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Electrochemical control of cell death by reduction-induced intrinsic apoptosis and oxidation-induced necrosis on CoCrMo alloy *in vitro*

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ABSTRACT

Electrochemical voltage shifts in metallic biomedical implants occur *in-vivo* due to a number of processes including mechanically assisted corrosion. These excursions may compromise the biocompatibility of metallic implants. Voltages can also be controlled to modulate cell function and fate. The *in vitro* effect of static voltages on the behavior of MC3T3-E1 pre-osteoblasts cultured on CoCrMo alloy (ASTM-1537) was studied to determine the range of cell viability and mode of cell death beyond the viable range. Cell viability and morphology, changes in actin cytoskeleton, adhesion complexes and nucleus, and mode of cell death (necrosis, or intrinsic or extrinsic apoptosis) were characterized at different voltages ranging from −1000 to +500 mV (Ag/AgCl). Moreover, electrochemical currents and metal ion concentrations at each voltage were measured and related to the observed responses. Results show that cathodic and anodic voltages outside the voltage viability range ($-400 < V < +500$) lead to primarily intrinsic apoptotic and necrotic cell death, respectively. Cell death is associated with cathodic current densities of 0.1 μA cm^{-2} and anodic current densities of 10 μA cm^{-2}. Significant increase in metallic ions (Co, Cr, Ni, Mo) was seen at +500 mV, and −1000 mV (Cr only) compared to open circuit potential. The number and total projected area of adhesion complexes was also lower on the polarized alloy ($p < 0.05$). These results show that reduction reactions on CoCrMo alloys leads to apoptosis of cells on the surface and may be a relevant mode of cell death for metallic implants *in-vivo*.

1. Introduction

The effect of electrochemical processes on cells on or adjacent to metallic surfaces is an important area of research, which falls under the general category of electrical stimulation of cells. These studies are mainly focused on either the electrical field effects on cells or the electrochemical processes happening at the cell/electrode interface. The electric effects on cells can be categorized into dielectric, electrophoretic, electrolytic, and cell/electrode interactive effects [1]. Electroporation and electrofusion of cells are well studied examples of the dielectric effect, in which a high voltage pulse of stimulation leads to dielectric breakdown of the membrane and formation of pores or fusion of the cellular membranes [2,3]. Electrophoretic effect, however, refers to the migration of cells in a solution down a potential gradient between two electrodes. On the other hand, electrochemical effects arise due to redox reactions of electroactive components of the solution at the electrode surface or the constituents of the cell. Irreversible electrochemical reactions of physiologically important substances on the cell membrane or electrochemical formation of active species such as reactive oxygen species (ROS) in the cell culture environment may lead to cell death (electrolytic effect). However, this is not the only outcome of applying voltage to cells cultured on electrodes. In fact, a proper electrode potential could create cell-electrode interactive effects that modulate cell viability [4], cellular proliferation [5–7], gene expression [7], protein production [8–10], morphology [11], and cytoskeletal organization [5,7,12] (cell/electrode interactive effects).

The study of voltage effects on cells has primarily been done on transparent indium tin oxide (ITO) electrodes using different cell types in potentiostatic and galvanostatic modes at anodic and cathodic potentials [4–16].

It is known that metallic implants undergoing mechanically assisted corrosion reactions [17], can experience large shifts in the

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