Cell Patterning

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Laser-Induced Cell Detachment and Patterning with Photodegradable Polymer Substrates**

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Degradable polymers are constantly being developed and are proposed for numerous uses in the biotechnological arena, for example, in the fields of drug delivery and tissue engineering as carriers for sustained or stimulus-mediated drug release and as scaffolds for tissue culture. Despite the many examples of materials that exhibit degradation upon exposure to (bio)chemical stimuli (i.e. a pH value, enzymes, etc.),[1] photodegradable polymers that are addressable in the biomedical context remain limited, mainly because of their very slow degradation rates and the high energy required for complete photodegradation. So far, research toward photosensitive polymers has been focused on shape-changing polymer actuators, polymers with pendant photolabile segments, and photodegradable polymer networks.[2] Surprisingly, the number of studies on polymers that exhibit fast and complete photodegradation in a backbone-breakdown manner at low irradiation energies is extremely limited. The development of polymers or other types of materials that degrade upon exposure to a light stimulus would be highly desirable for a number of applications in nanomedicine and biofabrication, such as drug delivery activated by exogenous stimuli[3] and the handling/manipulation of precious biological samples at the microscale (i.e. cell sorting,[4] on-chip patterning,[5] light-directed cell migration,[2d,6] etc.). Furthermore, photodegradable materials that undergo cell-compatible degradation could substantially improve existing laser-assisted cell-writing techniques (i.e. matrix-assisted pulsed laser evaporation, direct writing, laser-induced forward transfer, etc.), as the use of high-energy laser pulses and non-degradable materials severely affects the viability/functionality of cells and biomolecules.[7] However, to the best of our knowledge, a generic platform of materials that can be used in the biomedical field as photodegradable substrates has not been reported.

We set three important requirements as key points for the design of photodegradable polymers of practical biomedical interest: 1) ease of synthesis and processing to enable chemical versatility and use in a wide range of applications, 2) rapid photodegradation profiles at low irradiation energies to eliminate phototoxic events, and 3) low cytotoxicity of the initial polymer and the degradation products to enable their use as cell-culture substrates. Polyketals and polyacetals have already been used as pH-degradable polymers[8] that exhibit hydrolytic degradation under mildly acidic conditions. They have found application in targeted cancer therapy and controlled protein delivery. Herein, we report the photochemical degradation of polyketal and polyacetal polymers at low irradiation energies and their application as photodegradable substrates for laser-mediated cell harvesting and patterning in a process that rivals classic enzyme-mediated cell-detachment/sorting methods. Laser cell or tissue patterning/ablation techniques with the proposed polymers could potentially constitute an elegant means of accurately controlling the spatial arrangement of distinct cell populations on scaffolds for tissue regeneration or on living tissues (i.e. for wound healing, corneal repair, etc.) and the fabrication of highly ordered extracellular-matrix mimics.

We synthesized two model polymers comprising ketal (P1) or acetal (P2) repeat units as their main chain and characterized them by gel permeation chromatography (GPC) as well as UV/Vis and FTIR spectroscopy (see the Supporting Information). In initial photodegradation studies with an Hg–Xe exposure tool, we observed effective degradation of the P1 film at low doses (5.5 mJ cm^{-2}, 248 nm) and a decrease in the thickness of the film upon development with water (see Figure S1). In the case of P2, exposure under the same conditions did not result in a decrease in film thickness at comparable doses. Nevertheless, samples of both P1 and P2 that were exposed to higher irradiation doses exhibited a gradual increase in their absorbance at 248 nm, which suggested the possible formation of a carbonyl product (see Figure S2). Furthermore, FTIR monitoring of the exposed polymer samples revealed characteristic carbonyl and broad hydroxy peaks at 1723 and 3470 cm^{-1}, respectively. These peaks were attributed to the photodegradation products (see Figure S3). These studies support the proposed photolysis mechanism,[9] which involves the formation of zwitterion intermediates and their subsequent transformation into carbonyl and hydroxy products. This mechanism leads to complete polymer photolysis (Scheme 1). Finally, complete

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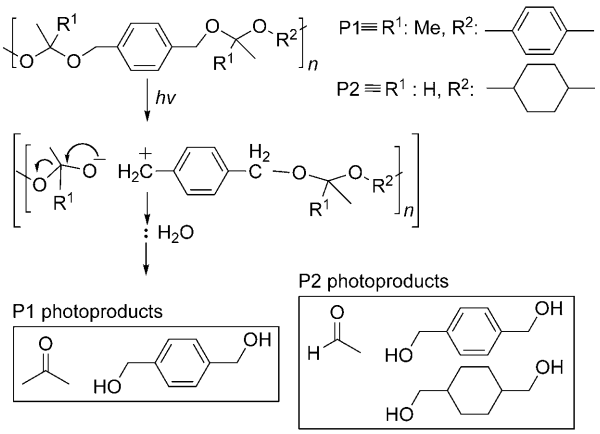

Scheme 1. Chemical structure of P1 and P2 and the proposed photodegradation mechanism.

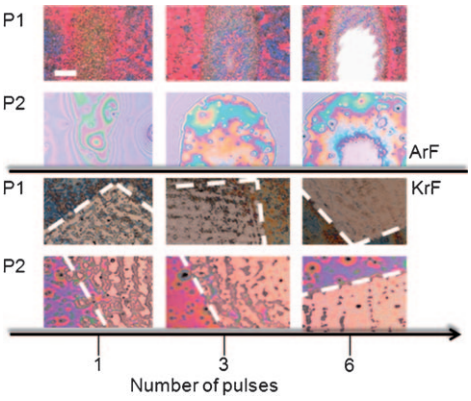

Figure 1. Progression of laser ablation as captured by optical microscopy with ArF (193 nm, 10 mJ cm^{-2} per pulse) and KrF (248 nm, 5 mJ cm^{-2} per pulse) laser sources. Dashed lines denote the boundaries of the ablated areas; scale bar: 500 μm.

main-chain scission was verified by GPC analysis of the irradiated polymer films (see Figure S4).

Encouraged by these results, we examined the photolysis rates of the polymers on the basis of laser-induced fluorescence (LIF) spectroscopy (see the Supporting Information). The ablation process was studied at 248 and 193 nm, at which wavelengths the polymers exhibit moderate and high absorption, respectively. Since the polymers fluoresce strongly when excited at 193 nm, we could directly monitor the reduction in film thickness as a result of the ablation process; however, direct monitoring was not possible for the ablation process at 248 nm. We therefore doped the polymers with iodonaphthalene (PhenI), which fluoresces strongly at 340 nm when excited at 248 nm, and used it as a reporting molecule to examine the ablation process at this wavelength.[10] Gradual material loss occurred in the ablated area as the pulse number increased, which resulted in a gradual decrease in the fluorescence recorded by LIF spectroscopy (see Figure S6). Both polymer samples were ablated by irradiation with pulses of very low energy (5 mJ cm^{-2} per pulse) at 248 nm, and a gradual decrease in the PhenI fluorescence was observed as the irradiation dose was increased (see Figure S6). Nearly complete film ablation was observed at a total fluence of approximately 25 mJ cm^{-2} for both polymers. It was also observed that irradiation with a KrF laser resulted in uneven and inhomogeneous material loss from the ablated areas, which led to residual polymer islands that were eventually removed as the ablation process progressed (Figure 1). We attributed this effect to cross-scattering of the laser beam owing to the poor absorption of the polymer at 248 nm and to inhomogeneous distribution of the energy across the laser spot.

Material loss progressed more smoothly when the ablation was carried out with an ArF laser owing to the strong absorption of the polymers at 193 nm. In this case, however, a higher energy per pulse (10 mJ cm^{-2} per pulse) was required for complete ablation (40 and 50 mJ cm^{-2} total fluence for P1 and P2, respectively; see Figure S6), as it is well-established that the higher the absorption of the target, the higher the energy required for efficient ablation.[11] We observed that material loss started from the center of the ablated area and progressed towards the edge of the sample as a function of the number of pulses, as expected from the Gaussian distribution of the energy of the particular laser beam across the focused area (Figure 1). The polymer films "feel" a higher dose in the center of the ablated area than at the edge. As a result, craterlike patterns formed.

As already mentioned, the ablation process at 193 nm takes place in a layer-by-layer manner, which significantly eliminates the nonspecific scattering of non-absorbed photons; this nonspecific scattering is observed to a much greater extent in the ablation process at 248 nm. Since the ablation process occurs at remarkably low energies, it is likely that photothermal and photomechanical mechanisms of material loss will have a negligible contribution to the ablation process. Therefore, photochemical degradation may be the dominant mechanism of the photolysis process. This particular photodegradation mechanism does not involve the production of free-radical intermediates that could cause severe oxidative stress to cells. Finally, since the majority of the photoproducts formed are water-soluble as a result of their low molecular weight and the rich carbonyl- and/or hydroxy-group content, they can be removed readily from the cell suspension by standard cell-centrifugation methods. Hence, the mild photodegradation conditions make P1 and P2 excellent polymer substrates for applications in laser-induced cell detachment and patterning.

We envisaged that the polymers could be used as photodegradable substrates to culture mouse fibroblasts, and that a mild laser ablation process could be used not only to harvest the cells from the substrate but also to grow patterns of cells directly on the cell-culture substrates in a postculture manner. We chose the ArF excimer laser with a 25 ns pulse duration at 193 nm because at this wavelength the polymer-ablation process takes place in a layer-by-layer manner, and the direct exposure of the cells to the laser beam is virtually eliminated owing to cross-scattering. Also, in the case of accidental direct exposure of the cells to the laser beam, the photons will be primarily absorbed by the protein-rich cytoplasm instead of by the cell nucleus; thus, potential light-induced DNA mutations can be completely avoided (proteins exhibit

stronger absorption at 193 nm than at 248 nm).[12] Furthermore, the use of short-pulse excimer lasers eliminates potentially harmful heat transfer from the substrate to the cells. Finally, ArF excimer lasers have already been used widely in the biomedical field, for example, in laser-assisted in situ keratomileusis,[13] angioplasty,[14] and tissue ablation.[15]

Cells were found to firmly adhere to both polymer substrates in similar manner to the adhesion of cells to tissue-culture polystyrene, without noticeable morphological discrepancies. We carried out laser cell-detachment experiments by a novel process comprising three consecutive steps, namely, cell seeding and growth onto the polymer substrate, direct laser photolysis of the polymer film, and cell harvesting and patterning (Figure 2).[16] The detachment experiments took place in a chamber that was made in-house (see

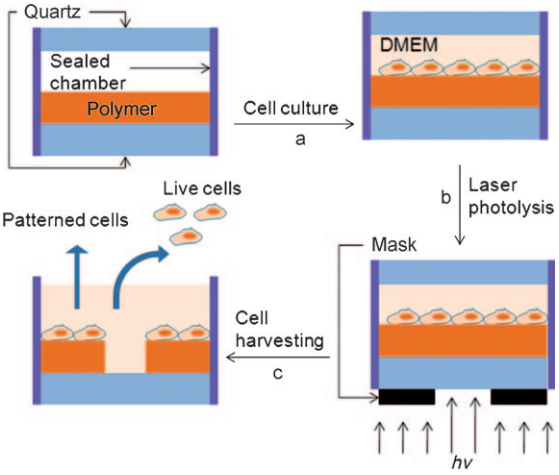

Figure 2. Proposed process for cell patterning based on laser-induced cell detachment from photodegradable substrates by a) cell seeding and growth, b) laser photolysis of the polymer substrate either directly or through a photomask, and c) simultaneous on-demand cell harvesting and patterning of the cultured cell sheet. DMEM = Dulbecco modified Eagle medium.

Figure S8), either by the direct ablation of large areas of the cell sheet (Figure 3a) or through the use of a photomask (Figure 3b, see also the Supporting Information). The energy dose used for polymer ablation was well below the cytotoxic light-dose threshold for UV radiation.[12,17] In the photodetachment process, the cells were removed as a result of ablation. The application of the mask enabled the formation of cell-free patterns directly on the preformed fibroblast sheet (Figure 3b). It was also possible to precisely control the number of ablated cells simply by varying the size of the ablation area for each detachment experiment. A linear correlation with acceptable accuracy was found between the number of detached cells and the corresponding ablated areas, although the accuracy was somewhat hampered by unavoidable inhomogeneities of the cell sheet (Figure 3c). Furthermore, the detached cells were found to be alive within the supernatant liquid, at comparable ratios to cells detached by trypsin-mediated detachment (Figure 3d). However, our technique is advantageous over classic trypsin-mediated or

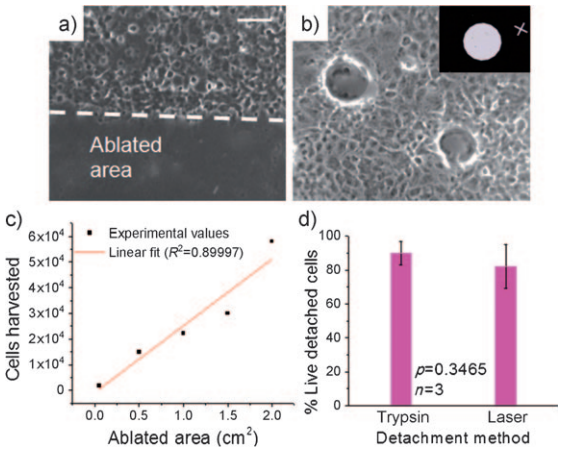

Figure 3. Optical microscopy images of a) a large cell-free P1[16] ablated area (dashed line denotes the boundary of the ablated area; scale bar: 20 μm; total ablation dose: 50 mJ cm^{-2}) and b) circular patterns formed on the cell sheet by the application of a photomask (part of the photomask is shown at the top right). c) Linear correlation of the number of harvested cells with the size of the ablated area. d) Viability rates of cells detached by treatment with a laser or trypsin. See also the Supporting Information.

even thermoresponsive[18] detachment, in which all cells in a flask are subjected to harvesting, because it enables more precise handling of specific cell populations. Furthermore, to the best of our knowledge, direct patterning on preformed cell sheets in a postculture manner has not been demonstrated previously. Therefore, our method substantially expands the repertoire of existing cell-patterning methods for use on prefabricated patterned surfaces.[19]

In conclusion, we have shown for the first time the photodegradation properties of novel polyketals and polyacetals with fast laser sources. Photochemical degradation was found to take place at very low energies, without the generation of free-radical intermediates, and led to low-molecular-weight by-products. Also, we demonstrated the principle of mild photolysis of photodegradable polymer substrates at remarkably low energies. This method enabled the precise and mild control of cell harvesting as well as cell-sheet patterning by postculture laser ablation. We anticipate that the polymers (and their photoproducts) presented herein can be generally regarded as safe (GRAS) materials for a variety of applications in the biomedical field, as they exhibit very low, nontoxic photolysis rates and acceptable biocompatibility in vitro. We are currently exploring the possibility of red-shifting the laser wavelength to the visible region by using ultrafast laser sources for two- or three-photon photoablation or by shifting the polymer absorption to the visible range of the spectrum.

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