MATERIAL SURFACE STRUCTURE INFLUENCES MACROPHAGE MORPHOLOGY AND DEGRADATIVE POTENTIAL

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INTRODUCTION

Monocytes are recruited to the site of an implanted biomedical device, differentiate into macrophages which subsequently fuse to form foreign body giant cells (FBGCs). Monocyte-derived macrophages (MDM) and FBGCs are believed to be the central cellular mediators of the chronic inflammatory response to biomedical material implants and have been shown to remain at the tissue-material interface for the lifetime of the device *in vivo*. Consequently, MDM and FBGC have been implicated in both surface oxidation and hydrolytic degradation leading to device failure.

In the present study, the influence of material surface chemistry on FBGC formation was investigated using an activated MDM cell system in order to relate their formation to cellular degradative potential. Biodegradation of PCNUs synthesized with either 1,6 hexane diisocyanate (HDI) or 4,4'-methylene bisphenyl diisocyanate (MDI), poly(1,6-hexyl 1,2-ethyl carbonate) diol (PCN) and 1,4-butanediol (BD), were compared in terms of FBGC formation and cell spreading of adherent MDM. In addition, protein synthesis was investigated through pulse labeling with ^{35}S-methionine and the secretion of two key hydrolytic enzymes involved in biodegradation (monocyte-specific esterase (MSE) and cholesterol esterase (CE)) were quantified through immunoblotting.

MATERIALS AND METHODS

PCNUs were synthesized with ^{14}C-HDI, PCN and BD or MDI, PCN and ^{14}C-BD in the ratio of HDI/PCN/BD (4:3:1) or MDI/PCN/BD (3:2:1) [referred to as HDI and MDI, respectively) [1]. Both polymers were also synthesized without a radiolabel for experiments not requiring radioactive assays. A polymer dimethylacetamide solution (10%w/v) was coated onto glass coverslips. Human monocytes were isolated from whole blood and underwent differentiation for 14 days on polystyrene (PS), followed by activation via trypsinization and re-seeding on either PS, HDI or MDI for 48h as previously described [2]. Biodegradation of HDI and MDI was assessed and compared at 48h through radiolabel release as measured by scintillation counting. Degradative potential was assessed through an assay for esterase activity in the cell lystate using p-nitrophenylbutyrate (PNB) as a substrate.

At 1 and 48h post re-seeding on PS, HDI and MDI, MDM were fixed for light microscopy and fluorescently labeled with rhodamine phalloidin and 4,6-diamidino-2-phenylindoledihydrochloride hydrate (DAPI) to enable visualization of F-actin and nuclei, respectively. FBGC populations were monitored by simultaneously visualizing the fluorescently labeled nuclei under phase contrast and counting the number of nuclei per cell. The percentage of FBGCs was determined by dividing total cell counts by FBGC counts (cells with > 3 nuclei). Cell spreading was evaluated 1 and 48h post-attachment to the three surfaces by measuring the surface areas of F-actin using the integrated morphometry analysis software, MetaMorph v. 4.01.

Protein synthesis and secretion was assessed after 1, 24 or 48h of cell-material interaction by pulse labeling the adherent MDM with ^{35}S-methionine for 1h and collecting the cell supernatant for analysis. *De novo* secreted protein was separated by electrophoresis and visualized by autoradiography.

Immunoblotting was carried out with antibodies against CE and porcine liver carboxyl esterase which cross reacts with human MSE. Protein bands were visualized through enhanced chemiluminescence and quantified with Quantity One software.

RESULTS

Biodegradation was measured through the amount of radiolabel released from a ^{14}C-labeled surface substrate by activated MDM for 48h. HDI was approximately 2.6-fold more degradable by cells than was MDI (Figure 1). In addition, there was a significant increase in esterase activity, as measured by PNB cleavage, in cells re-seeded on HDI versus MDI or PS (Figure 1). There was no significant difference in esterase activity between MDI and PS. Previously, it was shown that esterolytic activity was the most destructive enzymatic activity towards PCNUs [2] and could be detected in MDM conditioned media [3]. In order to further investigate the effect of different materials on MDM, the formation of FBGCs was assessed on PS, HDI and MDI. The number of nuclei per cell as well as the extent of cell fusion was evaluated at 1 and 48h post-attachment to the material surfaces. Approximately 20% of all cells were multinucleated FBGCs at 1h of cell-material interaction

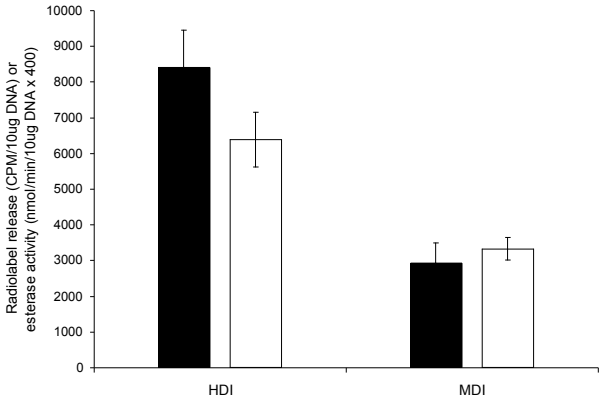

Figure 1 - Radiolabel release and esterase activity from MDM 48 hours post-attachment to either HDI or MDI. Monocytes were cultured on PS for 14 days, trypsinized and re-seeded on to the PCNUs before measuring radiolabel release (black bars) and esterase activity (white bars) (_ 400). The radiolabel release was related to the average DNA values, from the time of initial seeding to the time that the cells were lysed. The data were normalized to 10 µg DNA.

Time point	Surface	‡Average number of nuclei per cell	‡ FBGC formation (%)	¥Average cell surface area (mm^2)
1 h	PS	1.5±0.1	17.1±2.5	1.3± 0.1
	HDI	1.9±0.2	22.4±4.4	1.2± 0.2
	MDI	2.0±0.2	24.0±4.4	1.3± 0.2
48 h	PS	2.2±0.2	20.9±7.9	1.0 ± 0.1
	HDI	4.3±0.2*	72.7±4.9*	0.6±0.1*
	MDI	2.7±0.1	36.2±2.4	1.2± 0.1

Table 1- Characteristics of FBGCs on different material surfaces. Trypsinized MDM were re-seeded on PS, HDI or MDI and fixed at 1 and 48 h post-attachment to each surface. The number and size of FBGC, as well as the number of nuclei per cell were determined.

* $p < 0.05$ compared with PS and MDI

‡ To monitor cell fusion and FBGC populations, the number of nuclei per cell was counted by simultaneously visualizing cells labeled with DAPI under phase contrast. A minimum of 300 total cells were counted from 3 different donors. The percentage of FBGCs was determined by dividing total cell counts by FBGC counts (cells with > 3 nuclei).

¥ Surface areas of actin filaments were measured from a minimum of 200 total cells from 3 different donors using the integrated morphometry analysis software, MetaMorph ® 4.01.

and there was no significant difference between surfaces in either nuclei per cell or percentage of FBGCs. At 48h the percentage of FBGCs had increased approximately 3.5-fold on HDI. On that surface 72.7±4.9% of all MDM had fused to become FBGCs.

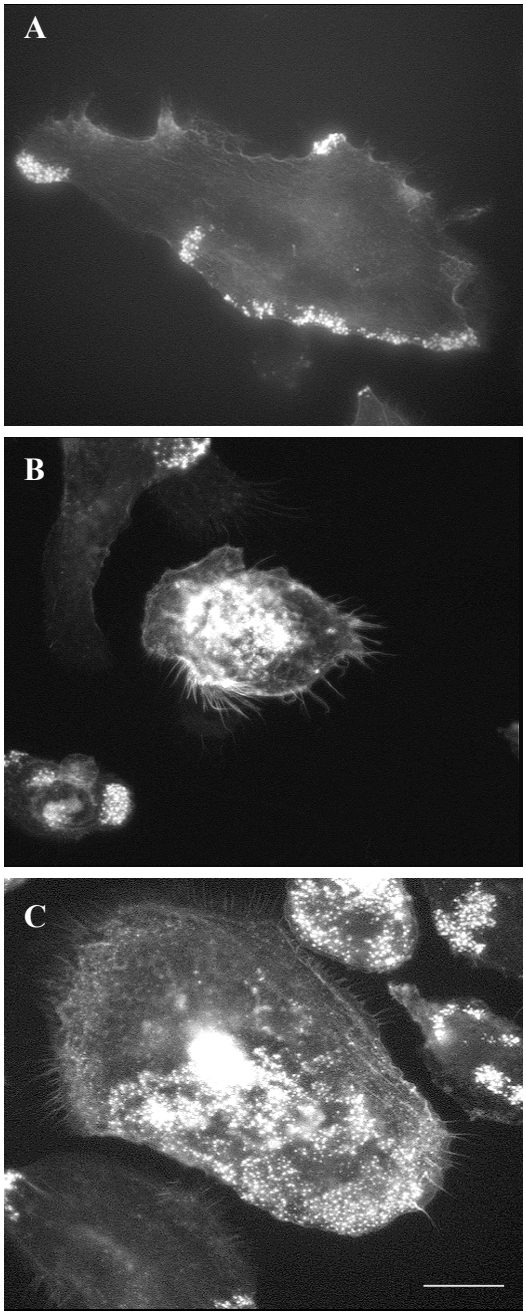

Figure 2 – Fluorescence micrographs of activated MDM labeled for F-actin. Monocytes were cultured for 14 days, trypsinized and re-seeded on PS (A), HDI (B) or MDI (C) for 48 hours before fixation and cytoskeletal staining. Bar represents 20 m.

However, on MDI there was only an increase from 24.0±4.4% to 36.2±2.4% multinucleated cells from 1 to 48h post-trypsinization (Table 1). There was no significant increase in FBGCs on PS over the 48h.

In addition, the number of nuclei per cell significantly increased over the 48h time period on all surfaces, the most pronounced difference being the 2-fold increase on HDI. Cells on HDI had an average of 1.99±0.15 nuclei per cell at 1h and 4.34±0.16 nuclei per cell at 48h (Table 1).

Using the fluorescence stain rhodamine phalloidin which labels F-actin, cell surface area was measured to look at the extent of cell spreading on the different surfaces. At 1h there was no significant difference in cell size on any surface tested. At 48h there was a distinct decrease in average cell surface area in cells re-seeded on HDI (1.2 ± 0.2 to 0.6 ± 0.1 mm^2) but there was no significant difference in average cell surface area from 1 to 48h on either PS or MDI (Table 1, Figure 2). The decrease in cell surface area in cells seeded on HDI may be due to a stress response that forced the cells to 'round up'. This behavior is seen in many cell types such as HeLa cells subjected to high pressures [4].

When *de novo* protein synthesis was investigated in activated MDM re-seeded on PS, HDI or MDI for 48h, the autoradiograph of newly synthesized secreted protein demonstrated the appearance of a ~50kDa band from cells re-seeded on HDI. Over a time course of cell activation the protein band increased in intensity and was secreted in a significantly greater amount from cells re-seeded on HDI versus cells re-seeded on PS or MDI (Figure 3).

Immunoblotting analysis was carried out with antibodies to CE and MSE. Blots of secreted protein demonstrated that cells re-seeded on HDI secreted approximately 5-fold more MSE than when re-seeded on PS or MDI (data not shown). Similarly there is also a significant increase in CE secreted from cells re-seeded on HDI versus cells re-seeded on the other surfaces (data not shown).

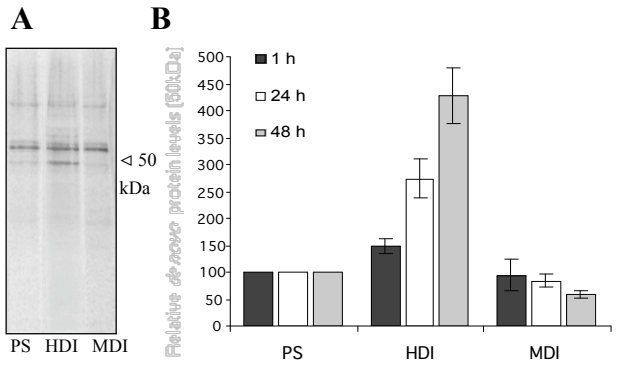

Figure 3 - (A) Autoradiograph showing *de novo* secreted protein at 48h (B) Graph depicting the relative secretion of a newly synthesized ~50kDa protein from MDM at 1, 24 and 48h of activation

DISCUSSION

The objective of this study was to demonstrate a relationship between cellular activation by material surface chemistry, FBGC formation and the biodegradation that is observed in long-term implant medical devices *in vivo*. The presence of FBGCs has been used as a histopathology marker for chronic inflammation; consequently, studies have indicated that FBGCs on implant surfaces represent a marker of poor biocompatibility [5]. Using an *in vitro* activated MDM cell system, it was shown that the most degradable surface tested induced the formation of more multinucleated cells as well as increased the secretion of *de novo* protein. In addition through fluorescence microscopy it was possible to demonstrate morphological differences in MDM and FBGC that were re-seeded on different PCNUs after differentiation on PS. This may indicate that biocompatibility influences the biostability of the material. Characterization of the cells' response to different material surfaces using this MDM cell system may provide insight into the MDM capacity for degradation of a synthetic biomaterial.

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