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In vitro studies on the effect of delaminated a-C:H film fragments on bone marrow cell cultures ☆

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Abstract

Amorphous hydrogenated carbon (a-C:H) films have many outstanding properties required for a protective coating material on load bearing medical implants. Recently, titanium doped a-C:H films have been evaluated regarding their effects on bone marrow cell cultures. But many materials that are well-tolerated in bulk form are able to induce toxic reaction if present particulate form. In order to further assess biocompatibility aspects of these two coatings, film delamination has been mimicked in exposure to fluids. In the present study, particles from a-C:H, a-C:H/Ti and a-C:H–a-C:H/Ti bilayer films were added to bone marrow cell cultures in vitro. The results showed that plain a-C:H and to a certain extent a-CH/Ti particles were inert. Both kinds of particles did not significantly stimulate the osteoclast-related enzyme tartrate resistant acid phosphatase (TRAP). A slight increase in cell proliferation and total culture TRAP was found in cultures treated by a-C:H–a-C:H/Ti bilayer films. Latter effect can probably be traced back by the relative high percentage of small particles of a size of around 2 μm. However, if corrected by the cell number also no differences between particle-treated and untreated control cultures could be found, indicating the absence of a toxic effect from delaminated a-C:H coatings.

Keywords: Particles; Bone marrow cells; a-C:H; Titanium; Biocompatibility

1. Introduction

Amorphous hydrogenated carbon coatings (a-C:H), also called diamond-like carbon (DLC) coatings have attracted attention as protective coating for load bearing implants, due to their high hardness, chemical inertness, good tribological characteristics and wear resistance [1–6]. Several studies carried out with different cell lines affirmed that a-C:H films induced no cytotoxic reactions in vitro and in vivo [7–13]. This makes that a-C:H coatings may be used for a broad variety of medical applications [14].

Recently, it could be shown, that the addition of titanium to the a-C:H matrix induced cellular reactions in bone marrow cultures in vitro, different from those observed in cell cultures on plain a-C:H films. A stimulation of cell proliferation as well as a reduction in the osteoclast associated enzyme TRAP has been observed [15,16].

Many implant materials are relatively inert in bulk form, but particles of these materials may cause adverse cellular reactions in surrounding bone, leading to a

Abbreviations: ALP, alkaline phosphatase; RBMC, rat bone marrow cells; PBS, phosphate buffered saline; TRAP, tartrate-resistant acid phosphatase; a-C:H, amorphous hydrogenated carbon

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reduction of implant lifetime [17–20]. It is now generally accepted that, in most cases osteolysis is a manifestation of an adverse cellular response to phagocytosable particulate wear and corrosion debris [21]. The biological response involves the accumulation and activation of inflammatory cells and subsequent bone resorption [22,23].

The rat bone marrow cell (RBMC) culture model used in the present work was selected because it is composed of heterogeneous cell populations which can advance along multiple differentiation pathways, one of which gives rise to the osteoblast lineage [24]. In addition, cells from the monocyte lineage can give rise to osteoclastic, bone resorbing cells [25]. Osteoclasts have been shown to phagocytose particles and to remain bone resorbing cells at the same time [26,27]. RBMC cultures permit to investigate the effects of particle exposure on the formation and consequent function of osteoblastic and osteoclastic cells.

Plain a-C:H films, a-C:H/Ti–a-C:H bilayer films, as well as pure titanium doped a-C:H films are considered potential candidates for coating several components in total joint replacements in contact to bone tissue. It is assumed that no particles are generated by wear of DLC sliding against DLC. The wear rate is below $10^{-4}\,mm^3$/year [28,29]. The extremely low amount of wear, which is believed to be generated in form of single carbon or hydrocarbon molecules, is removed out of the joints by the synovial fluid. Our main concern is a total delamination of the DLC films, perhaps due to DLC/implant interface corrosion [30] resulting in a possible particle release. Spallation resistance of some DLC films has already been shown to diminish when films are exposed to biological fluids [31]. This study focused on the effects of delaminated a-C:H, a-C:H/Ti–a-C:H bilayer and a-C:H/Ti particles on RBMC cultures in order to estimate their toxic potential in case where the adhesion of these coating would be affected by exposure to biological fluids.

2. Materials and methods

2.1. Film deposition and characterisation

a-C:H, pure titanium containing a-C:H (a-C:H/Ti) and a-C:H/Ti–a-C:H bilayer films (a-C:H/Ti on a-C:H) were deposited using a combined radio frequency (rf) plasma and direct current (DC) magnetron sputtering deposition process in an all stainless steel high vacuum system with a base pressure better than 2×10^{-6} Pa as described in Francz et al. [32]. The films were deposited onto polyethylene (PE) foil (Firma Lüscher & Co. AG) mounted on the rf powered electrode (for cell culture tests). Film thickness was regulated with the deposition time. Polyethylene foil was found to be an optimal substrate for deposition of films adhering weakly to the substrate. Particles were obtained by repeated and severe deformation of the coated PE foil, followed by washing with ethanol. Particle size distributions were assessed using a Beckman Coulter LS Particle Size Analyser. Particles were collected by centrifugation for 30 min at 950 g and subsequent removal of the supernatant. The remaining ethanol was allowed to evaporate under sterile conditions. The remaining particles were stored in α-minimal essential medium (α-MEM, Gibco) with 1% antibiotics (PSN, Gibco) without FCS at 4 °C until the beginning of cell culture experiments.

2.2. Cell isolation and in vitro particle exposure assay

Rat bone marrow cells (RBMC) were harvested and cultured using a previously reported method [33]. Briefly, femur and tibia were removed from adult male Wistar rats, cleaned from soft tissue and washed with phosphate buffered saline (PBS) containing 1% antibiotics (PSN, Gibco). The epiphyses were removed and RBMC were collected.

Tissue culture plates (24-well plates, Costar) were provided with a defined quantity of particles in 100 µl α-MEM with size distribution shown in Fig. 1 and with 5×10^5 particles being above 2 µm. The latter was determined using a Cell Coulter counter with counting range 2–200 µm. Afterwards, RBMC were added at a density of 5×10^5 cells/ml in α-MEM supplemented with 10% foetal calf serum (Gibco) and 1% antibiotics (PSN, Gibco) (final volume per well: 1 ml). Cultures without particles served as controls. Cells were cultured in a humidified atmosphere of 95% air, 5% CO_2, at 37 °C. Basic cell functions were determined after 14 days in

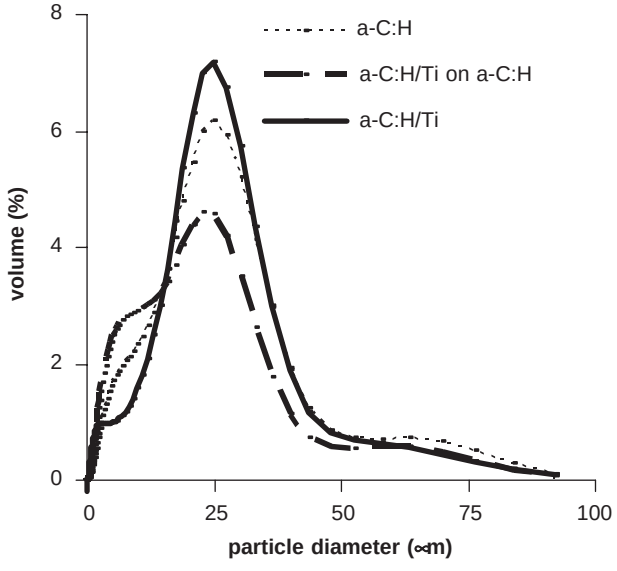

Fig. 1. Particle size distribution counted using a Beckman Coulter.