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In vitro and *in vivo* bioactivity of CoBlast hydroxyapatite coating and the effect of impaction on its osteoconductivity

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ABSTRACT

The novel non-thermal CoBlast process has been used recently to create a hydroxyapatite coating on metallic substrates with improved biological response compared to an uncoated implant. In this study, we compared the biological effect of coatings deposited by this process and the industrial standard technique — plasma-spray. Physicochemical properties of these two coatings have been found to be significantly different in that CoBlast HA is less rough but more hydrophilic than the plasma-spray HA as evidenced by data obtained from profilometry and goniometry. Mesenchymal stem cell attachment and adhesion are enhanced on CoBlast HA. Analysis by a combination of EDX and ICP suggests that the higher crystallinity retained by the CoBlast HA result in slower coating dissolution. Detailed *in vitro* evaluation reveals that plasma-spray HA might induce slightly faster cell proliferation and earlier osteogenic differentiation, but CoBlast HA becomes equivalent to it by the late osteogenic stage. PCR array facilitated the identification of differentially regulated genes involved in various functional aspects of *in vitro* osteogenesis by the CoBlast HA coating. The expression level of the functional protein products of these genes are in agreement with the PCR data. Coating metallic screws with HA significantly improves the *in vivo* osseointegration. By measuring of removal force using torque measurement instrument and analyzing the patterns found in X-ray images it is demonstrated that the two HA coatings elicit comparable osseointegration. Using simulated impaction model, CoBlast HA is shown to maintain better performance in cell attachment and mineralization than plasma-spray HA, especially following significant impactions. This might indicate a potentially greater osteoconductivity of CoBlast HA coating in shear-stress associated surgical applications. Collectively, it was demonstrated that CoBlast HA is an effective alternative to plasma-spray HA coating and a promising replacement for specialized surgical applications.

1. Introduction

Hydroxyapatite (HA), being chemically similar to the inorganic component of bone mineral, is one of the most popularly used bioactive ceramics in the surgical repair of hard tissue trauma and disease (Paital and Dahotre, 2009). HA's successful applications have been witnessed in a range of surgical specialties: bone substitute in bony defects restoration in orthopaedic surgery (Koshino et al., 2001), sinus obliteration (Moeller et al., 2010) and ossicular chain reconstruction (Pasha et al., 2000) in otolaryngological surgery, as well as craniofacial augmentation in plastic surgery (Quatela and Chow, 2008). In addition, HA has been extensively used as a thin film coating on titanium (Ti) alloys in load-bearing scenarios (Jaffe and Scott, 1996) and shear stress-susceptible applications (Sandén et al., 2002). The underlying rationale is to combine the high strength/weight ratio of the metallic alloy and the osteoconductivity and dissolubility of HA to achieve improved osseointegration. The outcome would be accelerated fixation of the implant by the adjacent newly formed bone tissue (Landor et al., 2007). Although various well-studied techniques exist to deposit HA onto Ti alloy substrate, plasma thermal spraying has been the industrial benchmark process owing to its high deposition rate, good biocorrosion resistance and substrate fatigue resistance of the coating, and capability to obtain various coating thickness (Sun et al., 2001). Nevertheless, the high thermal energy utilized in the plasma-spray process is its main drawback as described in the following series of events: (1) inevitable and unadjustable precipitation of crystal phases such as tricalcium phosphate (TCP) and tetracalcium phosphate (TTCP), (2) decreased crystallinity resulted in increased solubility of the coating, and (3) separation of the coating and possibly unsatisfactory *in vivo* bone fixation (Sun et al., 2003; Xue et al., 2004). Furthermore, the high temperature encountered

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eliminates the possibility of simultaneous deposition of protein or peptide based drugs such as antibiotics, anti-inflammatories and osteoinductive growth factors (Kazemzadeh-Narbat et al., 2010; Leonor et al., 2009).

Recently, an innovative room temperature microblasting coating process, namely CoBlast™, has been reported (O'Neill et al., 2009) and gained many interests by virtue of its promising performance during *in vitro* osteoconduction and *in vivo* osseointegration (O'Hare et al., 2010). CoBlast's technical versatility also allows improvement on the resultant coating's bioactivity by means of altering dopant and/or abrasive. A successful model is the creation of HA/bioglass composite coating by incorporating another extraordinary bioceramic-45S5 Bioglass (Tan et al., 2011). However, there has been no direct comparison between the HA coatings produced by this novel technique and the conventional plasma-spray technique. Therefore, one of the objectives of this study is to comprehensively compare the material properties, *in vitro* osteoconductivities and *in vivo* osseointegration on as-received HA coatings. Human mesenchymal stem cells (MSCs) have been chosen as the *in vitro* cellular model based for two reasons: (1) the bone marrow cavity, where HA coated femoral implant is inserted during total hip replacement, contains abundant pluripotent MSCs that is an unlimited self-renewal source to differentiate into osteoblastic cells (Bilezikian et al., 2008; Compston, 2002), (2) MSCs and HA-MSCs complex have already exhibited promising clinical potential in regenerative medicine (Adachi et al., 2005; Barry and Murphy, 2004). Understanding the interaction between MSCs and the HA coating is crucial, especially by bridging the cellular and mRNA levels in a pathway-specific pattern. Thus, we connected results from cell attachment/adhesion, osteogenic differentiation and PCR array with the material difference between the two HA coatings.

As mentioned above, HA coating's main usage is in load-bearing surgical applications such as the acetabular and femoral prosthesisina total hip replacement (THR) which can be accomplished in two approaches: cemented and cementless. The surgical technique applied in cementless THR to insert and fix the implant is called ‘press-fit’ during which impact forces are generally employed (Canale and Beaty, 2007). A HA coating that is less vulnerable to damage from impaction or shear stress would undoubtedly persevere longer *in situ* leading to the improved outcome of implantation. Cracking and delamination are not uncommon even in as-received and physiological fluid treated plasma-spray HA coatings (Lynn and DuQuesnay, 2002; Sun et al., 2003), but very little is known whether impaction or shear stress would worsen these features. Based on the morphological and physicochemical differences between the two as-received HA coatings, we hypothesized that their responses to the impaction would be different. Hence the second objective of our study is to answer a clinically relevant question: whether and how does physical impaction have an effect on the osteoconductivity of the HA coatings. In order to reproduce the ‘hammering’ commonly applied in cementless THR, we developed a simulated impaction system consisting of clamping and free-falling components. Changes in osteoconductivity by increasing number of impaction up to 16 times were analyzed in terms of cell attachment, osteogenic differentiation and *in vitro* matrix mineralization.

2. Materials and methods

2.1. Hydroxyapatite coatings deposition

HA coatings on Grade V Ti–6Al–4V alloy substrates were achieved on 20 mm × 20 mm × 1 mm coupons (Lisnabrin Engineering Ltd., Cork, Ireland) for *in vitro* examinations and on φ2.7 mm × 10 mm self-tapping cortex screws (Syntec Scientific Corporation, Taiwan) for *in vivo* study respectively. The *Ra* of the Ti alloy coupons were 0.32 ± 0.02 μm measured by optical profilometry based on 8 readings. All samples underwent pre-deposition processes including mechanical polishing, immersing in methanol and acetone, as well as brief ultrasonic cleaning.

Thereafter CoBlast (EnBIO, Cork, Ireland) HA coatings were deposited in a system shown schematically in Fig. 1. In brief, dopant – HA (S.A.I., Vaulx-en-Velin, France) and abrasive – MCD (Himed, New York, USA) were supplied by corresponding powder feeders and simultaneously blasted through separate nozzles onto metallic substrates. The essential deposition parameters including particle size, nozzle angle and height, powder feeder pressure and deposition direction are clearly illustrated in Fig. 1. All samples underwent post-deposition cleaning by blasts with clean dry air at 60 psi to remove non-adhered particles. They were then stored in a desiccator until autoclaved at 121 °C for 20 min prior to experiments.

On the other hand, plasma-sprayed HA coatings were attained on the same Ti alloy coupons and screws from APS Materials Inc. (Waterford, Ireland). The up-to-date APS master file conforms to the FDA guidance document titled ‘510(K) Information needed for hydroxyapatite coated orthopaedic implants’. In short, samples underwent grit blasting, substrate cleaning, HA plasma spray and removal of overspray.

2.2. Coating characterizations

Optical profilometry (Wyko NT1100, Veeco, Cambridge, UK) was conducted on the surfaces to obtain two fundamental surface roughness values (Ra and Rz). Hydrophilicity, expressed as water

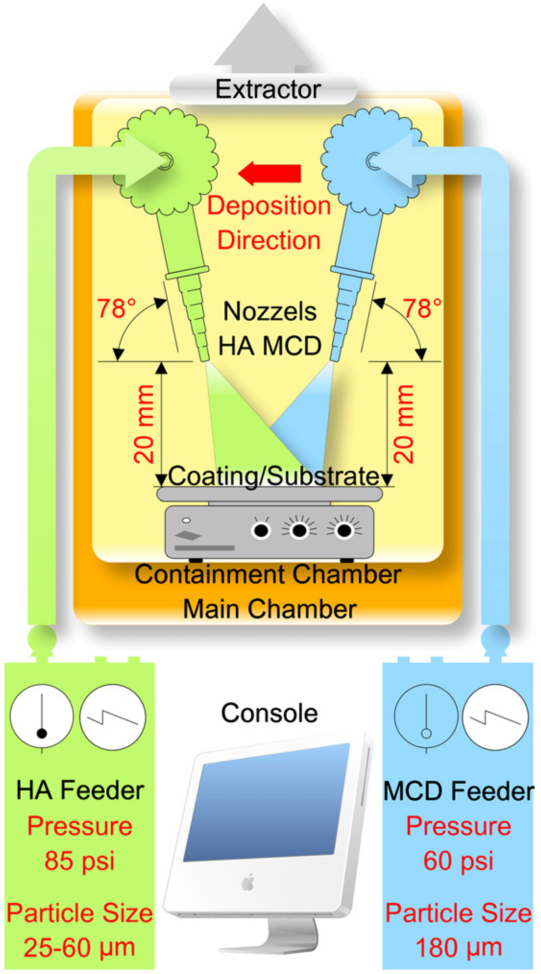

Fig. 1. Schematic of CoBlast deposition system. Black texts represent the core components of the system, and red texts refer to the essential parameters used to acquire HA coatings.