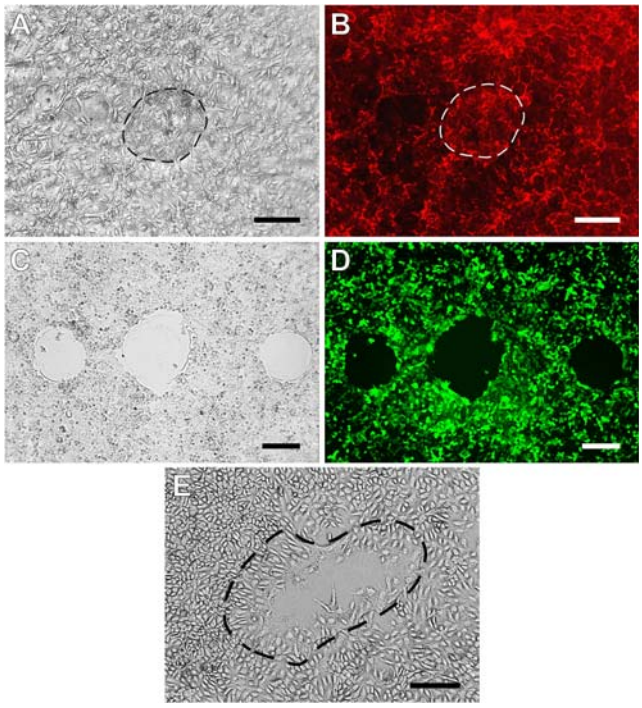

Fig. (6). Growth of cells on AFA-LIFT-treated surfaces. **A** and **B** show identical fields of astroglial cells plated on glass surface wich received small spots of collagen transferred by the AFA-LIFT method (broken lines indicate the borders of the spots). There is no guided or specific cell growth seen on the collagen spots (**A**: incident light illumination, **B**: Texas-Red immunofluorescence). In **C** and **D** neuroectodermal stem cells are shown as they attach to the untreated glass surface but do not grow onto the transferred serum spots (**C**: incident light illumination, **D**: FITC immunofluorescence). **E**: Growing endothelial cells reach the border of ECGS-coated spot (broken line) where they show signs of directed growth. Scale bars = 100 μm.

In the first set of our experiments we used pulsed laser deposition procedure to produce patterns of biological thin films and then plate living cells onto these coated areas. The advantages of this method are that using a direct mask on the substrate surface the parameters and the shapes of the required structures can be easily designed. The necessary cell growth-promoting materials are deposited directly without any supplementing materials (*i.e.* chemical or biological solvents) but the low concentration of the material requires the use of a vehicle. Moreover, PLD needs no chemical, mechanical or direct laser etching to form the required patterns. The thickness of the deposited layers can be controlled by the number of applied laser pulses with a submicrometer resolution up to an area of several cm^2. Cultured cells adhere to this surface without application of inkjet head, electromagnetic field, transferring laser beam, etc. and display highly oriented and facilitated growth. The plated cells do not suffer from any mechanical, photochemical or thermal damaging effects during the procedure. A disadvantage of this system is that the material deposition procedure requires

vacuum and femtosecond laser systems. Our experiments have clearly shown that some substances patterned by the PLD method onto the substrate induced a general growth-promoting and guiding effect on several cell types. Surprisingly, fibronectin proved to be so effective that the pattern was rapidly overgrown by the cells. This effect may be explained by the good solubility of fibronectin and its diffusion into the surrounding areas. In the case of specific substances, such as endothelial cell growth supplement controlled growth of endothelial cells was readily achieved. A very interesting growth-limiting interaction of glial cells on neuroblastoma cells was observed. This effect may be a) due to the well-known barrier function of astroglial cells in the intact and injured central nervous system and b) to the short term *in vitro* growth-inhibitory effect of astroglial cells on SH-SY5Y neuroblastoma cells. This latter view is supported by recent findings [36]. It is suggested that this *in vitro* barrier effect of the glial cells may be useful in controlling the expansive and uncontrolled growth of neuroblastoma cells.

In the rest of our experiments we used the AFA-LIFT technique to produce a growth-promoting matrix. The advantage of this method is its relative simplicity without using a vacuum system. This method works in a "non-contact" mode and is able to produce heterogenous patterns using different cell sources with lateral resolution in the micrometer range. The disadvantage of the technique is that the final concentration and thickness of transferred substances are more difficult to control than with PLD. We successfully transferred Na-alginate, endothelial cell growth supplement, rat serum and collagen from calf skin. Na-alginate and collagen induced an overall and non-patterned growth of the investigated cells while endothelial cell growth supplement produced a directed growth and arrangement of endothelial cells. On the contrary, undiluted rat serum, normally used for increasing the adherence of certain cell types, prevented the adherence and growth of astroglial and neuroectodermal stem cells. It can be argued that molecules transferred by these two methods induce slightly different results because of the way of deposition (thin film in case of PLD vs thicker spots for AFA-LIFT) and final concentration (controlled vs relative high final dry amount). We estimated that the amount of bioactive materials/μm^2 in the dried AFA-LIFT droplets was approx. 1000 times higher than that of in the PLD-induced thin layers computed according to the different initial concentration and the deposited thickness.

Based on the mode of obtaining the patterned surface, Pulsed Laser Deposition could be a competitor of the soft lithography, while the AFA-LIFT can be regarded as the laser-based equivalent of the microcontact printing.

These results may possibly be used in several biotechnological applications. One approach is to build up a given human tissue or tissues from cells in a system, where cellular growth was controlled and guided during the laser-based tissue engineering process. The other application is to repair injured tissues by specific coating and thus inducing guided growth of cells either from host or external sources. This latter could include epithelial, acute and chronic wound repair, ulcer healing, etc. The advantage of this approach may be that host cells could rapidly repopulate the injured areas coated with the appropriate cell growth-promoting substance and no use of cell transplantation is needed. However, at present neither the PLD nor the AFA-LIFT method is suitable for tissue engineering in clinical use but further development of these technologies may bring closer the future use of these promising applications.

A further application field could be the use of these techniques to produce cell based biosensors. In these biosensors physiological changes of living cells are monitored while the cells are subject to various stimuli [37]. There are many kinds of cell-based biosensors with different principles of operation and applications [38-41]. Getting more information about the multi-functional cellular processing of the detectable input- and output-signals in different cellular plants is essential for various biomedical applications. The methods presented in this study provide the opportunity to build simple or multifunctional cell-based biosensors, as precise localization of various cell types onto a substrate surface has been made possible.

CONCLUSIONS

We can conclude that biomaterials transferred by PLD or AFA-LIFT methods onto silicon plates successfully guide or occasionally prevent the growth of different cell types. Moreover, interactions between the competing cell types and between the substrate and the cells were observed in some cases. These findings suggest that the above methods are suitable to build an architecture of substrates which supports and directs the growth of cells. The guided growth may enable the cells to induce directed and rapid repair of injured tissues.

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