Standardized Methods for Characterizing Immune Responses of THA Revision Tissues

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Introduction: Within standardized analysis protocols for tissue retrieval, there are indications of multiple factors contributing to wear-mediated inflammation [1]. However, the clinical response of tissue has only been documented on a limited basis [2]. The purpose of this study was to establish a repeatable, more comprehensive approach to characterizing the immune response of retrieved periprosthetic tissue.

Methods: Tissue samples (n=30) were collected from 16 THA revision surgeries of uncemented polyethylene hip components, whose average implantation time was 6.89y (0.23-18.89y). The implants were revised for loosening (n=10), osteolysis (n=3), and polyethylene wear (n=3). Polyethylene components were classified into three cohorts: highly crosslinked annealed (n=7), highly crosslinked remelted (n=3), and historical (n=6). Tissue samples were retrieved from four distinct regions: capsule (n=15), retro-acetabulum (n=8), posterior femur (n=5), and intramedullary femur (n=2). Tissues were stored in paraffin wax at -20°C. At the time of analysis, 6 μm thick tissue slices were mounted on slides, dewaxed, rehydrated and stained with the following primary antibodies; CD68 (macrophages, M0814), CD3 (T cells, A0452), plasma cell (antibody-producing B cells, M7077) and MPO (neutrophils, A0398) (Dako, Carpinteria, CA) [1]. Appropriate primary-specific biotinylated secondary antibody was applied followed by streptavidine peroxidase, for amplification, and diaminobenzidine (DAB) for detection of positive signals with yellow-brown stain [1]. Slides were imaged at 10X with a Retiga Exi digital cooled CCD camera equipped with 3 color LCD filter (QImaging, BC, Canada) on a Nikon microscope Optiphot (Melville, NY). Image analysis was performed in ImagePro Plus (MediaCybernetics). Images of ten fields (750 μm^2) were acquired from each slide. The tissue samples were very heterogeneous for cellularity, both between samples and within given fields. To account for this variation, we created an automated analysis module within ImagePro. The module automatically outlined areas of high cellularity, performed a count of total and stained cells, then identified the remaining area as low cellularity and analyzed it in the same manner (Figure 1). A quantitative value of inflammatory response was then represented by the percent of positively stained cells to total cells from each density type.

Results/Discussion: Visual inspection of acquired images showed the collective presence of macrophages, T cells (Figure 2), B cells and neutrophils for all three cohorts. The image module accurately separated high and low cellular densities, and identified positively stained cells in a fast and repeatable manner.

Conclusions: In vivo studies have indicated a cellular response from polyethylene particle debris [3]. However, the combined incidence of these immunological markers has not been acknowledged. A quantitative assessment of these immunological responses is currently underway, and will provide the basis for a better evaluation of THA revision- and polyethylene-based inflammatory trends.

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References:

[1] ASTM F561-97. (Section 9.2 and 9.3). [2] Ingham E. Biomaterials. 2005;26:1271-86. [3] Reno F. Biomaterials. 2003;24:2895-2900.

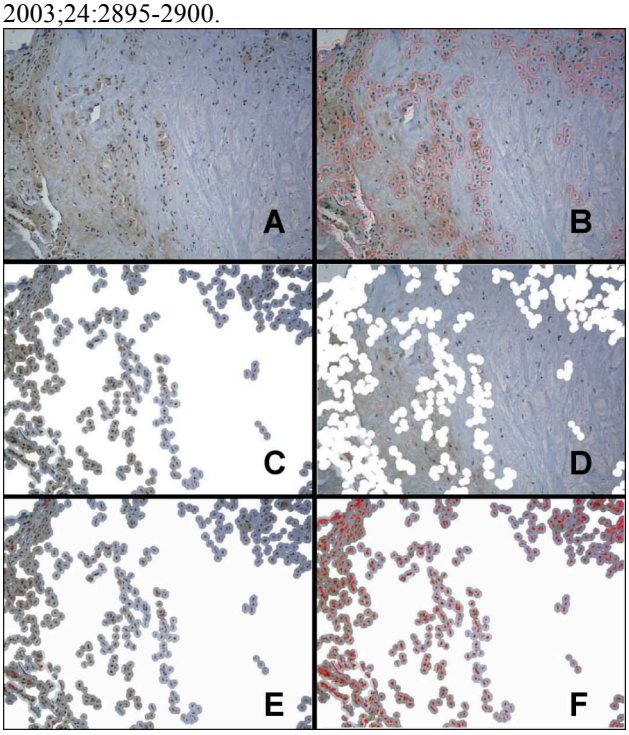

Figure 1: Example module output. [A. Original Image, B. High density outlines, C. High density mask, D. Low density mask, E. High density mask with positive cells, F. High density mask with total cells]

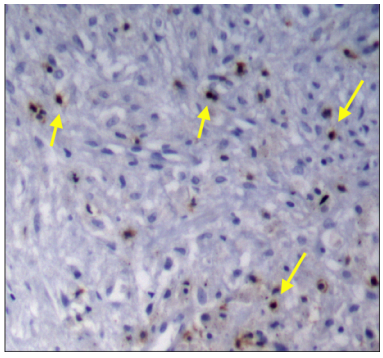

Figure 2: Historical implant tissue. Positive responses to CD3 antibody (T cells) are stained brown.