DIFFERENT REACTIONS OF MACROPHAGES CULTURED WITH THE SAMPLES FROM NEW AND OLD POLYETHYLENE CUPS OF THE HIP PROSTHESIS

Czajkowska B.*, Otfinowski J., Ptak M.*, Pawelec A.***, Lekki J.******

*Department of Immunology
Collegium Medicum of the Jagiellonian University in Cracow
**Department of Traumatology
Collegium Medicum of the Jagiellonian University in Cracow
***Department of Orthopaedics
Collegium Medicum of the Jagiellonian University in Cracow
****Institute of Nuclear Physics in Cracow

Abstract

We estimated release of cytokines IL-6 and TNFα and tested viability of macrophages cultured with the samples of polyethylene obtained from new acetabular cup of the hip prostheses and from the cups retrieved 4-6 years after implantation. The macrophages cultured with old polyethylene showed decreased viability and released more IL-6 and TNFα.

Key words: Hip prostheses, polyethylene, macrophages, inflammatory reaction

Introduction

Numerous observations have shown that polyethylene acetabular cups of the hip prostheses gradually undergo visible macroscopic changes associated with delamination and desquamation of the bearing surface, fracturing, and even fragmentation of the polyethylene [3,4,15,2,24,25]. Increasing number of reports have also showed that implanted high density polyethylene which is used for production of the acetabular cups may change over time its internal structure and its level of oxidation [16,17,28]. New polyethylene is generally regarded as biologically neutral but the question arises whether or not the macroscopic changes and alteration of the internal structure of the polyethylene can modify tissue response to the implanted material [11,12].

We have made an attempt to answer this question by testing the macrophages reaction to the samples from new, unused polyethylene acetabular cups, and to the samples obtained from old cups retrieved from the patients at the time of revision surgery. Macrophages are multipotential cells, which act in both, initial and final phases of the acquired immunity as well as in innate immunity. In contact with the material of appropriate size, macrophages begin the phagocytosis and when the object is too large, they attach to the surface and release many biologically very strong substances, such as IL-6 and TNFα. [2,6,7,8]. Macrophages are sensitive not only to chemical composition of the surface but also to its shape and size. The viability of macrophages on the tested materials and their excitation level measured as concentration of released IL-6 and TNFα were evaluated. To analyse the structure of the surface that was in contact with cultured macrophages we used scanning force microscope - SFM.

Materials and methods

We analysed two groups of samples of high-density polyethylene Chirulen DIN 58834 (Aesculap,Tuttlingen, Germany) of the same weight, obtained from both new and old acetabular polyethylene cups, removed 4-6 years after implantation because of loosening of the prostheses.

Macrophages were cultured with the samples of the polyethylene, their vitality was tested and the release of IL-6 and TNFα was estimated. The surfaces of each sample were analysed by means of scanning force microscope (SFM).

Determination of macrophages viability

The percentage of living cells was determined after 3 days of Mf culture without or with polyethylene samples. Viability was determined using a modification of MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye metabolism method [5] in the living cells mitochondria. One ml of supernatant from culture was removed and 100μl of 0.5% solution of MTT was added and left for 3.5 hours. The plate was then frozen and thawed twice and treated with 1,9 ml of isopropanol solution in HCl (100 ml of isopropanol + 4 ml 1M HCl). It was then shaken vigorously in order to completely break up the cells. The extracted supernatants were centrifuged and absorbance at 570 nm was measured. The results were presented as percentage of viable cells on polyethylene in comparison to Mf cultured alone.

IL-6 and TNFα tests

Cytokine concentration in culture supernatants was measured using capture ELISA.

For IL-6 the microtiter plates (Corning, NY) were coated with rat monoclonal antibody against mouse IL-6, Genzyme USA, (capture antibody) and biotinylated antibody against IL-6, Pharmingen USA, (detecting antibody). The ELISA was developed with a horseradish peroxidase streptavidin, Vector USA, followed by o-phenylenediamine and H_2O_2 as substrates. For TNFα a peroxidese-conjugated goat anti rabbit IgG, Sigma USA, was used to develop the reaction. The reaction was stopped with 3M H_2SO_4 and the optical density of each well at 492 nm was measured in a 96-well plate reader.

Recombinant murine cytokines were used as standards.

Results

The results showed marked difference in the behaviour of the macrophages cultured with the samples of new and old polyethylene. Forty percent of macrophages cultured with the samples of old polyethylene died after three days and only 20% of those cultured with the samples of new polyethylene (FIG.1). Both, the new and the old polyethylene fragments of acetabular cups of hip prostheses caused a release of interleukine from macrophages higher than that in control cells. The level of IL-6 and TNFα released from

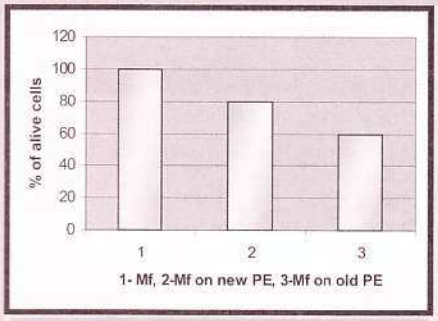

FIG. 1. Viability of macrophages line J774 on new and old polyethylene samples.

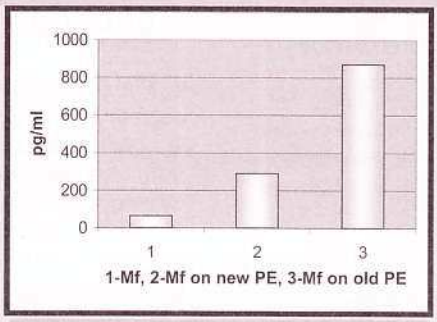

FIG. 2. IL-6 release from macrophages cultured on new old polyethylene samples.

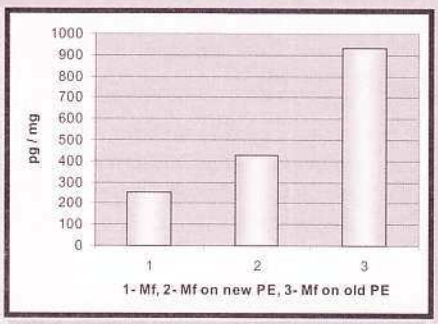

FIG. 3. TNFα release from macrophages cultured on new old polyethylene samples.

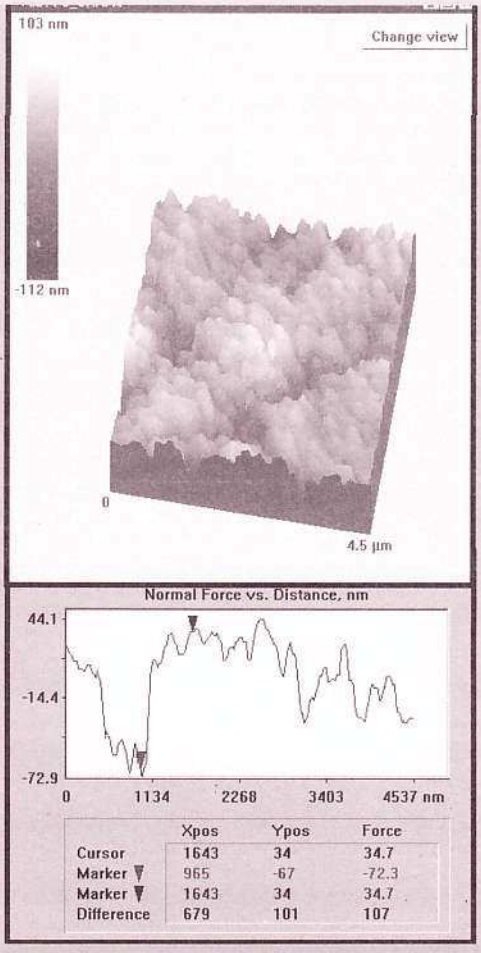

FIG. 4. Surface of new polyethylene (SFM).

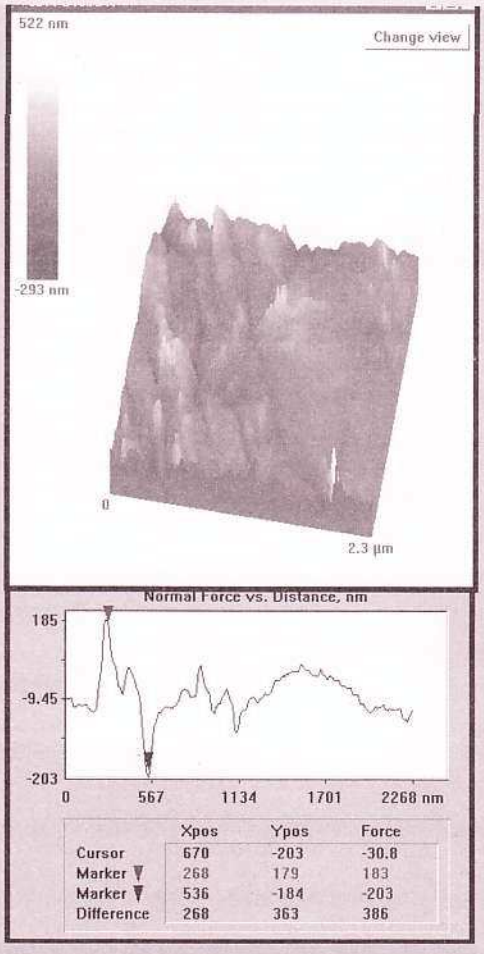

FIG. 4. Surface of old polyethylene.

cells cultured on the old polyethylene is nearly twice higher than on the new one. (FIGS.2, 3). The results of SFM studies (FIG.4) showed differences in the surface of estimated samples. It has been stated however, that the scanning force microscope (SFM) is not a suitable tool for the analysis of this type; the analysed area being very small compared with the whole surface of the sample subjected to the biological test.

Discussion

At present, polyethylene has become a commonly used implantation material in the joint replacement surgery. Years ago, when it was first introduced to orthopaedic surgery, it was regarded as biologically neutral. However, there have been increasing number of reports indicating that this material after long implantation can produce harmful biological reactions in the periprosthetic tissues [9,10,20,22,23,26]. This especially refers to small polyethylene fragments, which are rubbed off and released from the surface of the cup of the hip prosthesis and evoke an inflammatory reaction, induced by prolonged irritation of the surrounding tissues [13,14,18,19]. It is well known that the internal structure of polyethylene is non-uniform and contains crystalline and amorphous phases [1]. It is not unlikely that these two phases may cause different biological reactions in the periprosthetic tissues. Since we know that the ageing polyethylene changes its internal structure and degree of crystallisation [16,17,28] we may ask whether or not such alteration of the polyethylene structure could evoke different tissue reactions. The obtained results show that the viability of macrophages cultured with the samples of old polyethylene, from the retrieved cups, was lower and that

release of proiflammatory cytokines was increased. It cannot be excluded that the deep internal structural changes of polyethylene may have influence on the structure of the cup surface, which is in contact with the host body fluids and the cells responsible for inflammatory reactions. This could be an additional factor that, independently of the irritating influence of small polyethylene fragments, causes harmful inflammatory tissue reactions and provokes aseptic loosening of the hip prostheses.

Conclusions

1. Viability of macrophages cultured with the samples of polyethylene from retrieved hip prostheses is decreased.
2. Macrophages cultured with the samples of polyethylene from retrieved hip prostheses release more proinflamatory cytokines IL-6 and TNFα.
3. Decreased vitality and increased release of cytokines indicate that the ageing polyethylene causes a more pronounced biological reaction.

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