Adhesion of U-937 Monocytes on Amine-functionalised Parylene and Plasma Polymer Surfaces

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Abstract: Human U-937 monocytes are notoriously reluctant to adhere to normally cell-adhering surfaces, for example tissue-culture poly(styrene), TCPS. In earlier work, these laboratories observed that organic thin films prepared by plasma- or ultraviolet- assisted polymerisation, so-called PVP:N, did facilitate the adhesion and proliferation of U-937 under the condition that the concentration of primary amines exceed a critical value, $[NH_2]_{crit} \geq 4.2$ at.%. That criterion being satisfied by pristine Parylene diX AM, we have compared its performance with those of two particular types of PVP:N, L-PPE:N and UV-PE:N. Here, we report a study of aging of all these coating types in atmospheric air, then of time-dependent adhesion of U-937 cells, and of gene expression when possible. Although there are similarities, the coatings also manifest interesting differences that so far elude detailed understanding.

Keywords: Plasma- and UV polymers, Cell adhesion, Primary amines

1. Introduction

Novel applications of biotechnologies manifest an ever-increasing need for surfaces that permit control over a variety of cell functions, and that can provide solutions to particular challenges.[1] A good example of such a challenge is the *in vitro* culture of monocytes/macrophages, for example human U-937 monocytes.[2] The function of monocytes in the immune system is to replenish resident macrophages in their normal state, and in response to inflammation signals, in which case they can move quickly to sites of infection in the tissues and differentiate into macrophages in order to elicit an immune response. When these cells act in vivo, they adhere to tissues, but analogous cell lines tend not to reproduce this behavior on cell culture dishes, typically poly(styrene) (PS) that has been surface-modified by gas-plasma treatment. Thus, the extent to which monocyte cell lines can be used for *in vitro* studies is severely limited. One possible solution involves engineered surfaces that permit greater control of the cell environment, for example by directing cells to mimic *in vivo* behavior. In the case of monocytes, this can open the way to numerous important types of investigations.[2] We showed in a recent article [3] that these cells could be made to adhere and proliferate on PVP:N surfaces (for "Plasma- or VUV-Polymerized N-rich" materials) if at least one "simple" condition was met, namely that the surface-near concentration of primary amines, $[NH_2]$, exceed a certain critical value, $[NH_2]_{crit}$, 4.2 ± 0.5 (per 100 atoms measured by XPS, at.%). In a second part of that same article, we showed that the adhesion of U-937 monocytes to PVP:N surfaces induces a transient expression of cytokines, markers

of macrophage activation, as well as a sustained expression of PPARγ and ICAM-I, implicated in the adhesion and retention of monocytes.

Now, PVP:N films are not the only known amine-rich organic coatings candidates; a such material, one that is commercially available, is aminomethyl-[2-2]paracyclophane [4] (Parylene "diX AM", Kisco Conformal Coating LLC). Parylene, a diradical, can be deposited as thin coatings by sublimation and condensation under vacuum. The calculated value of N/C for diX AM, 6.3%, more than adequately satisfies the criterion for $[NH_2]_{crit}$ shown above, because 100% of the nitrogen on the precursor exists in the form of primary amine. The objective of the work reported here has been to compare the characteristics and performance of Parylene diX AM, with typical PVP:N coatings, N-doped "polymerized" ethylene deposited in a low-pressure radio-frequency glow discharge (L-PPE:N[3]), or by vacuum-ultraviolet photochemistry (UV-PE:N[3]). As was reported by Girard-Lauriault et al. [3], the latter two coating types can readily and reproducibly be prepared under conditions that lead to $[NH_2]_{crit} \geq$ 4.2 at.%, so a specific objective has been to compare the adhesion of U-937 cells on the Parylene, L-PPE:N and UV-PE:N surfaces under otherwise identical conditions, with the aim to shed further light on cell-adhesion mechanisms reported in [3], if possible. Knowing that all these film types are subject to chemical aging when exposed to ambient air,[5] gradual reduction of $[NH_2]$ with increasing storage time, a sub-objective has also been to investigate this aging phenomenon and its possible repercussions on the adhesion of U-937 cells. A final sub-objective has been to investigate whether gene-expression for U937 cells adhering to UV-PE:N is the same as the one we reported for the case of the structurally quite distinct L-PPE:N.[9]

2. Experimental Part

All coating types, Parylene diX AM, L-PPE:N, and UV-PE:N, were deposited on thin polymer substrates; in the first two cases this was biaxially-oriented polypropylene (BOPP), in the latter polyimide (DuPont Kapton(R)). Parylene coatings were prepared at the Fraunhofer Institute ("FhIGB") in Stuttgart, the latter two at École Polytechnique in Montreal. In order to carry out the above-mentioned aging experiments, the materials were stored in laboratory air in the dark, and samples were retrieved frequently (in some instances, on a daily basis, see Results section). Following this, they underwent surface analysis by X-ray photoelectron spectroscopy (XPS) before and after chemical derivatisation with TFBA (trifluoromethyl-benzaldehyde). [3]

Non-adherent human U-937 monocytes (ATCC, Rockville, MD) were expanded in suspension in Dulbecco's modified eagle medium (DMEM) high glucose supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were counted and 200 μl of cell suspension at 5 x 10^5 cells/ml were carefully pipetted onto 1 cm^2 samples of Parylene and at 5 x 10^6 cells/ml on 1 cm^2 samples of UV-PE:N films, which had previously been placed face-up on the flat bottoms of wells in 24-well cell-culture plates. Because this had already been done earlier for the case of L-PPE:N,[3] it was not repeated here. Cells were then incubated in humidified 5% CO_2 environment at 37°C for 1h before careful removal of the medium. We had previously demonstrated that the maximal adhesion of U-937 cells to PVP:N surfaces was reached within 1h,[3] and showed that the critical primary amine concentrations ($[NH_2]_{crit}$ = 4.2 ± 0.5 at.%) was necessary for U-937 cell adhesion under these conditions.[3] Non-adherent cells were then removed by gentle pipetting and fresh medium was pipetted into the wells (1 ml in each well) and again carefully removed to wash the cells. This washing procedure was repeated twice. Finally, optical photomicrographs of the surfaces were taken (time = 1h), and cells were incubated for a total of 24h before additional imaging.

3. Results

Figure 1A presents the results of $[NH_2]/[C]$ measurements on Parylene. Also shown, for each sample (i.e. same aging time) is the corresponding

value of [O]/[C], the oxygen concentration at the surface after the same period of aging (storage in ambient air, in the dark). In Figure 1B and 1C, we show $[NH_2]/[C]$ and [O]/[C] data for UV-PE:N samples corresponding to two different *R* values (*R* being the mixture ratio of ammonia/ethylene reagent gas flows [3,5]). In the cases of both (B) and (C), the results pertain to several nominally-identical fabrication runs with $R = 0.75$, and $R = 1.0$, respectively. The data show good reproducibility and relatively slight amounts of scatter. Contrary to Parylene diX AM and UV-PE:N (Figures 1A to 1C), we do not show equivalent data for L-PPE:N here, because those have recently been presented elsewhere,[5] also for different values of *R*. Those data, however, displayed considerably more scatter. In other words, L-PPE:N is apparently a less "well-behaved" (less reproducible) material than UV-PE:N.

Parylene is an homogeneous polymer coating; the "disappearance" of each primary amine group is accompanied by the uptake of exactly one oxygen atom in the film's chemical structure (within experimental precision of the available data). This statement is justified by the identical first-order kinetics of amine decay and oxygen take-up, with very similar time constants. A plausible reaction scheme for the observed aging is the conversion of the reactive primary amines into an oxygen-bearing functionality, for example an amide group.[5]

Figure 2 presents micrographs corresponding to a series of surfaces after 1h of culture of U-937 cells under the conditions described in the Experimental section above. Indeed, Figure 2A shows that U-937 cells adhered to the "pristine" surface ($[NH_2] \sim 6$ at.%). However, Figures 2B and 2C, corresponding to Parylene diX AM surfaces that had been aged for 6 and 10 days ($[NH_2] \sim 3.5$ at.% and ~ 2.5 at.%, respectively) both show significantly reduced numbers of adhering cells, meaning that cell adhesion decreases as $[NH_2]$ decreased. Figure 2D portrays a regular TCPS dish as a negative control, and it shows virtually no adhesion of U-937 cells. Even though there is still some adhesion when $[NH_2]$ is under $[NH_2]_{crit}$, this very graphically confirms our previous conclusions regarding the critical role of primary amine groups in the interaction of U-937 cells with bioactive surfaces and it suggests that Parylene diX AM acts in comparable manner to that of PVP:N coatings.

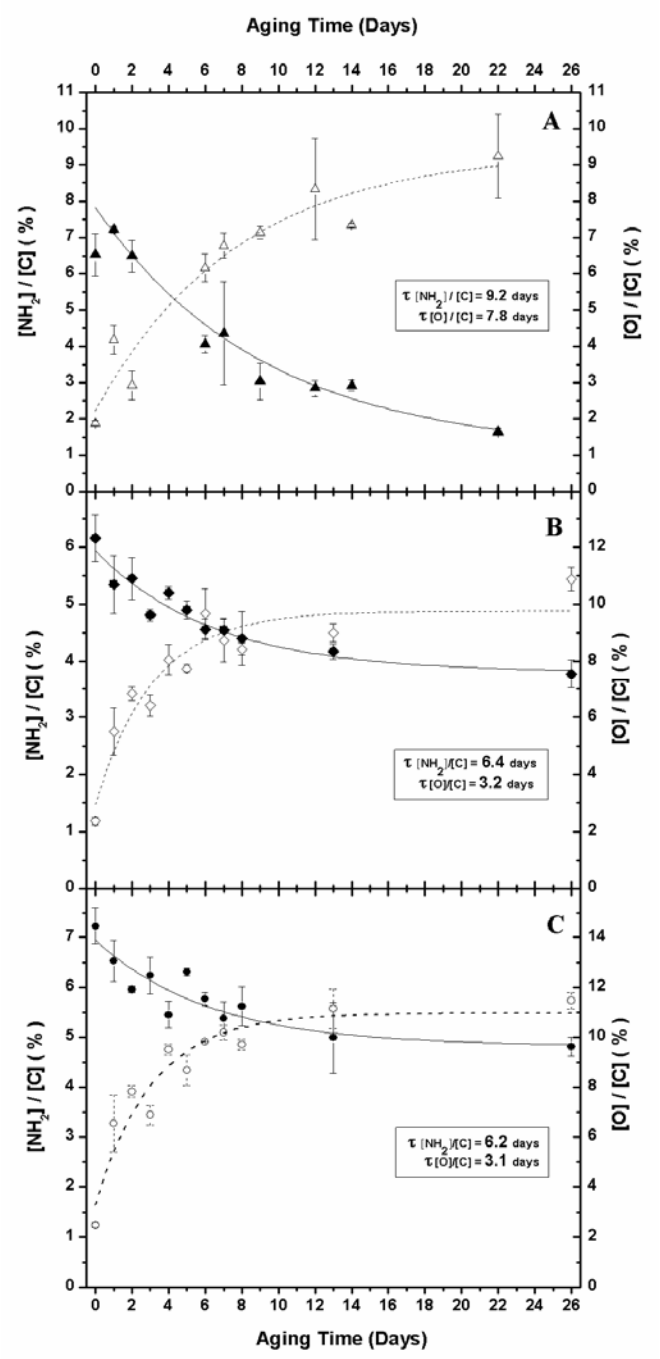

Figure 1. *Time-evolutions of the compositions of: A) Parylene diX AM; B) UV-PE:N (R = 0.75); and C) UV-PE:N (R = 1); as a function of the duration of storage in laboratory atmosphere in the dark. Open symbols represent [O]/[C].*

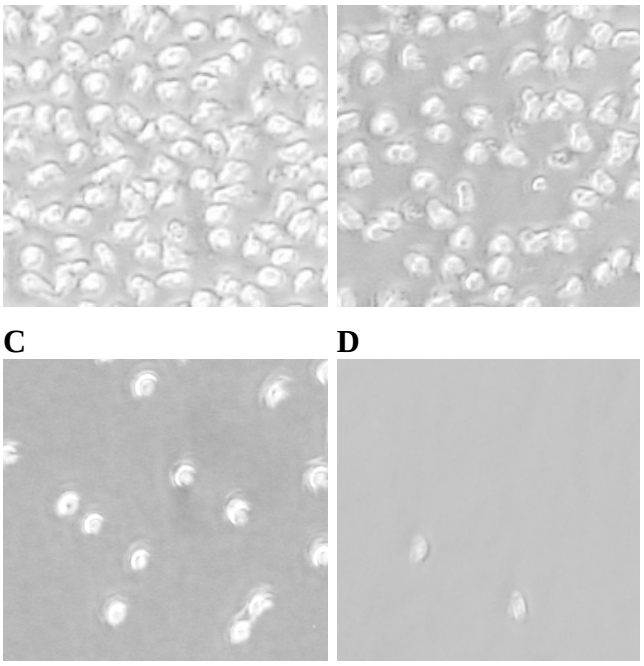

Figure 2. *Decrease in the adhesion of U-937 cells on Parylene surfaces with a decrease in [NH_2] after aging. U-937 cells were applied on 1 cm^2 of Parylene surfaces and incubated for 1h in a CO_2 incubator at 37ºC, on the pristine surface (A – ca. 6.0 at.% [NH_2]), after 6 days (B – ca. 3.5 at.% [NH_2]), and 10 days (C – ca. 2.5 at.% [NH_2]) of aging, resulting from exposure to atmospheric air in the dark. Surfaces were then gently washed 3 times with DMEM medium and pictures were taken (400X magnification). Cells on regular polystyrene Petri dish (D) served as negative control. Note the decrease in cell adhesion with decreasing [NH_2] resulting from aging in laboratory air.*

4. Discussion and Conclusions

We note that the measured value of [NH_2]/[C], 6.5 ± 0.6 %, is close to the calculated one, 6.3 %, based on the chemical structure of Parylene diX AM; hence, we are confident in the validity of all arguments based on numerical values of primary amine concentrations in the present as well as in our earlier work, where identical analytical procedures were employed.

Regarding the cell-biological results portrayed in Figure 2, U-937 cells were found to adhere to Parylene diX AM with [NH_2] ≥ [NH_2]$_{crit}$, but not to TCPS. In these regards the present observations are fully in line with those reported by Girard-Lauriault, [3] but there the similarities end:

(i) Those authors found no U-937 adhesion at all below [NH_2]$_{crit}$;
(ii) Cells came to confluence within 1h on any PVP:N surface above [NH_2]$_{crit}$;
(iii) Cells remained adherent and proliferated for more than 48h.

In contrast, Figure 2C ([NH_2] ~ 2.5%) suggests some minor degree of U-937 adhesion after 1h, and substantial detachment of previously adhering cells occurred after 24h.

The above-described differences between Parylene diX AM and all PVP:N family members may possibly be attributable to "synergistic" contributions from other functional groups to mediate interactions between the bioactive surfaces and integrin receptors on the U-937 cell-membranes, because all PVP:N coatings are known to possess a variety of such moieties, contrary to the case of Parylene diX AM, even after aging. However, this is currently speculation and subject to further investigations.

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