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Characterization and dissolution of functionalized amorphous calcium phosphate biolayers using single-splat technology

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ABSTRACT

New processing routes and characterization techniques underpin further growth of biomaterials for improved performance and multifunctionality. This study investigates the characteristics and solubility of amorphous calcium phosphate (ACP) printed splats. Splats made from 20 to 60 μm molten hydroxyapatite particles were classified for shape (rounded/splashed) and cracking. Recoil of the spread droplet created a bowl-shaped splat. This has previously not been observed and could be related to the longer solidification time associated with solidification to an ACP. A central depression was created from 20 μm particles, but a bowl-shaped splat from 60 μm particles. Cracking was more prevalent for splats that solidified with an edge discontinuity. Splats immersed in pH 7.3 tris buffer displayed dissolution followed by cracking. Cracking continued over a period of 15 min as dissolution induced more cracks. Further degradation occurred by delamination of splat segments. Delamination accelerated the process of splat removal. Applied to thermal spray coatings, this highlights topography and dissolution at the splat level. The use of separate splats can potentially be used as a biolayer where splats are separate, in a line or on top of each other.

1. Introduction

Hydroxyapaptite (HAp) based coatings are used extensively in the orthopedic industry to provide an osteoconductive surface to facilitate bone ingrowth and firm fixation of the implant. These coatings are built up by the sequential deposition of splats from individual powder particles, which together form a highly complex structure in terms of morphology and chemistry of the individual splats which is dependent on the thermal history of the droplet. Commercially, these coatings are most often produced by plasma spraying and this technique of coating HAp produces a diverse range of calcium phosphate phases (HAp, oxyhydroxyapatite, calcium oxide, tricalcium phosphate and tetracalcium phosphate) that appear concurrently, depending on the heating condition [1,2], thus preventing the analysis of separate phases. Therefore, due to the complex nature of coatings, including the varied morphology and chemistry even at a single splat level – the building blocks of coatings – in order to understand behavior of the complete coating, it is important to first consider the morphology and behavior of a single splat. Single splats have been produced by high velocity oxygen fuel [3] and plasma spraying [4], but these have always been splashed. Recently, we have shown more uniform splat geometries produced by flame spray [5].

Liquid flow from droplet impact produces a sequence of unique geometries during spreading that depend on surface conditions (e.g. surface energy, temperature and roughness) and droplet characteristics (e.g. viscosity, surface energy, kinetic energy). Droplet spreading has been investigated to avoid splashing, which in turn leads to coatings with less porosity and stronger adhesion. Little attention has been given to the range of droplet geometries that can be frozen through rapid solidification. Previous work has shown that a hemispherical droplet forms from a more viscous liquid [5]. A symmetric shape can be changed to an off-center shape with faster movement of the torch. The majority of work is focused on more fluid droplets that flatten to a disk. This work will address a splat with a higher rim and determine the change in shape with droplet size.

The importance of this work extends to thermal spray processes (air and vacuum plasma spraying, high velocity oxy fuel and flame spraying), pulsed laser deposition and low-temperature processes such as electrostatic spray deposition and printing. A higher substrate temperature promotes slower solidification [6], leading to a denser top layer [5], a pathway to a multifunctional inorganic printed deposit. Conditions here are chosen to maintain an amor-

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phous calcium phosphate (ACP) to separate solidification from the droplet spreading process. It is proposed that if solidification is delayed, the initial droplet kinetic energy is converted to surface energy of the spread droplet that drives the recoil process.

Splashing of the droplet depends on the substrate temperature, with little splashing on titanium surfaces preheated above 100 °C [7]. Preheat removes absorbed moisture and assists the bond formation with the underlying surface [8]. Hydroxyapatite has a low fracture toughness and so residual stress manifests in the formation of cracks. The population of cracks has not been analyzed to date since previous studies were on splashed splats that were not reproducible and did not contain a single phase. Cracking of rounded splats and those with splashes will be examined in the as-sprayed condition and after immersion in a tris buffer solution in order to simulate the in vivo environment.

Previous work on solidified splats has examined a splashed flattened droplet with micro-Raman spectroscopy to identify the phases undergoing dissolution from the splat [9]. A period of 2 h was previously found to remove a solidified splat [10], but there has been no discussion of the splat removal mechanism. Previous work on commercial coatings with partially molten particles has shown faster dissolution of the resolidified portion. This solid core then provides a site for remineralization [11] and may become dislodged upon more severe degradation [12,13]. The in vitro degradation process of a single splat containing a single phase has not yet been determined.

This study investigates single flattened solidified splats and provides a new classification system that includes not only shape, but also degree of cracking. Use of powder with a narrow particle size range has produced droplets with a similar melt viscosity and thus produced a population of flattened solidified disks with the same thermal history. The shape and topography of the micron-thick splats are first characterized and then tested for solubility. A mechanism of degradation will be given with respect to the splat characteristics, emphasizing the importance of controlling the characteristics in the splat.

2. Materials and methods

2.1. Sample preparation

Commonly used powders exhibit a wide size distribution and lead to different degrees of particle melting and fluid viscosity, thus producing an array of rounded/splashed splats with different splat heights and shapes. This study chose a narrow particle size to completely melt all particles and heat droplets to a set liquid viscosity for deposition onto a surface. Hydroxyapatite powder (CAM implants, Netherlands) was sieved and 20–60 μm powder used for spraying. Characterization has been documented in a different paper that has used the same powder [14]. The sieved powder was fed into a flame spray torch (Metco 5P, Sulzer Metco, Switzerland) and a manual flame spraying system was used with acetylene as the fuel gas combusted in oxygen gas, and compressed air as the powder carrier gas. Polished disks (10 mm diameter, 2 mm thick) of annealed, 99.6% pure titanium (Goodfellow, UK) were prepared by grinding on 800, 1000, 1200 SiC paper and then polished on a Dur surface (Struers, Denmark). This produced a roughness less than 1 μm R_a. Substrates were vertically mounted and preheated to 100 °C with the flame spray torch. Splats were formed by spraying powder at 2 g min^{-1} at a spray distance of 8 cm onto both substrates.

2.2. Splat classification

The entire surface with splats was imaged using optical microscopy (BH2-UMA, Olympus, Japan) to classify each splat. Classifications were determined based on cracking and edge uniformity. Splats were divided between rounded and splashed splats, and a further division was made to differentiate those with cracks. The extent of cracking varied; however, this was not differentiated in the classifications.

2.3. Splat topography

Each classification of the droplet was carbon coated to enhance electron surface conduction and imaged by scanning electron microscopy (XL 30 Phillips). Atomic force microscopy (MFP-3D, Asylum Research) was performed to determine the topography of a deposited HAp splat.

2.4. In vitro behavior

The dissolution of single splats was observed using optical microscopy. Splats were identified and optical images were taken prior to dissolution. The titanium disks covered in single splats was placed in 30 ml of 0.1 M tris-(hydroxymethyl)-aminomethane solution (Tris buffer) (Sigma Chemicals, USA), titrated to pH 7.3 at 37 °C using 1 M HCl, stirred at 200 rpm in a water bath. Deionized water was used. A PHM250 Ion Analyzer with a calcium selective ion electrode (Radiometer Analytical, Lyon, France) was calibrated using calcium standard solutions (0.04, 0.4, 4, 40 ppm) prepared from $CaCl_2 \cdot 2H_2O$ (MERCK Pty Ltd, Australia) and 0.1 M Tris buffer. The calcium electrode monitored calcium concentration in the solution over time. The concentration was normalized to the initial weight of splats adhered to the surface of a disk (calculated to be approximately 1 mg) or amount of powder (20 mg).

Splats were imaged at 0, 2, 5, 10, 15, 20, 30, 45, 60 and 90 min to visually monitor the changes in a rounded splat during the dissolution process. A single splat was chosen and the numbers of cracks at different time periods were calculated to determine the crack formation over time. Energy-dispersive spectroscopy was performed on a range of splats, monitoring the calcium and titanium distributions.

3. Results

3.1. Classification of flattened solidified droplets

Observation of more than 300 splats revealed variation in the shape and cracking of the splats on six polished titanium coupons. A system was developed to classify and quantitate splats from flame spraying. The classification system was based on the shape of the deposit as well as the presence of cracks. Splats with a uniform, circular edge/outline were defined as Class I, while those with discontinuous edges were Class II (Fig. 1). Splats with no cracks and those with cracks were further subdivided. The majority of ACP depositions were Class I with uniform, circular edges and an absence of cracking. Splats with a circular edge represented at least 65% of all those samples.

3.2. Topography of a single splat

The topography of splats suggests complete melting of the HAp powder particle during the thermal spray process rather than the retention of a solid crystalline core that is often reported [15]. Flame spraying of HAp produced ACP splats, as confirmed previously by Raman spectroscopy (not shown here). Scanning electron microscopy shows that splats are thinner in the center than at the edges producing a flat central area with a raised perimeter (Fig. 1). The rim thickness is uniform for similarly sized splats, suggesting a consistent and reproducible rate of solidification. However, it was