Material surfaces affect the protein expression patterns of human macrophages: a proteomics approach.

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Background: Monocyte-derived macrophages (MDM) are inflammatory cells that form part of the foreign body response to implant materials. MDMs have been shown to change in morphology and function following exposure to polycarbonate-urethanes (PCNU)s[1]. Changes in actin cytoskeleton, multinucleation and cell size occurred in response to small alterations in PCNU surface chemistry. Although PCNU chemistry has an influence on *de novo* protein synthesis[1], no assessment of the protein expression profiles for MDM have yet been reported. The current study adopted a proteomics approach which applied 2-dimensional electrophoresis (2-DE) combined with MALDI-ToF (matrix assisted laser desorption ionisation–time of flight) mass spectrometry, to determine the influence of PCNU on MDM protein expression.

Methods: Monocytes were isolated from healthy human volunteers and differentiated to mature MDM. In a well established model of PCNU degradation, MDM elicited maximal degradation when differentiated for 14 days on tissue culture grade polystyrene (PS), followed by gentle trypsinization and reseeding to candidate surfaces for 48 hours[2]. In the current study, MDM protein profiles were assessed at two time points (7 day differentiation and 24 hours post-reseeding) which represent time points during differentiation of MDM on PS, and initial PCNU biodegradation by mature MDM. MDM were cultured on either PS or a model PCNU synthesized with 1,6-hexane diisocyanate, polycarbonate and 1,4-butanediol in a stoichiometric ratio of 4:3:1. At the above two time points, MDM cell lysates were collected and proteins were precipitated. IPG strips were passively rehydrated with equal amounts of resolubilized protein samples and focused for 100 kVh. Focused strips were loaded and proteins separated on 8-18% gradient SDS-PAGE gels which were then fixed and stained (Sypro Ruby) to obtain images, followed by Coomassie staining to detect protein spots to be excised for identification by MALDI-ToF. Gel pieces were destained, dried and reswelled with trypsin to achieve in-gel digestion. Tryptic digests were then concentrated and desalted (C_{18} ZipTips) prior to peptide mass fingerprinting (PMF) by MALDI-ToF analysis. Resulting peptide masses were submitted into a SwissProt database using MASCOT software.

Results and Discussion: The results of these studies generated four 2-DE gel MDM protein maps [1) differentiation on PS, 2) differentiation on PNCU, 3) reseeded on PS, 4) reseeded on PCNU] that were used to successfully identify more than 60 proteins by MALDI-ToF. Protein spots that showed differential expression between PS and PCNU were visually compared and contrasted. This outlined two broad groups of proteins that differed for each surface. Examples of protein spots that were differentially expressed are shown in Figure 1 for MDM differentiation (day 7) and in Figure 2 for MDM during biodegradation (24 hr post-reseeding). Identified proteins that were differentially expressed included 1) proteins responsible for cell structure and actin remodeling and 2) proteins involved in protein trafficking and protein structure modification. Cell structure proteins included: β-actin and vimentin (cytoskeleton), macrophage capping protein and cofilin-1 (actin remodeling), nuclear transcription factor Y (β-actin gene regulation), diaphanous protein homolog (recruits profilin (actin polymerization) to the cell membrane) and lamin A/C (nuclear lamina component). Protein trafficking and protein structure modification proteins included: cathepsin D (intracellular protein breakdown), calreticulin (chaperone), 27 kDa golgi SNARE protein (protein transport) and protein disulphide isomerase (disulphide bond rearrangement).

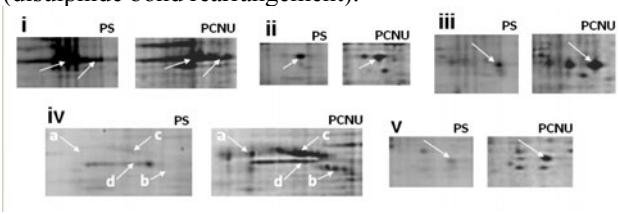

Figure 1 – Identified proteins spots that differed in relative expression between MDM differentiated for 7 days on PS or PCNU- i) β-actin, ii) macrophage capping protein, iii) cathepsin D precursor, iv) [a] nuclear transcription factor Y [b] lamin A/C [c] diaphanous protein homolog 3 [d] calreticulin, v) 27kDa golgi SNARE protein

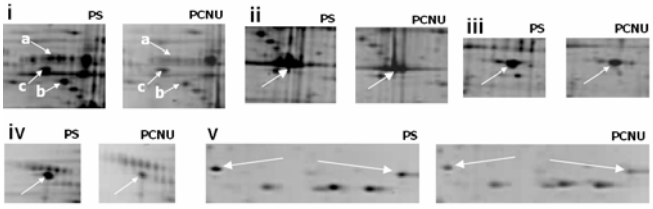

Figure 2 – Identified protein spots that differed in relative expression between MDM differentiated on PS for 14 days and reseeded to either PS or PCNU for 24 hours – i) [a] vimentin [b] β-tubulin [c] protein disulphide isomerase, ii) β-actin, iii) macrophage capping protein, iv) calreticulin, v) cofilin-1.

Conclusions: The above proteomics approach allowed for the broad characterization and comparison of protein profiles associated with human MDM cultured on PS or PCNU surfaces during differentiation and biodegradation time points. Results indicated that MDM respond to material chemistry by cytoskeletal remodeling in addition to intracellular protein modulation, implicating changes in proteins responsible for trafficking and protein structure modification. The proteomics approach used in the current study provided an innovative method to elucidate the mechanism of cell-biomaterial interactions and the proteins that may be involved in the cellular response.

References: 1) Matheson LA et al (2004) J Cell Physiol 199: 8-19. 2) Labow RS et al (2001) Biomaterials 22: 3025-33.

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