Macrophage Adhesion on Conventional and Nanophase Alumina

Peishan Liu-Snyder, Thomas J Webster
Division of Engineering Box D, Brown University, 184 Hope Street, Providence, RI 02912

Statement of Purpose: Extensive interactions of inflammatory cells (such as macrophages) with biomaterials at the host-implant interface are often blamed for failure of implanted biomedical devices [1]. While previous studies have shown increased in vitro and in vivo bone cell responses on nanophase ceramics [2], few (if any) studies have been conducted elucidating inflammatory cell responses on such novel materials. Nanophase ceramics are intriguing materials for orthopedic implant applications since they mimic the nanometer grain size of natural bone. In this study, we report altered macrophage adhesion on nanophase compared with conventional alumina imperative for the future design of nanophase materials for orthopedic applications.

Materials and Methods:

Substrate preparation: Alumina (Al_2O_3) samples (circular disks 12 mm in diameter and 2 mm thick) were prepared by compacting nanophase (45 nm grain size) alumina (γ-phase) powders (Nanophase Technologies Corp.) in a tool-steel die via a uniaxial pressing cycle, (0.2 to 1 GPa over a 10 min period). Nanophase alumina samples were obtained by sintering (in air at 10 °C/min) the 45 nm grain size alumina compacts from room temperature to 1100 °C and by maintaining 1100 °C for 2 h to obtain materials with grain sizes less than 100 nm. Conventional alumina samples were obtained by sintering (in air at 10 °C /min) the 45 nm grain size alumina compacts from room temperature to 1200 °C and by maintaining this temperature for 2 h to obtain materials with grain sizes greater than 100 nm [3].

Surface characterization by scanning electron microscopy (SEM): Sintered conventional and nanophase alumina samples were mounted on stubs and sputter-coated with gold prior to examination using a Leo 1530-VP scanning electron microscope.

Cell culture: IC-21 macrophage cell line was purchased from ATCC. Cells were cultured according to ATCC instructions in a 37 °C, humidified, 5% CO_2/95% air environment.

Cell adhesion assay: Macrophages (3500 cells/cm^2) were seeded per substrate and allowed to adhere in a 37 °C, humidified, 5% CO_2/95% air environment for 4 h. Glass and petri dish substrates served as controls. After 4 h, non-adherent macrophages were removed by rinsing in phosphate buffered saline (PBS). Macrophages adherent on substrates were fixed with 4% formalin in PBS and stained with DAPI (Sigma). Zeiss Axiovert 200M light microscope was used to take cell images. The adhesion experiments were run in triplicate and repeated at three different times per substrate type. Cell density (cells/cm^2) was determined by averaging the numbers of adherent cells in five random fields per substrate. Cell adhesion density was analyzed statistically using standard analysis of variance (ANOVA) techniques; statistical significance was considered at $p < 0.05$.

Results/Discussion:

SEM microscopy of the surface of alumina

SEM images of the surface of conventional and nanophase alumina are shown in Fig. 1. The grain size of Al_2O_3 nanoparticles in conventional alumina (187.4 nm) is much larger than that of nanophase alumina (97.7 nm).

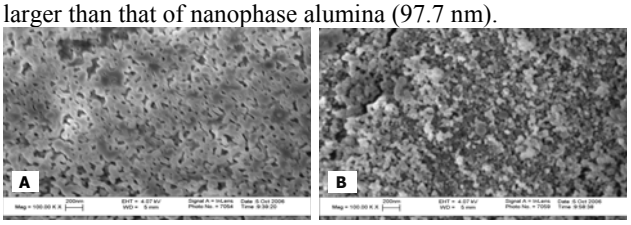

Figure 1. Scanning electron microscopic images of conventional alumina (A) and nanophase alumina (B). Scale Bar= 200 nm

Macrophage adhesion on different substrates

Macrophage adhesion on the conventional alumina was significantly greater than not only on the glass ($p < 0.01$) (1763 versus 1312 cells/cm^2) but also on the nanophase alumina ($p < 0.05$) (1763 versus 1399 cells/ cm^2) after 4 h (Fig. 2). In contrast to enhanced osteoblast adhesion on nanophase ceramics [4], macrophage adhesion was significantly less on nanophase alumina.

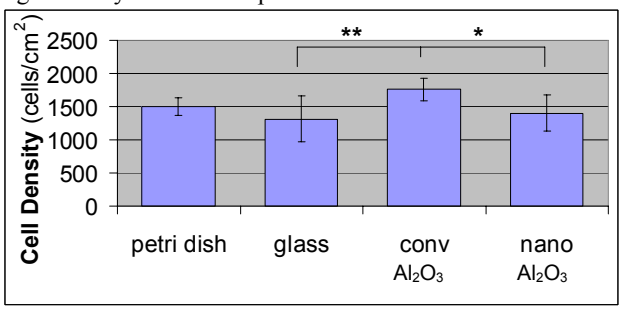

Figure 2. Macrophage adhesion (4 hours) on petri dish, glass, conventional and nanophase alumina (**$p < 0.01$, *$p < 0.05$)

Conclusions: In this study, we found that macrophages adhered greater on the conventional alumina than glass and nanophase alumina within a 4 h period. Such selective adhesion needs to be tested for a longer period. Macrophage functions and their phagocytotic activity on these different substrates will be examined in the future. The results from this study provide clearer evidence that macrophage responded differently based on conventional and nanophase alumina roughness, which is important for their potential biomedical implant applications.

References

[1]Gristina AG Clin Orthop Relat Res. 1994;298:106-118.
[2]Webster TJ Tissue Eng. 2001;7:291-301.
[3]Wu SJ J Am Ceram Soc. 1996;79:2207-2211.
[4]Webster TJ J Biomed Mater Res. 2000;5:475-483.