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Antibacterial Property of Cold-Sprayed HA-Ag/PEEK Coating

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The antibacterial behavior of HA-Ag (silver-doped hydroxyapatite) nanopowder and their composite coatings were investigated against *Escherichia coli* (DH5α). HA-Ag nanopowder and PEEK (poly-ether-ether-ketone)-based HA-Ag composite powders were synthesized using in-house powder processing techniques. Bacteria culture assay of HA-Ag nanopowder and their composite powders displayed excellent bacteriostatic activity against *E. coli*. The antibacterial activity increased with increasing concentration of HA-Ag nanoparticle in these composite powders. These nanocomposite powders were subsequently used as feedstock to generate antibacterial coatings via cold spray technology. The ratios of HA-Ag to PEEK in their composite powders were 80:20, 60:40, 40:60, and 20:80 (wt.%). Microstructural characterization and phase analysis of feedstock powders and as-deposited coatings were carried out using FESEM/EDX and XRD. Antibacterial nanocomposite HA-Ag/PEEK coatings were successfully deposited using cold spraying parameters of 11-12 bars at preheated air temperature between 150 and 160 °C. These as-sprayed coatings of HA-Ag/PEEK composite powders comprising varying HA-Ag and PEEK ratios retained their inherent antibacterial property as verified from bacterial assay. The results indicated that the antibacterial activity increased with increasing HA-Ag nanopowder concentration in the composite powder feedstock and cold-sprayed coating.

Keywords characterization of HA biomaterial, cold gas dynamic spraying of nanopowders, nanopowders, spray deposition

1. Introduction

Cold spraying is part of the large family of thermal spray processes and is now widely known as a materials deposition process for relatively small particles (ranging in size from approximately 1 to 50 micron in diameter). Upon deposition, spray materials experience little change in microstructure with little oxidation and decomposition. Most metals such as Cu, Al, Ni, Ti, and Ni-base alloys can be deposited by cold spray (Ref 1-4), and even cermets (Ref 5) and ceramics can be embedded into a substrate to form thin-layered coatings. A potential advantage of this process over thermal spray is the ability to generate dense coatings while retaining their feedstock material chemistry and phase composition.

Recently, the antibacterial activity of some ceramic powders has been highlighted and much attention is now directed toward using them as a new substitute over conventional organic agents (Ref 6), which tend to contain noxious materials harmful to humans. Ceramic powders of zinc oxide (ZnO), calcium oxide (CaO), and magnesium oxide (MgO) have been found to show a marked antibacterial activity even without the presence of light. Moreover, the use of these ceramics has the advantage of containing mineral elements that are essential to the human body as well as a strong antibacterial activity, even when present in small concentrations (Ref 7). Al-ZnO coatings were earlier deposited on glass at room temperature using cold spraying parameters (Ref 8). The results indicated that the antibacterial activity increased with increasing ZnO nanopowder concentration in the composite powder feedstock and cold-sprayed coating.

In previous works, the antibacterial ceramics based on hydroxyapatite (HA) were successfully prepared in a wet chemical process with additions of $AgNO_3$ (Ref 9). Feng et al. cited that Ag-doped HA coatings on implants exhibited strong antibacterial effects (Ref 10). In addition, the incorporation of Ag+ ions into microporous HA coatings can be used as an effective bioactive delivery systems for the slow release of antibiotics (Ref 11).

PEEK (poly-ether-ether-ketone) has become one of the most attractive polymeric materials used in industry because of its excellent thermal stability, friction reduction, and wear resistance (Ref 12). To meet the stringent demand of engineering and design driven by ecological and economical reasons, some researches have recently considered using thermally sprayed PEEK coating on lightweight metallic substrates for friction reduction and antiwear applications (Ref 13).

In the present work, the deposition characteristics of the cold-sprayed HA-Ag/PEEK coating was examined using microstructural analysis of as-sprayed coatings comprising various ratios of HA-Ag/PEEK composite

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powders. The biofunctionality of HA-Ag/PEEK coating was also evaluated using bacterial assay to ascertain the killing rate against *Escherichia coli*.

2. Experimental Procedure

2.1 Materials

$AgNO_3$, H_3PO_4, and $Ca(OH)_2$ were purchased from Sigma-Aldrich, Germany. NH_4OH was purchased from Merck. PEEK powder was purchased from Victrex Pte Ltd. (UK). *Escherichia coli* DH5α was provided by the Department of Biological Science of the Nanyang Technological University, Singapore.

2.2 Synthesis of Ag-Doped Hydroxyapatite

Silver ion-doped HA was prepared by substituting calcium with the silver ion from $AgNO_3$ (Sigma-Aldrich, Germany). The percentage of metal ion present in the HA was varied according to the experimental requirements.

The amount of metal salt substitution was governed by the following molar equation. For this experiment, to produce 5% of Ag-doped HA, the calculation was made.

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

Considering 0.12 moles of H_3PO_4 was used, the amount of $Ca(OH)_2$ required for pure HA was 0.2 moles. Since 5% of calcium was expected to be substituted with silver, only 0.19 mole of $Ca(OH)_2$ was used and 0.01 mole of $AgNO_3$ was required. The concentration of $AgNO_3$ is 0.1 N; therefore, 100 mL of $AgNO_3$ was used.

The synthesis of metal-doped HA is similar to pure hydroxyapatite. The aqueous solution of $Ca(OH)_2$ was heated and maintained at 98 °C. Aqueous solution of H_3PO_4 was slowly dripped into aqueous solution of $Ca(OH)_2$ using a peristaltic pump at the flow rate of about 2 L/h. A magnetic stirrer was used to ensure the suspension was well mixed at all times. When the initial pH of $Ca(OH)_2$ began to drop sharply from 12, the metal salt solution was added into the suspension. The pH of the suspension will be very low after discharging all the solution of aqueous H_3PO_4 into the bath. Aqueous ammonia, NH_4OH (Merck), was added to increase the pH to about 8. The suspension was left to stir for 2 h at 98 °C. The suspension was left overnight to settle and thoroughly washed (at least 3 days) before drying in the oven at 100 °C. The dried HA cake was then crushed into powder using mortar and pestle before further use.

The samples were analyzed with JEOL JSM 6700-F Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive X-ray Spectrometer (EDX) for compositional analysis. Their antibacterial properties were tested against *E. coli* in Luria-Bertani broth and agar (both from Sigma-Aldrich).

2.3 Cold Spray HA-Ag/PEEK Powders Preparation

HA-Ag nanopowder has an irregular morphology as shown in Fig. 1. The commercially available PEEK powder (−45 mesh) was used as the ductile phase. The PEEK powder has irregular morphology as shown in Fig. 2.

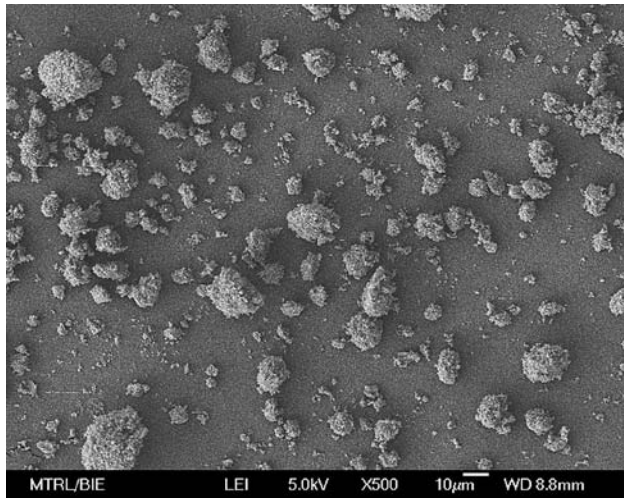


Fig. 1 SEM micrograph of HA-Ag powder

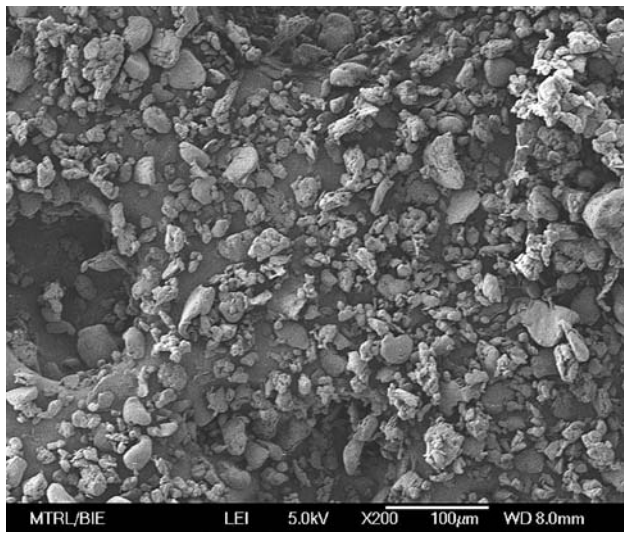


Fig. 2 SEM micrograph of PEEK powder

Both powders that were used to generate the coatings were sieved to less than 45 micron before blending in a ball mill for about 24 h. The HA-Ag and PEEK nanopowders were mixed in the ratios of 80:20, 60:40, 40:60, and 20:80 (wt.%).

2.4 Cold Spray Parameters of HA-Ag/PEEK Powders

The cold spray system was obtained from the Russian Academy of Science, Siberia and co-developed with ST Kinetics (Singapore) Engineering Company. An ABB Robot (IRB2400) was used in conjunction with the cold spray system. Glass slide will be cleaned with acetone and dried with pure air before using it as coating substrate.

Pure air was used as the accelerating and carrier gas operating at pressures of 11-12 bars in the prechamber. The gas was preheated to a temperature of 150-160 °C. The standoff distance from the gun exit was 15 mm. The traverse speed of the gun relative to the substrate was 50 mm/s. The microstructure of the HA-Ag/PEEK coating was analyzed using a Field Emission scanning electron microscope (JEOL JSM-6700F).

2.5 LB Agar Plate and LB Broth Preparation

LB agar plate was prepared by dissolving LB agar powder in deionized water. The mixture was stirred and heated until the agar was completely dissolved. The solution was then autoclaved for 15 min at 121 °C to obtain a yellow solution. The agar solution was poured onto Petri dishes until fully covered. These agar plates were left uncovered inside a BioSafety cabinet for drying. After which, the Petri dishes were then covered, stacked together, and sealed in plastic packaging. They were further placed inside a 37 °C incubator for 24 h to ensure that no bacteria contamination occurred during agar preparation. These agar plates were then stored in a 4 °C refrigerator.

LB broth was prepared by mixing LB broth powder with deionized water. The broth was stirred and dispensed into 10 mL test tubes. The solution was autoclaved for 15 min at 121 °C to obtain a yellow solution. The broth solution was stored in a 4 °C refrigerator.

2.6 Tests of Antibacterial Activity

2.6.1 Bacterial Qualitative Test. To perform qualitative analysis of the antibacterial properties of the metal ions-substituted HA coating, LB agar solution was poured onto Petri dishes after the cold-sprayed sample was placed perpendicular to the base of the Petri dish. Only half the sample was covered by the LB agar. The *E. coli* was introduced onto the LB agar surface by using a micropipette to spread the bacteria over the agar surface. The agar plates were placed upside down in a 37 °C incubator for 24 h before further analysis.

2.6.2 Bacterial Quantitative Test. The *E. coli* stock was stored inside microcentrifuge tubes at −80 °C. When required, the tubes were taken out and thawed at room temperature. A sterile wire loop was used to plate the bacteria by streaking lightly on the surface of agar-coated Petri dishes. Figure 3 shows the procedure of the first, second, and third streaking strokes on a single agar plate.

The agar plates were placed upside down in a 37 °C incubator for 24 h. Using a sterile wire loop, a single colony was extracted and streaked on to another agar plate. The plates were further incubated upside-down at 37 °C for another 24 h. Using a sterile wire loop, an isolated colony was placed into a test tube containing 10 mL of broth, and vortexed for 60 s. With a micropipette, 1 mL of *E. coli* solution was taken out and put into: (a) pure broth 9 mL as a control, (b) broth 9 mL + sample 1, (c) broth 9 mL + sample 2, (d) broth 9 mL + sample 3, etc. This was followed by a number of serial dilutions, six times (up to 1:1,000,000).

Hundred microliters of solution was plated on to agar dishes using a spreader. Where necessary, more than one plate was replicated for each concentration. The plates were again incubated at 37 °C (upside down) for 24 h. Choose and count the number of colonies in plates containing 30-300 colonies per plate. One colony represents a colony-forming unit (CFU).

Fig. 3 Schematic diagram of streaking bacteria

3. Results and Discussion

3.1 Microstructure of Cold-Sprayed Coatings

Figure 4 shows the cold-sprayed coating samples of (a) HA-Ag 20/PEEK 80, (b) HA-Ag 40/PEEK 60, (c) HA-Ag 60/PEEK 40, and (d) HA-Ag 80/PEEK 20, respectively. The coating thicknesses of all samples were in the same range of about 30-40 micron.

SEM picture of HA-Ag/PEEK coating is shown in Fig. 5: (a) HA-Ag 20/PEEK 80, (b) HA-Ag 40/PEEK 60, (c) HA-Ag 60/PEEK 40, and (d) HA-Ag 80/PEEK 20. These images revealed that the surface of the cold-sprayed coating consisted of HA-Ag powder embedded in a continuous PEEK matrix.

EDX results confirmed the presence of both HA-Ag/PEEK contents on the coating after cold spraying as shown in Table 1.

The negligible difference in HA-Ag/PEEK contents suggested minimal preferential deposition of phases in a composite powder during the cold spray process. Thus, it can be stated that the composition of the coating of the

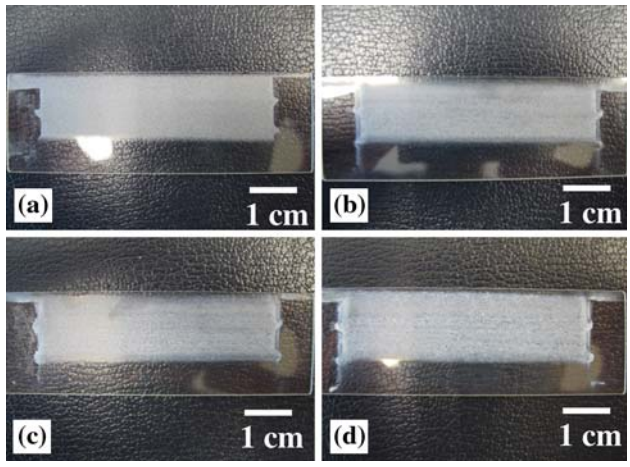


Fig. 4 Cold-sprayed coating samples

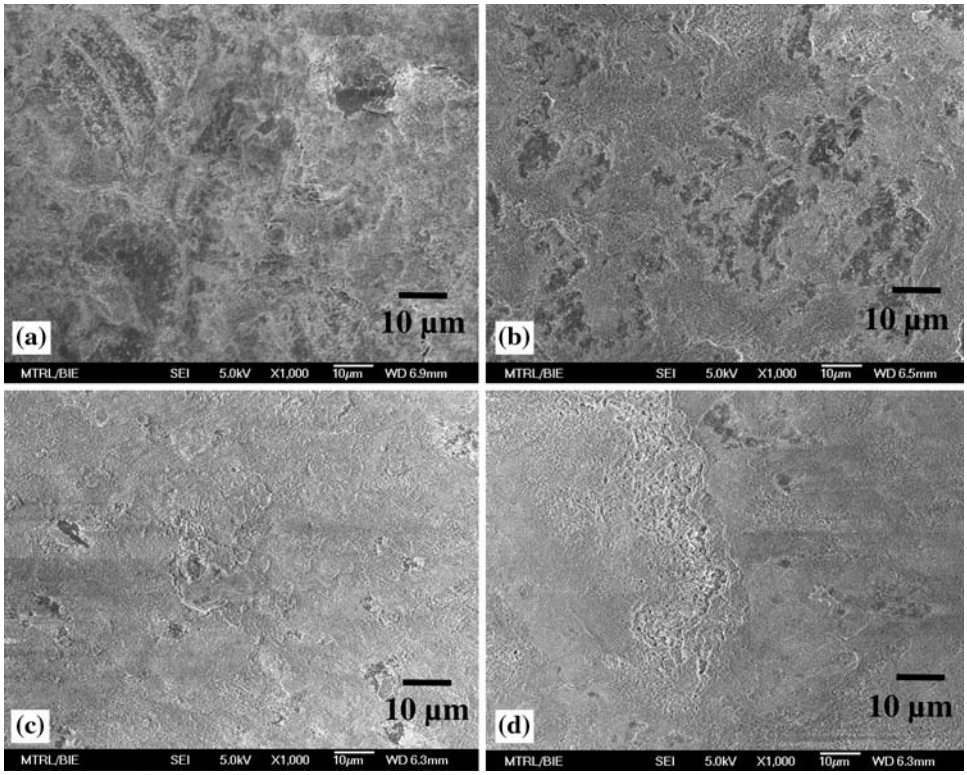
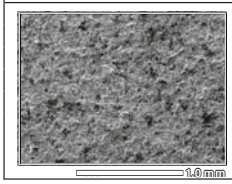


Fig. 5 SEM picture of HA-Ag/PEEK coating

Table 1 EDX result of HA-Ag60/PEEK 40 coating

SEM picture	ZAF Method Standardless Quantitative Analysis		
	Element	Mass %	At %
	C	27.06	41.48
	O	33.76	38.85
	Si	3.15	2.07
	P	11.38	6.76
	Ca	22.97	10.55
	Ag	1.67	0.29
	Total		**100**

cold spraying was almost the same as that of the starting feedstock powder as shown in Table 2.

The formula used for calculation (wt.%) of HA-Ag and PEEK from EDX results has been shown below.

$$\text{HA-Ag (wt.\%)} = \frac{\%\ \text{mass of [P + Ca + Ag]}}{\%\ \text{mass of [P + Ca + Ag + C]}} \times 100$$

$$\text{PEEK (wt.\%)} = \frac{\%\ \text{mass of [C]}}{\%\ \text{mass of [P + Ca + Ag + C]}} \times 100$$

According to the backbone structure of HA-Ag and PEEK, phosphorus (P), calcium (Ca), and silver (Ag) were used for calculation HA-Ag (wt.%) while carbon (C) was used for calculation PEEK (wt.%).

Oxygen (O) was ignored for calculation because it was presented in both HA-Ag and PEEK structures. Moreover, it was found as the part of SiO_2 which was the main chemical structure of glass slide.

Table 2 The HA-Ag/PEEK contents (wt.%) in the powder feedstock and coatings

	Powder feedstock		Coating	
Powder composition	**HA-Ag**	**PEEK**	**HA-Ag**	**PEEK**
HA-Ag 20/PEEK 80	20	80	28	72
HA-Ag 40/PEEK 60	40	60	45	55
HA-Ag 60/PEEK 40	60	40	64	36
HA-Ag 80/PEEK 20	80	20	81	19

3.2 Antibacterial Results of Cold-Sprayed Coating

3.2.1 Bacterial Qualitative Test. Figure 6 shows that all cold-sprayed samples have a very distinctive killing effect on *E. coli.* A lack of *E. coli* colonies growing in the region surrounding the HA-Ag-coated samples was observed and appeared as clearance zone. The area of the clearance zone increased with increasing HA-Ag nanopowder concentration in the composite powder feedstock and cold-sprayed coating, showing that the killing effect on *E. coli* increased with increasing amount of HA-Ag.

3.2.2 Bacterial Quantitative Test. Figure 7 shows that all cold-sprayed samples displayed an even more pronounced killing effect on *E. coli*. The killing rate increased with increasing HA-Ag nanopowder concentration in the composite powder feedstock and cold-sprayed coating.

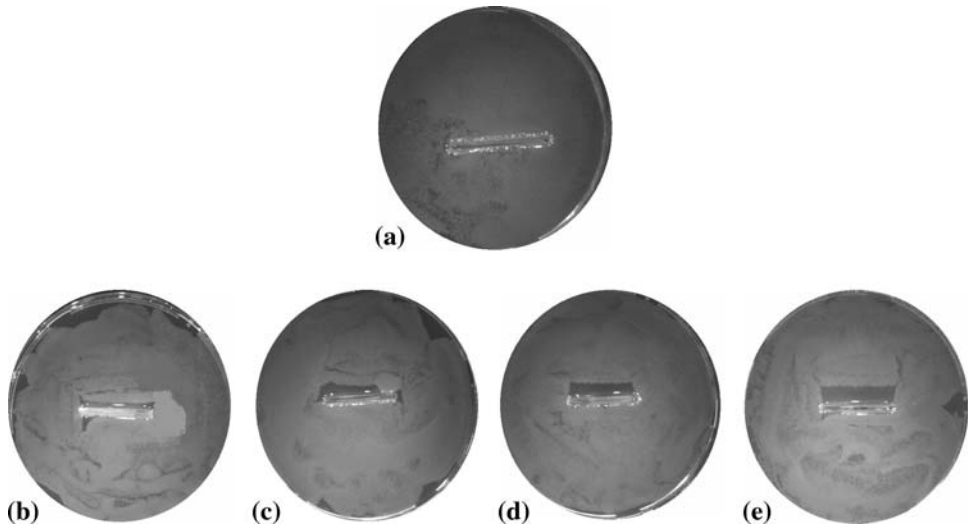


Fig. 6 Qualitative analysis on the antibacterial properties of HA-Ag/PEEK coatings (a) glass without coating (b) HA-Ag 20/PEEK 80 (c) HA-Ag 40/PEEK 60 (d) HA-Ag 60/PEEK 40 (e) HA-Ag 80/PEEK 20

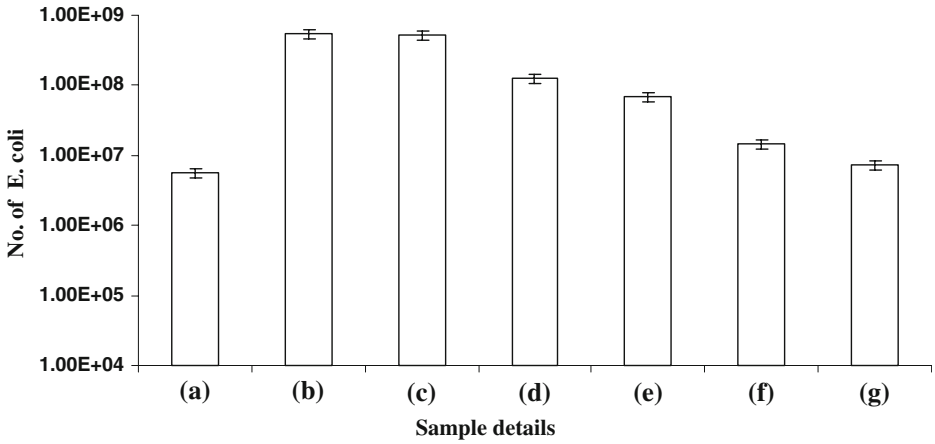


Fig. 7 Quantitative analysis on the antibacterial properties of HA-Ag/PEEK coatings (a) E coli @ 0 hr (b) E coli @ 24 hr (c) pure glass (d) HA-Ag 20/PEEK 80 (e) HA-Ag 40/PEEK 60 (f) HA-Ag 60/PEEK 40 (g) HA-Ag 80/PEEK 20

4. Conclusions

HA-Ag/PEEK coatings were successfully deposited on glass at room temperature using a range of cold spraying parameters. EDX analysis verified comparable HA-Ag/PEEK contents in the starting powders and as-sprayed coatings. It can, therefore, be inferred that the powder characteristics in terms of phase compositions and ratio remained unchanged during the coating deposition. These as-sprayed coatings of varying HA-Ag/PEEK composition retained their inherent antibacterial property as clearly verified from bacterial assay. The results indicated that the antibacterial activity increased with increasing HA-Ag/PEEK nanopowder concentration in the composite powder feedstock and cold-sprayed coating. The study has thus demonstrates the ability of cold spraying to deposit a ceramic material (HA-Ag), a nanophase, and a composite powder (HA-Ag/PEEK), and yet retain and elicit a coating functionality (Bio) similar to that of the starting material.

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