Silver doped hydroxylapatite plasma sprayed coatings – antimicrobial properties and cytotoxic effect on human osteoblasts.

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Statement of purpose: Plasma thermal spraying, commonly used to apply wear and heat-resistant barrier coatings, has also demonstrated its capability in applying bio-deposits. HA and other related calcium phosphate and calcium silicate minerals have been extensively utilised as implants coatings due to their excellent biocompatibility, bone bonding ability and also due to their structural and compositional similarity to that of the mineral phase of hard tissue contained in human bones. This is especially true in regards to the bioactive properties of these ceramic layers which are important as they promote chemical fixation on the implant-host tissue interface and therefore eliminating the use the bone cements in some cases [1].

Another significant problem that medical personnel have to face is the postsurgical infections as they are one of the most common reasons for revision surgery. Due to poor blood circulation in osseous defect sites; drugs, such as antibiotics, antimicrobials and growth factors applied conventionally (orally or intravenously) have shown limited therapeutic action. The solution to this problem may be through the introduction of various drug delivery systems (DDS) directly onto the implant prior to implantation. This can be achieved by incorporation pharmacologically active bioceramic coatings on the metal implant surface. Apart from the traditional antibiotics and antifungal agents used in medicine; silver-based compounds are a group of therapeutic agents with a wide spectrum of antimicrobial properties. Silver has been used in medicine for thousands of years but recently it has gained more attention due to its unique properties. However it has been reported that increasing the concentration of silver ions in the body can have toxic effect on living cells [2, 3].

The aim of this research is to introduce silver ions onto plasma sprayed hydroxyapatite coatings by exposing them to silver-containing solutions. These samples were then evaluated to assess their antibacterial and antifungal properties within the obtained bioactive layers and cytotoxic effects were measured on living, mammalian cells in contact with human osteoblasts (HOB).

Materials and methods: Hydroxyapatite was deposited onto Titanium alloy Ti6Al4V substrate discs (10 mm in diameter x 2 mm in thickness) as per typical femoral cementless type implant procedures. Prior to spraying the substrates were cleaned in an ultrasonic bath and using acetone as a cleaning liquid medium. The powder used for the deposition was Captal 60-1 Hydroxyapatite Powder (Plasma Biotal Ltd., UK) with average particle size of 45 μm, a powder typically used in thermal spraying. Coatings were deposited by using 9MB Plasma Thermal Spray gun (Sulzer Metco) controlled by a Sulzer Metco 9MCE system. Silver was incorporated onto the obtained coatings by applying a thin layer of silver nitrate solution mixed with distilled water (0.1M and 0.01M) onto the deposits. The liquid evaporated overnight and then samples were treated in a flame to remove nitrates. The morphologies of obtained coatings were evaluated using SEM technique. Chemical and phase compositions were evaluated using FTIR, XRD and EDAX analysis. The antimicrobial and biological test samples were sterilized in a plasma. Three different micro-organisms were used during the antimicrobial test: *S. aureus* (MRSA), *E. coli* and *C. Albicans*, (supplied by DSMZ Germany). Human osteoblasts (HOB) were used in the preliminary biological evaluation of obtained materials (PromoCell, Germany). Passage number 4 was used for this study with a cell density of $3x10^4$ /cm^2. At selected times (24 and 72 hrs), the viability of the cells were estimated using the WST-1 assay test and total protein concentrations were established. Alkaline phosphatase assessment were used to estimate the differentiation of the cells.

Results: SEM observations showed that uniform, continuous layer of hydroxyapatite were obtained via plasma spraying. Elemental analysis confirmed that after exposing the HA coatings to silver nitrate solutions silver ions were detected in the coating structure, also confirmed by XRD analysis.

HA-Silver ion coatings show antibacterial properties against *E. coli* and *S. aureus* and antifungal properties against *C. albicans*. The cell culture study showed that the obtained materials did not show cytotoxic properties when contacted with human osteoblasts, rather they promoted growth and maturation into the osteocytes.

Conclusions: Obtained results indicate that the described method is useful for obtaining: biocompatible, bioceramic coatings with antibacterial and antifungal properties. Thus this can be promising alternative to postsurgical prevention of infections.

References:

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