Determination of the effects of biomaterials on human peripheral blood mononuclear cells (PBMC)

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1. Introduction

Biomaterials produced from ceramic, metallic, polymeric and composite materials [1] improve human health and welfare of the society. In addition to desired physical, chemical and mechanical properties of biomaterials, their biocompatibility is important for the treatment or replacement of body parts [2]. In this study, the effects of metallic materials (polished and unpolished stainless steel (316L) and titanium alloy (Ti-6Al-4V)), ceramic materials (Hydroxyapatite (HA), Alumina and Zirconia at different sintering temperatures), ceramic-ceramic composites (HA-Alumina and HA-Zirconia sintered at 1250°C) and polymeric material (Ultra High Molecular Weight Polyethylene (UHMWPE)) on the viability and proliferation of PBMC as well as on the secretion of proinflammatory cytokines IL-1α and IL-6 from PBMC were studied.

2. Materials and methods

Ficoll density gradient centrifugation method was used to isolate human PBMC [3]. The cells (2.5×10^5 cells/well) were incubated with ceramic, metallic and polymeric samples (15 mm in diameter) in 24-well plates for 24, 48 and 72 h at 37°C and 5% CO_2 in a humidified atmosphere. The percent viability of cells was determined by Trypan blue exclusion method. The effects of ceramic samples (5 mm in diameter) on the proliferation of PBMC with and without Concanavalin A (200 μg/ml) were determined by using Biotrak^{TM} Cell Proliferation ELISA kit (Amersham Life Science). This test is based on the incorporation of 5-bromo-2'-deoxyuridine (BrdU) instead of thymidine into the DNA of proliferating cells. After incubation of PBMC (2.5×10^5 cells/well) with biomaterials in 24-well plates for 17 h in the presence and absence of lipopolysaccharide (LPS) (10 μg/ml), the secretion of human IL-1α and IL-6 were examined by using Quantikine® ELISA kits (R&D Systems).

3. Results

HA samples sintered at low temperatures (800°C, 900°C and 1000°C) caused 1.6, 1.7 and 1.4-fold decrease, respectively, in the viability of PBMC in comparison to the control culture (without biomaterials) after 24 h incubation. After 48 h, the viability of cells decreased to 30%, 35% and 59% in the cells treated with HA 800°C, HA 900°C and HA 1000°C pellets, respectively. At the end of 72 h, the viability reduced to 30% and stayed around at the same level for all HA pellets sintered at 800°C, 900°C and 1000°C. HA pellets sintered at 1100°C and 1250°C, Alumina 1450°C and Zirconia 1450°C samples did not affect the cell viabilities at all the time points examined. Polished metallic samples showed the highest percent cell viability at 24 and 48 h. However, after 72 h incubation, stainless steel, polished stainless steel, titanium alloy, polished titanium alloy and UHMWPE resulted in 22%, 5%, 10%, 4% and 16%, decrease in the viability of PBMC, respectively. In addition, unlike HA 1250°C, Alumina 1450°C and Zirconia 1450°C, the samples of HA 800°C, HA-Alumina 1250°C and HA-Zirconia 1250°C showed inhibitory effects on the proliferation of PBMC which were stimulated with mitogenic agent Concanavalin A.

Cytokine secretion analysis after treatment of PBMC with biomaterials indicated that HA 800°C, HA-Alumina 1250°C and HA-Zirconia 1250°C pellets led to 80%, 69% and 49% respectively, decrease in IL-1α secretion in the presence of LPS. Stainless steel, titanium alloy and UHMWPE samples caused 30%, 10% and 23% respectively, decrease in IL-1α secretion from stimulated PBMC. In addition, HA 800°C pellets, metallic samples and UHMWPE led to secretion of low IL-6 levels with respect to the control cultures with and without LPS stimulation.

4. Conclusion

In conclusion it can be stated that HA 800°C, HA-Alumina 1250°C and HA-Zirconia 1250°C samples resulted in negative effects on PBMC. Ceramic samples sintered at low temperatures have porous structures and a relatively high surface area. The released ions or particles of ceramics due to dissolution in the culture medium or adsorption of some molecules from culture medium may change the pH and conductivity of medium that affect directly the cell viability, proliferation and stimulatory effects of mitogens on the secretion of cytokines from cells.

References

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