

The novel use of waste animal bone from New Zealand agricultural sources as a feedstock for forming plasma sprayed hydroxyapatite coatings on biomedical implant materials

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ABSTRACT: *This study presents the feasibility of using animal bone-derived hydroxyapatite (HAP) as feedstock powders for plasma spraying. Bovine, cervine and ovine bone from abattoirs was boiled in a pressure cooker to remove blood, fat and adhering meat tissue. The bone was then placed in a muffler furnace, pyrolyzed at approximately 1000 °C to remove collagen and residual organics, cooled and subsequently ground to a powder then digested in nitric acid. Sodium hydroxide was added to the digest to reprecipitate the HAP. Ageing of the precipitate followed by filtration, extensive washing and drying produced the white powder used as the feedstock. X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR) confirmed the powder to be poorly crystalline HAP with low-level carbonate. Out of several batches of the sieved powders, one batch was plasma sprayed to produce adherent HAP coatings; therefore, demonstrating that animal bone-derived HAP powders can be seriously considered as a feedstock powder, subject to the powder being processed for the correct rheological characteristics for easy flowing within the plasma spray flow lines. The phase composition of the successful plasma sprayed HAP coatings on both stainless steel and titanium were found by XRD to be mainly HAP with minor contributions from α -tricalcium phosphate, tetracalcium phosphate and CaO; therefore, demonstrating that feedstock decomposition on its passage through the plasma spray torch was insignificant under the conditions employed. Scanning electron microscopy (SEM) micrographs of the coatings indicated that their morphology featured the classical heterogeneous and splat-like appearance expected of plasma sprayed coatings. Young's modulus and Vicker's microhardness tests conducted on the coatings revealed values in the range, respectively, 22-87 GPa and 166-287 (HV₂₀₀) indicating high strength plasma spray HAP coatings had been produced from the feedstock powder. (Journal of Applied Biomaterials & Biomechanics 2004; 2: 96-104)*

KEY WORDS: *Animal bone, Reprecipitated hydroxyapatite, Feedstock, Plasma spray coatings, Biomedical*

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INTRODUCTION

Calcium phosphate based compounds are well-established biomaterials (1-7) in current medicine because of their excellent biocompatibility. Research in this area is intense with much published on calcium hydroxyapatite (HAP) ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) use, in particular, as bone replacements in non-load bearing applications and as coating mate-

rials to improve the biocompatibility of titanium-based load bearing prosthetics such as hip implants (8-12). Although HAP powder manufacture for implants, prostheses and feedstocks is standard practice in the USA, Europe or Japan, the cost of these materials to countries distant from the HAP-manufacturing country or to countries with developing economies remains very high; therefore, requiring a cheaper local HAP source. Animal bone from

abattoir processing, in particular from New Zealand, where the incidence of bovine spongiform encephalopathy (BSE) or other serious animal maladies like Foot and Mouth Disease is non-existent, could provide a niche market as a potentially cheap local source of extractable HAP especially if it can be used as a feedstock for plasma spraying onto titanium based implants. In Europe, animal derived-bone xenograft use is subject to strict regulation due to possible BSE transmission from livestock. For example, a product known as "LUBBOC T650" manufactured by a French company known as Ost Développement (and now owned by Osteotech Inc.) and marketed by various distributors (13) is stated to be a highly purified bovine xenograft (14-16). Its manufacture required processing by stringent protocols under current French and European law. The Directives (13) with which it was stated that xenograft's manufacture had complied were: 1) French Directive III/3298/91-FR concerning the risk of the agent responsible for BSE and; 2) European Directive III/8115/89-EN concerning validation procedures for the elimination and inactivation of viral agents. Finally, the T650 LubboC product was stated to have undergone final sterilization by accelerated electrons at 25 kGy in accordance with standard EN 552 followed by batch sterility control.

We dealt briefly, previously, with the issue of using cattle bone-derived powder for plasma spraying (17); however, no other research group has reported any study on the specific use of animal bone-derived HAP powders as plasma spray feedstocks. Given that plasma spray research is well-established in material science (8-12), the main purpose of this study (extending previously published studies and using a different method for bone processing) was to assess the feasibility of processed abattoir-derived animal bone as a novel feedstock powder for plasma sprayed HAP coatings on medical grade titanium and stainless steel substrates.

MATERIALS AND METHODS

Raw bovine, ovine and cervine femurs and ribs were obtained from New Zealand Ministry of Agriculture and Fisheries approved local commercial butcheries and processing plants. The cut and uncut bones remained frozen until further processing. A lapidary push saw was used to cut the large bovine femurs. Cutting the smaller ovine and bovine femurs was unnecessary. Raw bone pieces were boiled for 6 hr in a 7.5 L domestic pressure cooker with the water replaced every 2 hr, to remove meat, bulk surface

fat and connective tissue. In contrast to bone-derived HAP prepared in an earlier published study (17) (which had been boiled, defatted and then dissolved directly in acid for eventual reprecipitation), bone in this study was dried for 24 hr at 120 °C and then calcined at approximately 1000 °C in a muffle furnace to remove all collagen and other organics. The resultant white bone was coarsely ground using a Bico adjustable pulverizer producing a coarse powder, which is referred to as "ground pyrolyzed bone powder". This powder was ground further in Germany to a talcum powder like fineness (and called "further ground pyrolyzed bone powder") in order that it could be tested as a plasma spray feedstock. The "reprecipitated bone powder" samples tested for plasma spray feedstocks were made by digesting ground pyrolyzed bone powder in 15% (w/v) HNO₃ within a 10 L round-bottomed quick-fit flask with the filtration and then the precipitation of the digest solution in NaOH with Ca(NO₃)₂ added in a nitrogen atmosphere. After a period of ageing, the voluminous white precipitate was filtered, thoroughly washed to remove nitrate and dried to produce a fine white powder. Several batches of reprecipitated pyrolyzed bone powder were prepared for use in Germany.

The powders were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Malvern laser light scattering particle size analyzes. For the elemental analysis, the elements Fe, Cu, Mg, Na, Ni, Ca, Mn, Zn, Cd, K, Co, Cr, Hg, Pb, Al, As, Li, Sb, Sr, Ti and ions NO₃⁻ and PO₄³⁻ were analyzed in powder using inductively coupled optical emission spectrometry (ICP-OES), inductively coupled mass spectrometry (ICP-MS), molybdovanadate colorimetry (for PO₄³⁻ determination only), and flow injection analysis (FIA) (for NO₃⁻ nitrogen detection). Element extraction from the powder samples involved, in most cases, HCl/HNO₃ digestion, except for Pb, Cr and Ni, which involved HClO₄/HNO₃ digestion. Nitrate ion was extracted with water from the powders, reduced to nitrite ion, and subsequently analyzed by FIA. The volume-based particle size distributions in the plasma spray feedstocks were measured using a Malvern Mastersizer S Long Bed with a MS17 sample introduction unit. The lens range was 300RF mm giving a size range of 0.05-900 μm. Beam length was 2.4 mm. Obscuration was between 10% and 20%. The data was analyzed using a polydisperse model with an assumed HAP particle density of 3.16 g cm⁻³ (18). Malvern measurements were taken on both ultrasonicated and static (no ultrasonication applied) samples. XRD analysis was carried out by Industrial Research Ltd (Lower Hutt,

New Zealand) and performed on both the plasma spray feedstock powders and the plasma sprayed coatings on the stainless steel and titanium substrates using a Philips PW1700 series automated powder X-ray diffractometer with a graphite-diffracted beam monochromator, running at 40 kV and 35 mA using cobalt radiation. Unit cell refinements to obtain information on unit cell dimensions were carried out using software by Industrial Research Ltd (as above). The feedstock powders were shipped to Germany where they were dried at 100 °C for several days prior to use. Pre-sieving using RETSCH-brand wire sieves connected to a vibrating apparatus was carried out on the powders in order to select particle agglomerates between 63 and 100 µm for plasma spraying. Plasma spray coating was performed at the Technical University of Chemnitz, Germany, using a Plasma Technik Lab 1200 VPS plasma coating instrument. Implant grade stainless steel and pure titanium disks (40 x 10 mm) gritblasted with alumina particles immediately prior to coating were used as substrates. Table I summarizes the parameters used in the VPS. Powders were stored in heated, internally stirred canisters immediately prior to supply into the plasma spray lines.

Coatings produced were examined by SEM/energy dispersive X-ray analysis (EDX), first, using a Jeol 840 (Germany) and subsequently using a Hitachi S100 FESEM (New Zealand) equipped with an IXRF system for EDX measurements. EDX measurements performed with the Hitachi unit were measured relative to a calcium and phosphorus standard embodied in fluorapatite manufactured by Microanalysis Consultants (UK) Ltd.

Mechanical testing (Nanyang Technological University, Singapore) was performed on coated samples labeled "Coat5", "Coat 7", "Coat15" and "Coat17" consisting of approximately, respectively, 348 µm, 184 µm, 100 µm and 125 µm thick plasma sprayed HAP coatings from reprecipitated cattle bone on medical grade stainless steel (Coat5, Coat7 and Coat17) and titanium (Coat15). Young's modulus (E) of the coatings was measured through an indentation test using Vickers micro-indentation equipment (CSEM® MHT, Switzerland). The testing was performed and rechecked twice using initially loadings of 50 mN and 300 mN for confirming values with a 100 mN/min loading rate and for 10 sec. The unloading rate was 100 mN/min. During the indentation test for Young's modulus determination, every two points had a distance of 50 µm, which was sufficiently long to avoid the influence of previous loading on the following indentation. The microhardness (Vicker's) of the coatings was measured from their polished cross-sections using an

HMV-2000 Shimadzu microhardness tester. A 200 g load (a larger load caused coating fracture) was employed for 15 sec. For each plasma spray coated sample tested, 10 readings were collected for an average value.

RESULTS AND DISCUSSION

Characterization of the powder feedstocks

The ground pyrolyzed bone powder (prior to its further grinding treatment in Germany) was coarse in texture and had a grainy salt-and-pepper appearance. FTIR spectra of the powder indicated it was composed of crystalline HAP. XRD of the further ground pyrolyzed bone powder confirmed that the bulk of the powder consisted of crystalline HAP containing approximately 7% carbonate (occurring most likely via atmospheric CO₂ uptake while cooling after pyrolysis completion) with a small amount of periclase (MgO). Malvern measurements of the statically sampled ground pyrolyzed bone powder gave an average particle size represented by the volume weighted mean diameter, i.e. De Broucker mean, D[4,3] of 405 µm. Ultrasonication of the sample caused the D[4,3] value to drop to 357 µm. The further ground pyrolyzed bone powder had average particle sizes determined by the Malvern Mastersizer in the range 21.5-23 µm for ultrasonicated and static sampling. However, there were no coatings prepared from the further ground pyrolyzed bone powder because it failed to flow through the plasma spray powder flow lines and into the plasma gun.

In contrast, the reprecipitated bone powders were

TABLE I - INSTRUMENTAL PARAMETERS USED FOR PRODUCING PLASMA SPRAY CALCIUM PHOSPHATE COATINGS IN THE PLASMA TECHNIK LAB 1200 VPS

Chamber pressure	600 mbar Ar
Plasma gas	60 L min ⁻¹ Ar, 12 L min ⁻¹ H ₂
Plasma current	600 A
Plasma voltage	60-63 V
Plasma power	36,000-37,800 W
Spraying distance	130 mm
Carrier gas	Ar
Burning velocity	5000
No. of passes	8
Nozzle	Dusche type 8 mm

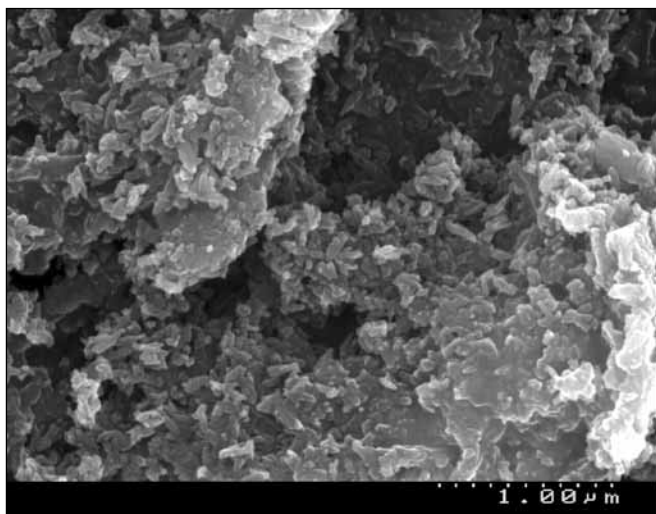


Fig. 1 - SEM showing the typical appearance of the reprecipitated pyrolyzed bone powder particles used for plasma spraying.

white and fine in texture. The XRD and FTIR traces of the powders indicated a mainly HAP phase with approximately 3% carbonate of poorer crystallinity than the HAP in the further ground pyrolyzed bone powder. SEM analysis of the reprecipitated bone powders showed the typical short rod-like morphology expected for HAP powders (Fig. 1). Statically sampled Malvern Mastersizer measurements of the reprecipitated bone powders before and after sieving demonstrated that the un-sieved powder had a higher D[4,3] value of 64.7 μm than the sieved powder, which had a D[4,3] value of 38.6 μm . Although the direct comparison of particle size values obtained from metal sieving with those obtained from Malvern measurements was not experimentally valid, the difference in the average particle sizes (between sieved and un-sieved powders) from the statically sampled powders can be regarded as a consequence of the 63-100 μm metal sieving, which eliminated larger particles from the as-prepared powders. Interestingly, if Malvern Mastersizer measurements of sieved and un-sieved powders were acquired with ultrasonication, the average particles size D[4,3] values were close at, respectively, 29.50 μm and 23.92 μm . This indicated that the primary particle sizes after allowing for the breakup of aggregates in solution by the ultrasonication of the two samples were similar.

Elemental profiles as measured by ICP-OES, ICP-MS, FIA and molybdovanadate colorimetry of the further ground pyrolyzed bone HAP, reprecipitated HAP before sieving and reprecipitated HAP after sieving are shown in Table I. In general, these demonstrated low levels of any potentially toxic heavy metals such as Hg, Cd, and Pb. The Ca:P

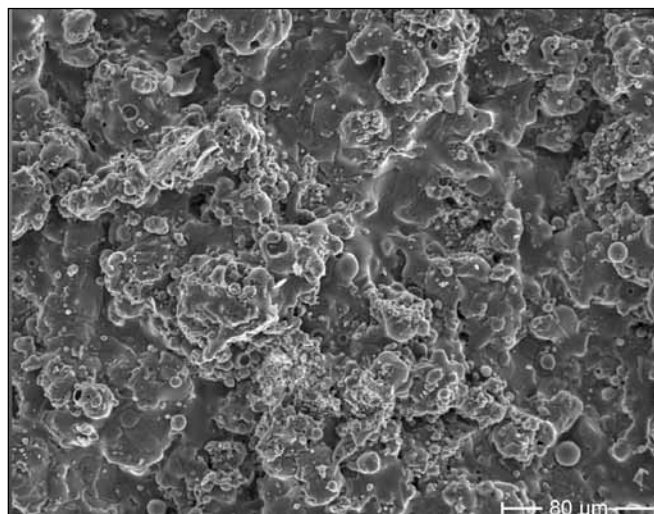


Fig. 2 - SEM of the surface of a plasma sprayed HAP coating on medical grade stainless steel from reprecipitated New Zealand animal bone (mixture of cervine, ovine and bovine bone). This represents a coating produced by eight passes of the plasma spray gun over the surface.

mole ratio (commonly used to indicate the calcium phosphate phase present) for the powders ranged from 1.70-1.75. This demonstrated that the dominant phase in the powders was HAP ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, theoretical Ca:P mole ratio = 1.67), although the fact that Ca:P mole ratios were >1.67 indicated carbonated apatite presence in the powders, which agreed with the FTIR and XRD analyzes (see above). The presence of 90-120 ppm nitrate (Tab. II) in the reprecipitated pyrolyzed bone samples was a direct consequence of using nitric acid to digest the pyrolyzed bone powders. The increase in Cu, Fe, Cd, Cr, Ni and Ti levels between the un-sieved and sieved reprecipitated bone powders shows the effect of using metal sieves. Although every effort was made to use cleanly dusted sieves, obviously sieving can introduce unwelcome metal contamination in the powders either from the actual sieve itself or possibly from tiny traces of residual powders clinging to the metal screens from previously sieved samples.

Plasma spraying trials

Initial trials establishing the usability of several reprecipitated pyrolyzed bone powder feedstock batches eliminated all but one of the powders on the basis of the flowability from the storage canisters on the VPS instrument to the supply lines that took the powders directly to the plasma spray nozzle. Therefore, data pertaining to coatings in this study are derived from the use of one batch of

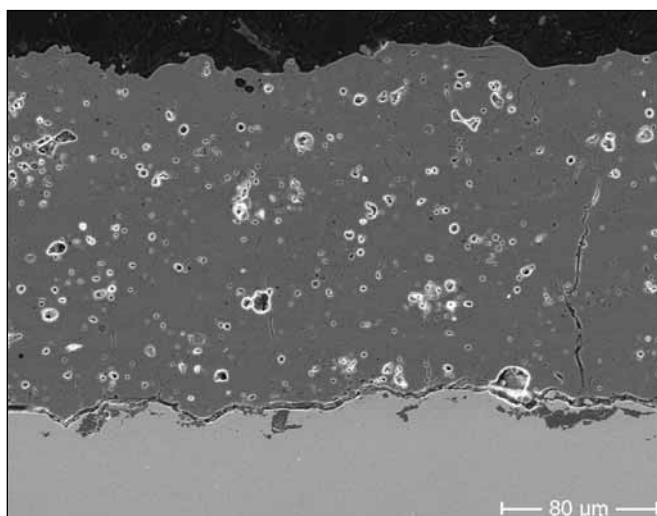


Fig. 3 - SEM of a metallographically prepared cross-section of a plasma sprayed HAP coating on medical grade stainless steel from sieved, reprecipitated New Zealand animal bone (mixture of cervine, ovine and bovine bone). This represents a coating produced by eight passes of the plasma spray gun over the surface. Coating thickness ~ 267 μm.

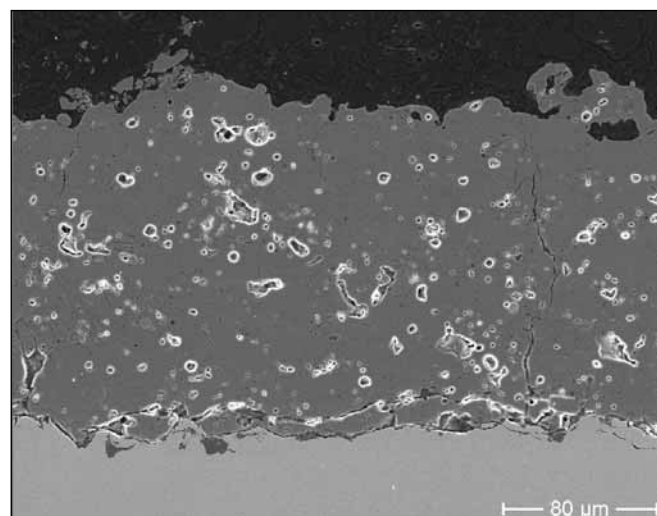


Fig. 4 - SEM of a metallographically prepared cross-section of a plasma sprayed HAP coating on medical grade titanium from sieved reprecipitated New Zealand animal bone (mixture of cervine, ovine and bovine bone). This represents a coating produced by eight passes of the plasma spray gun over the surface. Coating thickness ~ 100 μm.

TABLE II - ELEMENTAL PROFILES OF BONE AFTER PYROLYSIS AT 1000 °C AFTER CRUSHING AND FURTHER GRINDING, AND HAP POWDERS REPRECIPITATED FROM ACID-DIGESTED PYROLYZED BONE BEFORE AND AFTER SIEVING

Element sample	Further ground pyrolyzed bone	Reprecipitated bone before sieving	Reprecipitated bone after sieving
Ca (%)	40	40	38.4
P (%)	17.76	17.64	17.46
Ca:P mole ratio	1.74	1.75	1.70
Mg (%)	0.67	0.65	0.53
Na (%)	0.95	1.61	0.99
K (ppm)	550	<30	<30
Fe (ppm)	107	172	239
Cu (ppm)	34.9	5.1	35.6
Mn (ppm)	1	3	2
Zn (ppm)	25	42	50
Al (ppm)	37	137	122
As (ppm)	1	1.3	1
Cd (ppm)	0.04	0.06	0.44
Cr (ppm)	2	1	21
Hg (ppm)	<0.05	<0.05	<0.05
Ni (ppm)	13	2	611
Pb (ppm)	1.6	2.8	1.7
Li (ppm)	15.1	8.2	7.1
NO ₃ -N, nitrate nitrogen (ppm)	1	120	90
Sb (ppm)	1.7	2.1	2.1
Sr (ppm)	379	192	230
Ti (ppm)	<4	<4	90

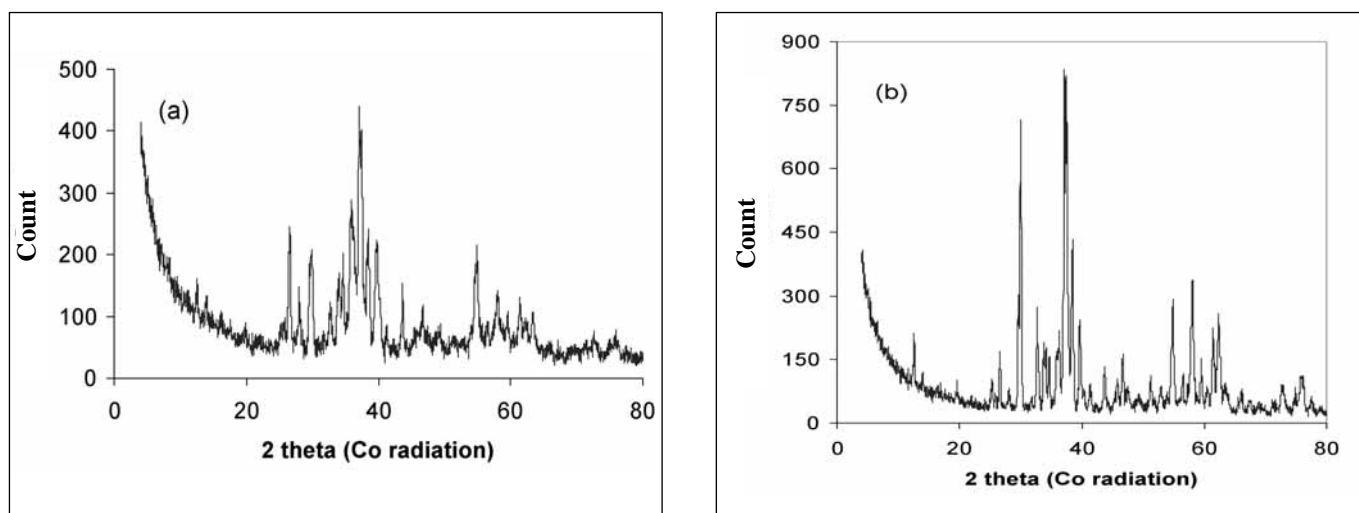


Fig. 5 - XRD patterns of plasma sprayed HAP coatings from reprecipitated animal bone formed on a) stainless steel substrates and b) titanium substrates.

sieved, reprecipitated pyrolyzed bone powder. The failure of the other powders was perceived to be a rheological problem, possibly caused by agglomeration of the fine particulate matter in the powders, which precluded their smooth flow into the plasma spray lines. This impasse occurred at the powder entry point from the bottom of the storage canisters into a revolving plate containing a shallow groove into which powder from the canisters was meant to drop.

Evaluation and characterization of the plasma sprayed coatings produced

In general, plasma sprayed coatings produced on both titanium and stainless steel substrates were adherent not exhibiting any outwardly obvious signs of flakiness or spalling. The texture of the coatings was rough in nature and possessed a slight brown discoloration believed to be caused by dust impurity uptake during cooling or from other feedstock materials used in the spraying chamber during earlier spraying experiments. Coating thickness (as measured, approximately, by the differences in the micrometer-measured thicknesses of the disks before and after the plasma sprayed coating application) ranged from 100-400 μm . Figure 2 shows a SEM illustrating the typical appearance of a plasma sprayed HAP coating on medical grade stainless steel produced from the reprecipitated bone powder. The surface demonstrated the characteristic molten splat-like appearance expected of plasma sprayed HAP coatings (19). SEM micrographs of the surface coatings on both the stainless steel and the pure titanium disks showed practically identical

morphological characteristics. The SEM micrographs of metallographically prepared cross-sections of the coating on stainless steel in epoxy resin (Fig. 3) demonstrated porosity and some cracking, although not to the extent to cause any spalling of the material from the substrate. The cross-sections of the plasma sprayed coatings on the titanium disks were similar in nature apart from the appearance of relatively more cracking and porosity (Fig. 4). Quantitative EDX analyzes and Ca:P mole ratio calculations of the plasma sprayed calcium phosphate coating surfaces mainly gave values in the range 1.6-1.8, additional evidence that the coating material consisted largely of HAP (theoretical Ca:P molar ratio = 1.67).

XRD analysis of the plasma sprayed coatings of the reprecipitated pyrolyzed bone plasma sprayed onto stainless steel substrates and onto titanium substrates are illustrated in, respectively, Figure 5a and b. Coatings prepared on stainless steel for the three samples examined gave essentially identical diffraction patterns indicating that the same phases had been consistently formed during spraying. The phases formed were found to be HAP (major phase), α -tricalcium phosphate, tetracalcium phosphate ($\text{Ca}_4\text{O}(\text{PO}_4)_2$) and CaO (all minor phases), in agreement with results obtained from a preliminary study (17) on plasma spraying using differently prepared animal bone-derived feedstock powders.

The XRD pattern of plasma sprayed coatings prepared on titanium substrates, despite being slightly different in appearance to those of the coatings on stainless steel (Fig. 5b), showed that the coatings had the same phase distribution. The difference in

spectral appearance was caused by the HAP phase exhibiting pronounced preferential orientation with the "c" axis normal to the titanium surface (this is obvious from viewing the unusually high intensity of the peak at approximately 30° , 2θ in Figure 5b relative to the peak at the same 2θ value in Figure 5a). It was not clear why this preferential crystallite orientation occurred on titanium and not on stainless steel, but it could be caused by the crystallographic texture of the particular grade of titanium used due to its manufacture, which could predispose HAP coatings to form with a specific epitaxy form on the surface. The preferred orientation phenomenon was not observed in plasma sprayed coatings of animal derived calcium phosphate on the thin rectangular titanium plates used in an earlier preliminary study (17).

Young's modulus and Vicker's microhardness measurements on four coated specimens consisting of plasma sprayed stainless steel and titanium substrates showed that the coatings produced had good mechanical characteristics (Fig. 6). The values obtained for both Young's modulus and Vicker's microhardness were noticeably influenced by the overall thickness of the coatings produced. The thicker coatings (Coat5, Coat7 samples with a thickness of approximately $348\ \mu\text{m}$ and $185\ \mu\text{m}$ respectively) gave high Young's modulus values of 85-87 GPa, whereas the Coat15 and the Coat17 samples (with a thickness of approximately $100\ \mu\text{m}$ and $125\ \mu\text{m}$ respectively) gave lower Young's modulus values of 22-24 GPa. For Vicker's microhardness, the HV_{200} values obtained from the Coat5/Coat7 and Coat15/Coat17 samples were in the range, respectively, 286-275 and 168-176.

The thicker coated samples gave exceptionally high Young's modulus values compared to values of 19-31 GPa reported in earlier studies of plasma spray coated HAP (20). This suggests that the cattle bone-derived feedstock powders could produce coatings that acquire very favorable mechanical characteristics with sufficient thickness.

In conclusion, these studies demonstrated that animal bone-derived HAP powders are viable feedstock powders, which can be successfully applied to medical grade titanium and stainless steel by plasma spraying to produce mechanically strong coatings. Under the conditions employed in this study, plasma spraying led to a coating that mainly consisted HAP with some minor phases such as α -tricalcium phosphate, tetracalcium phosphate and CaO. The importance that the prepared powders possess the correct rheological characteristics was also highlighted in this study, with several of the prepared feedstock powders not used for plasma

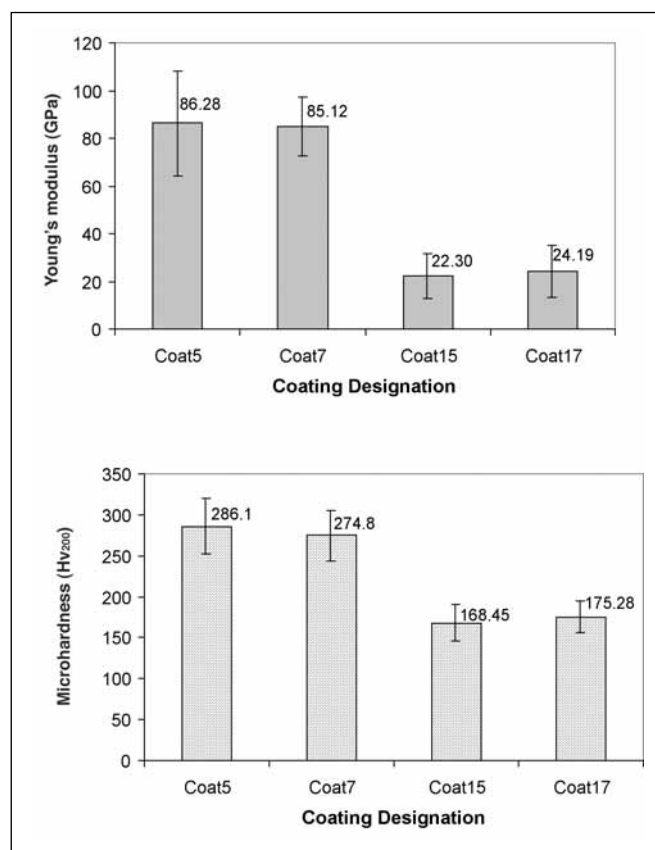


Fig. 6 - Summary of (top) Young's modulus and (bottom) Vicker's microhardness data for HAP derived from reprecipitated sintered cattle bone plasma spray coated on stainless steel (Coat5, Coat7, Coat17) and on titanium (Coat15) (see text for details). Error bars in the measurements are shown.

spraying due to the mechanical agglomeration of particles, which prevented them from flowing into the plasma spraying powder flow lines. This study also demonstrated that animal bone can be easily turned into HAP for coating biomedical implants; therefore, adding value to a previously low value waste material and, in addition, benefiting the environment by preventing or reducing the unnecessary dumping of abattoir-derived bone. Potential limitations or increases in processing costs could include bone supply and the need to ensure rigorous processing to exclude organic traces such as fat and protein. For example, although New Zealand has no BSE incidence in its cattle, it could be necessary in any commercial processing protocol to ensure that the material is subjected to prolonged dry heat $>600\ ^\circ\text{C}$ to remove any traces of proteinaceous matter to allay concerns about prions (21). However, the presence of a base reprecipitation step in this study coupled with sintering or hypochlorite treatment of the bone more than satisfies this condition

for the removal of all organic or proteinaceous matter that risks BSE transmission (22). A major challenge in the manufacture is to produce from naturally variable bone products, a consistent feedstock powder with the correct rheological properties to flow reliably into plasma flow supply lines and form uniform thick coatings.

Finally, the true biocompatibility of plasma spray coated implants made from animal-derived HAP feedstocks requires testing. Given that the material is effectively plasma sprayed with reprecipitated calcium deficient HAP, the likely biocompatibility is expected to be comparable, if not identical, to similar coatings derived from synthetically sourced HAP. In fact, the hydroxycarbonate apatite xenotransplants developed in our laboratories from defatting and deproteination of New Zealand bovine cancellous bone cubes have been recently clinically tested (23) and found to be osteoconductive in a sheep model. This paints a very positive picture of cattle bone materials as candidates for plasma spray feedstocks and promises to be an issue worth pursuing in future studies in this area.

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REFERENCES

1. de Groot, K. Clinical applications of calcium phosphate biomaterials: a review. *Ceramics International* 1993; 19: 363-6.
2. Song L, Gosain AK, Amarante MT, et al. Optimising osteoinduction with hydroxyapatite biomaterials. *Surg Forum* 1998; 49: 663-5.
3. Suchanek W, Yoshimura M. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *J Mater Res* 1998; 13: 94-117.
4. Rey C. Calcium phosphates for medical applications. In: Amjad Zahid ed. *Calcium Phosphates in Biological and Industrial Systems* 1998: 217-351.
5. Gosain AK, Persing JA. Biomaterials in the face: benefits and risks. *J Craniofac Surg* 1999; 10: 404-14.
6. Chow LC. Calcium phosphate cements: chemistry, properties, and applications. *Materials Research Society Symposium Proceedings (Mineralisation in Natural and Synthetic Biomaterials)* 2000: 27-37.
7. Vallet-Regi M. Ceramics for medical applications. *J Chem Soc Dalton Trans* 2001: 97-108.
8. Gottschling S, Kohl R, Engel A, Oel HJ. Characterisation of plasma sprayed hydroxyapatite coatings on titanium tooth root implants. *Ceramic Transactions* 1995; 48: 201-13.
9. Kweh SWK, Khor KA, Cheang P. Plasma sprayed hydroxyapatite (HA) coatings with flame-spheroidised feedstock: microstructure and mechanical properties. *Biomaterials* 2000; 21: 1223-34.
10. Sun L, Berndt CC, Gross KA, Kucuk A. Material fundamentals of plasma sprayed hydroxyapatite coatings: a review. *J Biomed Mater Res* 2001; 58: 570-92.
11. Lee TM, Wang BC, Yang YC, Chang E, Yang CY. Comparison of plasma sprayed hydroxyapatite coat-

- ings and hydroxyapatite/tricalcium phosphate composite coatings: *in vivo* study. *J Biomed Mater Res* 2001; 55: 360-7.
12. Carayon MT, Lacout JL. Study of the Ca/P atomic ratio of the amorphous phase in plasma sprayed hydroxyapatite coatings. *J Solid State Chemistry* 2003; 172: 339-50.
 13. Information obtained from the websites: www.maitrise-orthop.com and www.ebedent.com.
 14. Bertrand G, Aubert H, Boyer J. The use of T650 in bony filling: experimental and clinical results. *Acta Stomatol Belg* 1994; 91: 197-216.
 15. Morax S, Hurbli T, Smida R. Bovine heterologous bone graft in orbital surgery. *Annales de Chirurgie Plastique et Esthetique* 1993; 38: 445-50.
 16. Poumarat G, Squire P. Comparison of mechanical properties of human, bovine bone and a new processed bone xenograft. *Biomaterials* 1993; 14: 337-40.
 17. Johnson GS, Mucalo MR, Lorier MA, Gieland U, Mucha H. The processing and characterization of animal-derived bone to yield materials with biomedical applications. Part II: milled bone powders, reprecipitated hydroxyapatite and the potential uses of these materials. *J Mater Sci Mater Med* 2000; 11: 725-41.
 18. Aoki H. *Medical Applications of Hydroxyapatite*, Ishiyaku EuroAmerica Inc. Tokyo, 1994.
 19. McPherson R, Gane N, Bastow TJ. Structural characterization of plasma-sprayed hydroxyapatite coatings. *J Mater Sci Mater Med* 1995; 6: 327-34.
 20. Kweh SWK, Khor KA, Cheang P. An *in vitro* investigation of plasma sprayed hydroxyapatite (HA) coatings produced with flame-spheroidized. *Biomaterials* 2002; 23: 775-85.
 21. Brown P, Rau EH, Johnson BK, Bacote AE, Gibbs CJ Jr, Gajdusek DC. New studies on the heat resistance of hamster-adapted scrapie agent: threshold survival after ashing at 600 degrees C suggests an inorganic template of replication. *Proc Natl Acad Sci USA* 2000; 97: 3418-21.
 22. Taylor DM. Inactivation of BSE agent. *Developments in Biological Standardisation* 1991; 75: 97-102.
 23. Mucalo MR, Johnson GS, Worth A, et al. The use of New Zealand animal bone as bioceramic bone xenografts: Concept to clinical study. *Key Engineering Materials* 2003; 240-242: 427-30.