In Vitro and *In Vivo* Characterizations of Induction Plasma Sprayed Hydroxyapatite coating on Ti for Load Bearing Implants

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Introduction: Plasma spraying is the most widely used commercial technique to coat metallic implants with hydroxyapatite (HA) to improve the osteoconductive property of metallic implants. Problems associated with conventional plasma sprayed HA coating includes decomposition of HA, amorphous calcium phosphate (ACP) formation, and cracking [1,2]. The ***objective*** of this research is to study the effect of plasma nozzle design of radio frequency (RF) induction plasma spray on HA coating crystallinity, phase decomposition, and mechanical properties along with their *in vitro* and *in vivo* response. Our ***hypothesis*** is that the RF induction plasma processing can produce a homogeneous and crack free coating, which will improve the *in vivo* stability of the coated implant. The ***rationale*** is that the clear understanding of the plasma processing and phase decomposition can facilitate the design and tailoring of surfaces of load bearing implant with improved *in vivo* lifetime. We prepared HA coatings on titanium (Ti) metal using supersonic and normal plasma nozzle. The coatings were characterized for phase purity and bond strength. The *in vitro* and *in vivo* biocompatibility of the coatings was evaluated using human fetal osteoblast (hFOB) and rat distal femur model, respectively.

Methods: Commercial grade HA powder was used to coat 2 mm thick Ti substrate (President Titanium, MA, USA) using a 30 kW inductively coupled RF plasma spray system (Tekna Plasma Systems, Canada), equipped with normal and supersonic plasma nozzle and were named as NHA and SHA, respectively. Coatings were prepared with the optimized parameters of 25kW plasma power and at 110 mm working distance. Phase compositions of the coatings were studied using X-ray diffraction (XRD) and Fourier transformed infrared spectroscope (FTIR). Bond strength of the coating was evaluated using ASTM C633 methods. *In vitro* biocompatibility study using hFOB cell line was aimed to evaluate any possible toxic effect of plasma processing on HA coating. MTT assay was used to quantify the cell proliferation. The HA coating was implanted in rat femur to determine the *in vivo* biocompatibility. After 2 weeks, the rats were sacrificed and samples were prepared for histological studies.

Results: HA was identified as the major phase in both of these coatings along with other minor phases, such as α-TCP, TTCP, and CaO. Phase decomposition and amorphous phase formation was predominant in NHA coating compared to SHA coating, as shown in Figure 1. FTIR results also indicated higher crystallinity of HA in SHA coating. The major differences between these two nozzles are (1) exit velocity, and (2) the place for powder discharge which made the residence time 5 ms and 290 µs for HA particles in normal and supersonic plasma nozzle, respectively, and contributed to the higher phase decomposition and dehydroxylation of HA in NHA coating. The bond strength of the coating was found to be as high as 24 Mpa.

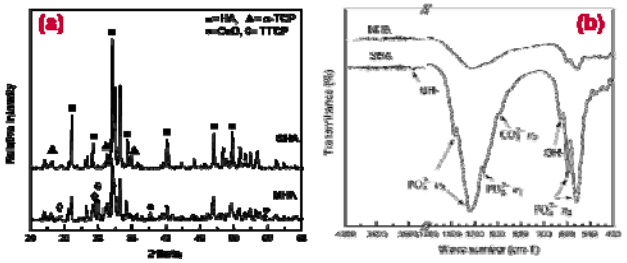

Figure 1. (a) X-ray diffraction and (b) FTIR spectra of induction plasma sprayed HA coating.

Initial attachment, growth and spreading of hFOB cells on uncoated Ti and plasma sprayed HA coating were analyzed using FESEM and is shown in Figure 2. Compared to coated surface, the uncoated Ti showed very little cell spreading at day 3. After 11 days of culture, a dense and confluent cellular layer was formed on plasma spayed HA coating [3]. The cell proliferation was determined by MTT assay, which showed improvement in cell proliferation from uncoated Ti to plasma sprayed HA coating.

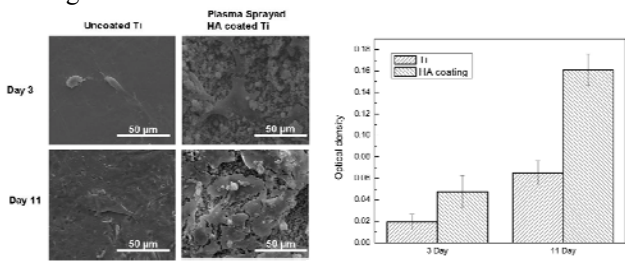

Figure 2. hFOB cell morphology and MTT assay after 3 and 11 days of culture.

Histological analysis of the *in vivo* HA coated samples in rats, indicated a close integration of the HA layer and bone whereas, a thick layer of fibrous tissue encapsulated the uncoated Ti surface.

Conclusions: Crystalline HA coatings were prepared on Ti using RF induction plasma spray equipped with supersonic nozzle. The coated surfaces showed excellent hFOB cell attachment and proliferation. The immunohistological analysis showed better bone cell integration to HA surface compared to Ti in rat distal femur model. The authors gratefully acknowledge the financial support from NIH (Grant # Ro1EB 007351).

References:

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