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Epoxy resin-based ultrafine dry powder coatings for implants

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ABSTRACT: Ultrafine dry powder coating technology creates biocompatible polymeric coatings for implants. Nanoparticles ($nTiO_2$) modify flow to prevent agglomeration and create homogenous coatings. Since polyester-based coatings require the potentially harmful 1,3,5-triglycidyl isocyanurate (TGIC) curing agent, this study's objective was to develop alternative TGIC-free formulations. Epoxy and epoxy/polyester (1:1) hybrid mixtures were enriched with CaO (5% w/w) and $nTiO_2$ (0.5% w/w), as functional additives and flow modifiers, respectively. Epoxy-TiO_2 and Hybrid-TiO_2 mixtures were prepared with micron-sized TiO_2 (25% w/w) to enhance biocompatibility. Polymer chips and additives were combined in a high-shear mixer and passed through a sieve (35 μm) to yield ultrafine particles that were sprayed (20 kV) onto metal sheets and cured (200 °C). Particle size analyses showed that all formulations were ultrafine (D 0.5 < 35 μm), and epoxy/polyester/TiO_2 mixtures were the smallest (D 0.5 = 16.34 μm). Angles of repose, avalanche and resting indicated reduced flowability when epoxy was enriched with TiO_2 and/or polyester, although all formulae were highly flowable. Elemental mapping of coatings showed a predominance of carbon (C) and oxygen (O) from resin polymer, and elevated titanium (Ti) in the TiO_2 enriched surfaces. However, calcium (Ca) clusters were higher on the epoxy/polyester Hybrid coatings. Optical microscopy showed human mesenchymal cells (ATCC CRL-1486) attached and spread out, and Alizarin Red staining showed mineral deposits in 2–4 week cultures, particularly on epoxy/polyester/TiO_2 Hybrid surfaces. These epoxy resin-based formulations were effective TGIC-free substitutes for ultrafine dry powder coatings on implants. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43960.

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INTRODUCTION

Dental and orthopedic implants have attained considerable success in replacing damaged tissues. Orthopedic implants alone in the U.S. have reached a $14 billion market share, while maintaining steady annual growth of nearly 10%.[1] Yet, the success of these intraosseous implants can be compromised by complications that occur at the tissue-implant interface due to a failure of biocompatibility and osseointegration.[2,3] In addition, their immediate post-surgical stability is largely dependent on both the quality and quantity of surrounding osseous tissues, to provide sufficient anchorage for the prosthesis. Inadequate bone at the surgical site is likely to cause a subsequent loosening and failure of the implant.[4]

Therefore, considerable efforts have focused on studying tissue-implant interactions, and on enhancing the biocompatibility and osteo-inductivity of the biomaterial surfaces so as to optimize the cellular response.[5,6] Studies have shown that various surface additives can enhance cell survival, attachment, metabolism, proliferation, and differentiation,[7–10] and that surface modifications such as grinding and chemical etching can improve the biocompatibility of implants.[11] Similarly, a novel surface coating technique was developed, whereby the surfaces of titanium implants can be enhanced with a polyester-based ultrafine dry powder coating. The polymeric powder coatings were enriched with titania and bioactive agents such as calcium containing mineral oxides. These coatings showed significant improvements in biocompatibility and osteo-inductivity when compared with the unmodified commercially pure titanium (cpTi) surfaces.[10,12–15]

However, an essential component of the polyester-based coatings is 1,3,5-triglycidyl isocyanurate (TGIC), which is a low molecular weight multifunctional crosslinker that acts as the curing agent. This curing agent has been used in dry powder coating for over 40 years. Industry claims are that the powders melt and chemically react during heat curing to form a stable polymeric matrix in which TGIC is chemically bound to the polymer, and cannot contaminate the environment (Powder Coating Institute). However, exposures to TGIC during

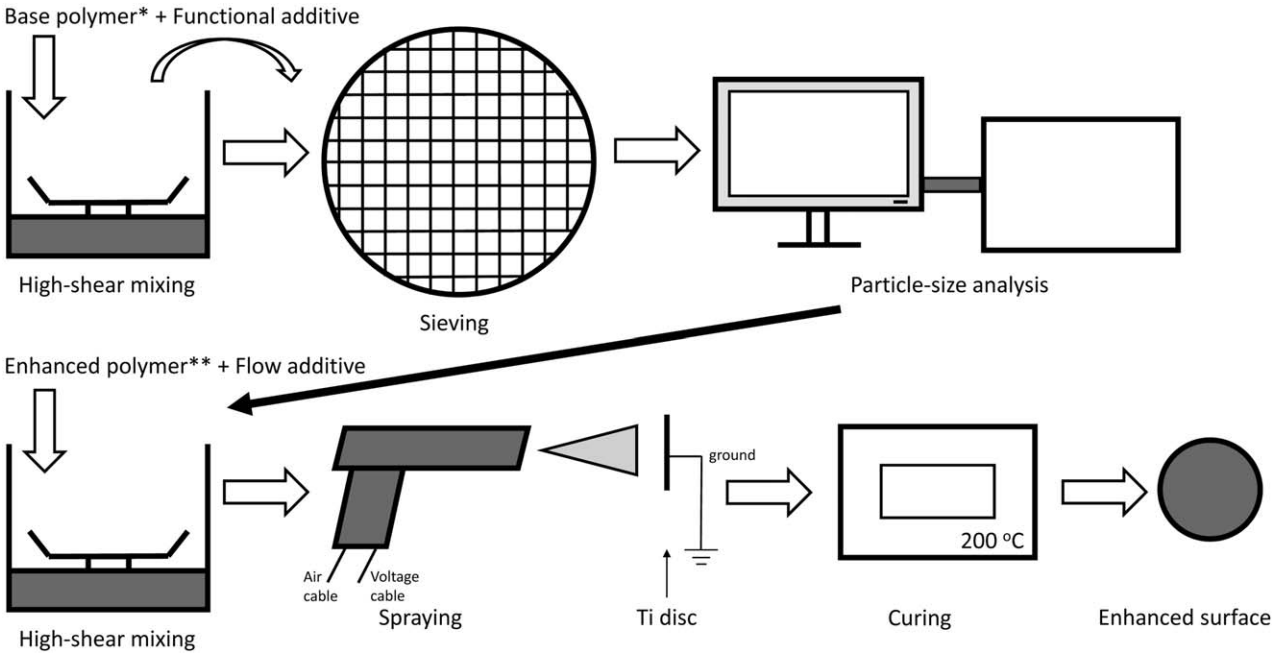

Figure 1. An ultrafine dry powder coating technique was used to create novel biocompatible surface coatings that are enriched with bioactive agents.

manufacturing procedures have been identified as hazardous to workers, so that authorities recommend body protection and proper ventilation in the spraying area.[16] Subsequently, it is present at very low concentrations in the polyester resin coated materials, so that its potential for toxicity is limited.[17] Nevertheless, the application of ultrafine dry powder coating for implantable biomaterials necessitates an optimization of biocompatibility and a complete minimization of cytotoxicity. Therefore, TGIC-free mechanisms for the dry powder coating process are being investigated.

In this study, epoxy resin-based polymer mixtures that are devoid and independent of TGIC as the curing agent were carefully prepared and assessed for their suitability in the ultrafine dry powder coating process. The epoxy resin is chemically stable and utilizes only minute quantities of dicyandiamide as the curing agent, which is a compound that has been used in food and pharmaceuticals without safety concerns. In addition, epoxy/polyester (1:1) hybrid mixtures have the capability of intercuring between the epoxy and polyester polymers, which completely eliminates the need of any curing agents. These resin base polymers were then enriched with micron-sized titanium dioxide (TiO_2, 25% w/w) and calcium oxide (CaO, 5% w/w), which have been shown to promote the cellular response.[12] Ultimately, the polyester-based ultrafine dry powder coatings were analyzed for cell-surface interactions and the initiation of biomineralization by human mesenchymal cell cultures *in vitro*.

EXPERIMENTAL

Preparation of Ultrafine Particles for Coatings

The ultrafine particles were created by using a novel and patented technique (Figure 1).[10,12–15,18] The base polymer powders (Table I) were prepared as either Epoxy pure or epoxy/polyester Hybrid formulations, which were either with or without micron-sized TiO_2 (Luanchuan Yuxing Chemicals, China). All of the formulations were enriched (Table II) with TiO_2 nanoparticles (0.5% w/w, Degussa) as a flow additive to improve flowability, and CaO (5.0% w/w; Sigma-Aldrich, Oakville, Canada) as a bioactive agent to enhance biocompatibility. These powder mixtures were thoroughly mixed in a high-shear mixer and passed through a sieve (35 μm).

Table I. Composition of Ultrafine Base Polymer Powders

Constituent (Weight %)	Epoxy Pure	Epoxy-TiO_2	Hybrid Pure	Hybrid-TiO_2
Epoxy resin	3000 g (96.0%)	2250 g (72.3%)	2000 g (50.0%)	1150 g (37.7%)
Polyester resin	–	–	2000 g (50.0%)	1150 g (37.7%)
Dicyandiamide	120 g (3.8%)	90 g (2.9%)	–	–
2-Methylimidazole	4 g (0.1%)	4 g (0.1%)	–	–
TiO_2	–	750 g (24.2%)	–	750 g (24.6%)

Epoxy resin: Anhui Shanfu New Material Technology, China. Polyester resin: Haining Guanghua Chemicals, China. Dicyandiamide: Hubei Nice Chemicals and New Materials, China. 2-Methylimidazole: Source Chemical, China. TiO_2 (micron-sized): Luanchuan Yuxing Chemicals, China.

Table II. Composition of Ultrafine Powders for Coating

Constituent (Weight %)	Epoxy Pure	Epoxy-TiO_2	Hybrid Pure	Hybrid-TiO_2
Base polymer powder	250.10 g (94.8%)	250.15 g (94.8%)	250.12 g (94.8%)	200.03 g (94.7%)
CaO	12.52 g (4.7%)	12.51 g (4.7%)	12.53 g (4.7%)	10.04 g (4.8%)
$nTiO_2$	1.24 g (0.47%)	1.25 g (0.47%)	1.27 g (0.47%)	1.11 g (0.50%)

Base polymer powder: Table I. CaO: Sigma-Aldrich, Canada. $nTiO_2$ (nanoparticles): Degussa.

Characterization of Ultrafine Particles

The ultrafine dimensions of the particles were verified by a Laser Particle Analyzer (BT-9300s; Ningbo Yinzhou Hybers, China). This involved suspending the particles in an aqueous media, and creating a diffraction pattern with a laser beam, which was then reflected onto a detector and analyzed by computer software to determine their size distribution.

The flowability of these ultrafine powder particles was assessed by examining their angles of repose, avalanche angles, and resting angles. The angle of repose (AOR) was measured by using a powder characteristic tester (PT-N Powder Characteristic Tester, Hosokawa Micron Powder Systems, Summit NJ). This involved slowly dispensing powder samples onto a flat surface to create a pile, and then measuring the angle between the surface of the pile and the flat surface as the angle of repose. This was repeated six times for each sample. The avalanche angle (AVA) and resting angle (RA) were measured by using a rotating drum (Revolution Powder Analyzer, Mercury Scientific, Sandy Hook CT). This involved placing a known amount of particles (120 mL tapped volume) into a transparent rotating drum (0.6 rps), and measuring the maximum angle that the powder achieved before it collapsed to the bottom of the drum (avalanche angle), as well as the angle of the pile formed after each avalanche (resting angle). This was repeated 200 times for each sample.

Dry Powder Coating Titanium Surfaces

The ultrafine powder formulations were used to create dry powder coatings on titanium surfaces, by using a well proven technique (Figure 1).[10,12–15] During this process the ultrafine particles were charged (20 kV) and sprayed with a Corona Gun (Nordson, Westlake, OH), so that they were electrostatically attracted to grounded titanium surfaces. Commercially pure titanium (cpTi) sheets (McMaster-Carr, Cleveland, OH) were used, which had been cut into circular disks (Grade 2, 0.5 mm thick, 24 mm diameter). The dry powder-coated disks were then cured (200 °C, 10 min) in a high performance air flow oven (Sheldon Manufacturing, Cornelius, OR).

Characterization of Coated Surfaces

The composition of the coated surfaces was analyzed using scanning electron microscopy (SEM, S-4000; Hitachi, Pleasanton, CA) to conduct energy dispersive X-ray spectroscopy (EDX) on the coated surfaces.[10,12] The coated disks were mounted onto metal stubs, secured with adhesive carbon tape and sputter coated with gold nanoparticles (10 nm). The SEM was configured (working voltage 15 kV, working distance 15 mm) so that each surface element could be identified (minimum detection limit = 0.1%), and its presence and distribution mapped across the entire surface.

Surface Cleansing, Disinfection and Sterilization

The coated surfaces were then prepared for cell culture assays. This involved cleaning and disinfecting the surfaces chemically, enzymatically, and sonically, followed by sterilization with UV light. Initially, the coated disks were rinsed (3x) with phosphate buffered saline (PBS, Gibco, pH 7.4, calcium chloride and magnesium chloride free) and then rinsed (3x) with trypsin (0.25%, Gibco). They were then submerged in a polypropylene conical tube (50 mL, BD Falcon) and sonicated in fresh trypsin (60 mins) and then sonicated in sodium hypochlorite (2.6% bleach, 60 mins). Finally the coated surfaces were washed (10x) with distilled water, washed (2x) with autoclaved distilled water, rinsed (3x) with ethanol (70%) and PBS, and then sterilized under the UV light (30 mins each side) in a tissue culture hood.

Human Mesenchymal Cell Culture

The coated disks and control (uncoated cpTi) surfaces were assessed for their capacity to support cell attachment and spreading in short term *in vitro* cultures.[10,12–15] They were placed individually into each well of 6-well tissue culture plates (BD Falcon) and then covered with tissue culture media. The media contained DMEM (Gibco, 4.5 g/L D-Glucose, L-Glutamine, and 110 mg/L sodium pyruvate) supplemented with fetal bovine serum (10%, FBS, Gibco) and Antibiotic–Antimycotic (1%, Gibco). At the center of each disk surface, human embryonic palatal mesenchymal cells (HEPM, ATCC CRL-1486) were carefully seeded, and the plates were then transferred to an incubator (37 °C, 5% CO_2).

Following 24 h of incubation, the disks and their cell cultures were carefully rinsed (3x) in PBS (5 min/rinse) to remove unattached cells. The cells that remained attached to the disks were then fixed (24 h at room temperature) with paraformaldehyde (4%, Fisher Scientific). The disk surfaces were then examined with an optical microscope to observe attached cells (100x magnification) and their morphology (400x magnification).

Biomineralization of Cultures

The coated disks and control surfaces were assessed for their capacity to support the initiation and progression of biomineralization in long-term cell cultures. In these extended cultures, the cells that had been seeded onto disk surfaces were maintained in fresh media (replenished every 3 days) for either 2 or 4 weeks of growth. The media was then carefully removed by vacuum suction, the disk surfaces rinsed in PBS, fixed (1 h) in formalin (4%), and washed (2x) in Calcium-free Nanopure water. They were then stained for mineral deposits with Alizarin Red S (2%, 10 mins, room temperature), and washed with Ca-free Nanopure water to remove excess stain.

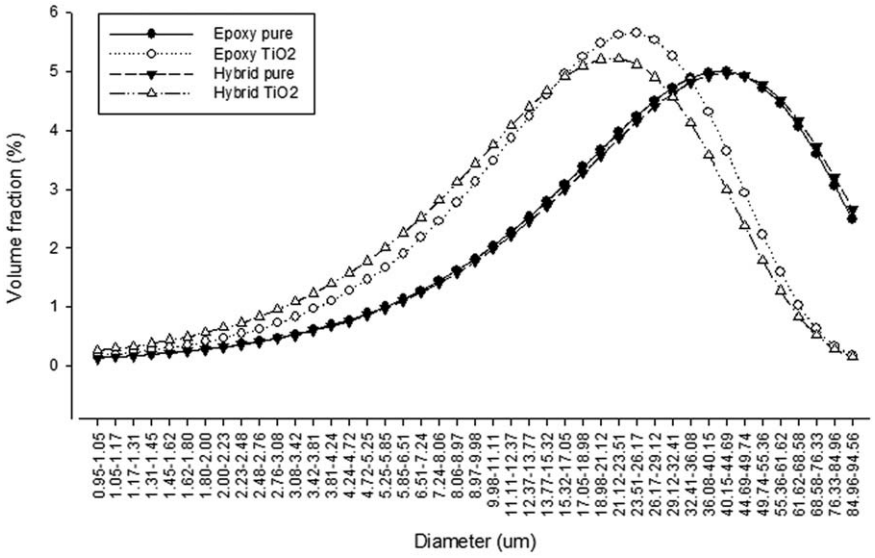

Figure 2. The powder mixtures were analyzed for their particle-size distribution.

Statistical Analyses

Quantitative data were analyzed by using SigmaPlot 12.0 (Systat Software, Chicago IL). Their mean and standard error values were calculated, examined graphically and analyzed statistically. Differences were identified by one-way ANOVA, and post hoc comparisons were performed by the Holm–Sidak method ($\alpha = 0.05$).

RESULTS

Epoxy, Polyester, and TiO_2 Mixes Produced Analogous Ultrafine Powders

The pure epoxy (Epoxy), epoxy with micron-sized TiO_2 (Epoxy-TiO_2), epoxy/polyester hybrid (Hybrid), and epoxy/polyester hybrid with TiO_2 (Hybrid-TiO_2) mixtures were created

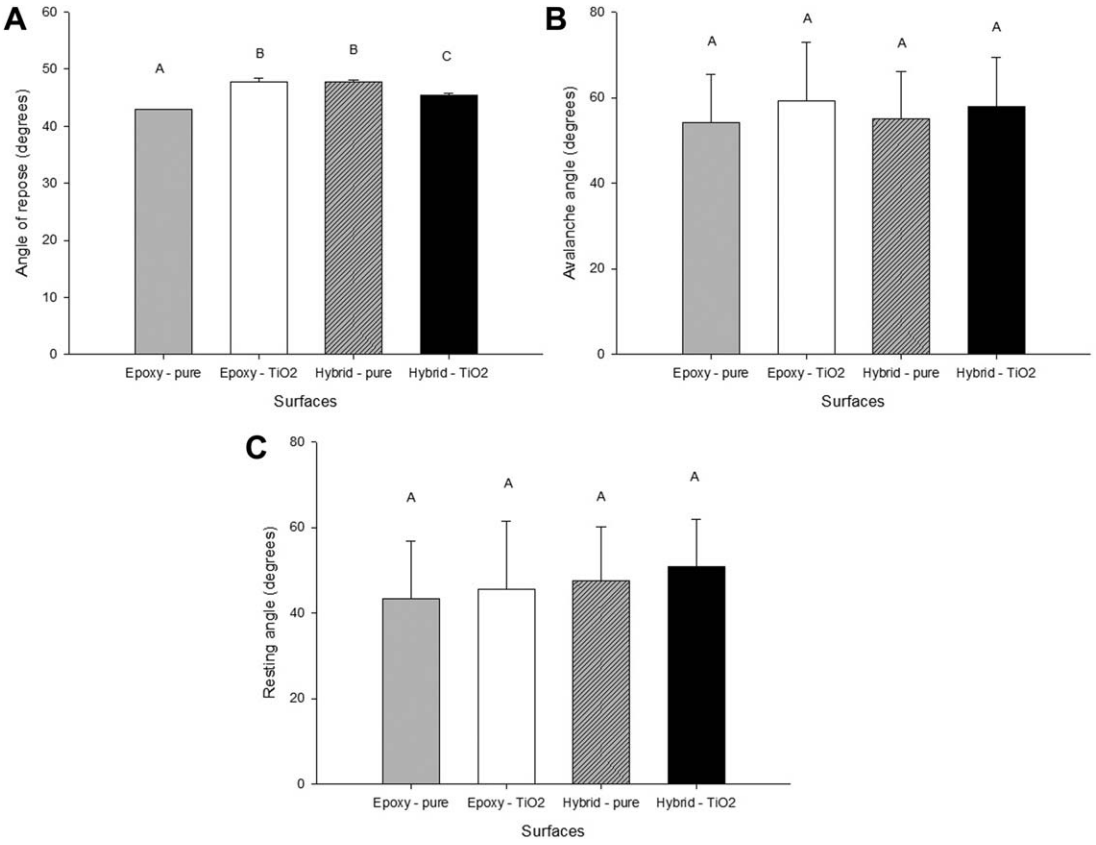

Figure 3. The ultrafine particles flow properties were accessed from their angles of repose (A), avalanche angles (B), and their resting angles (C) after each avalanche.

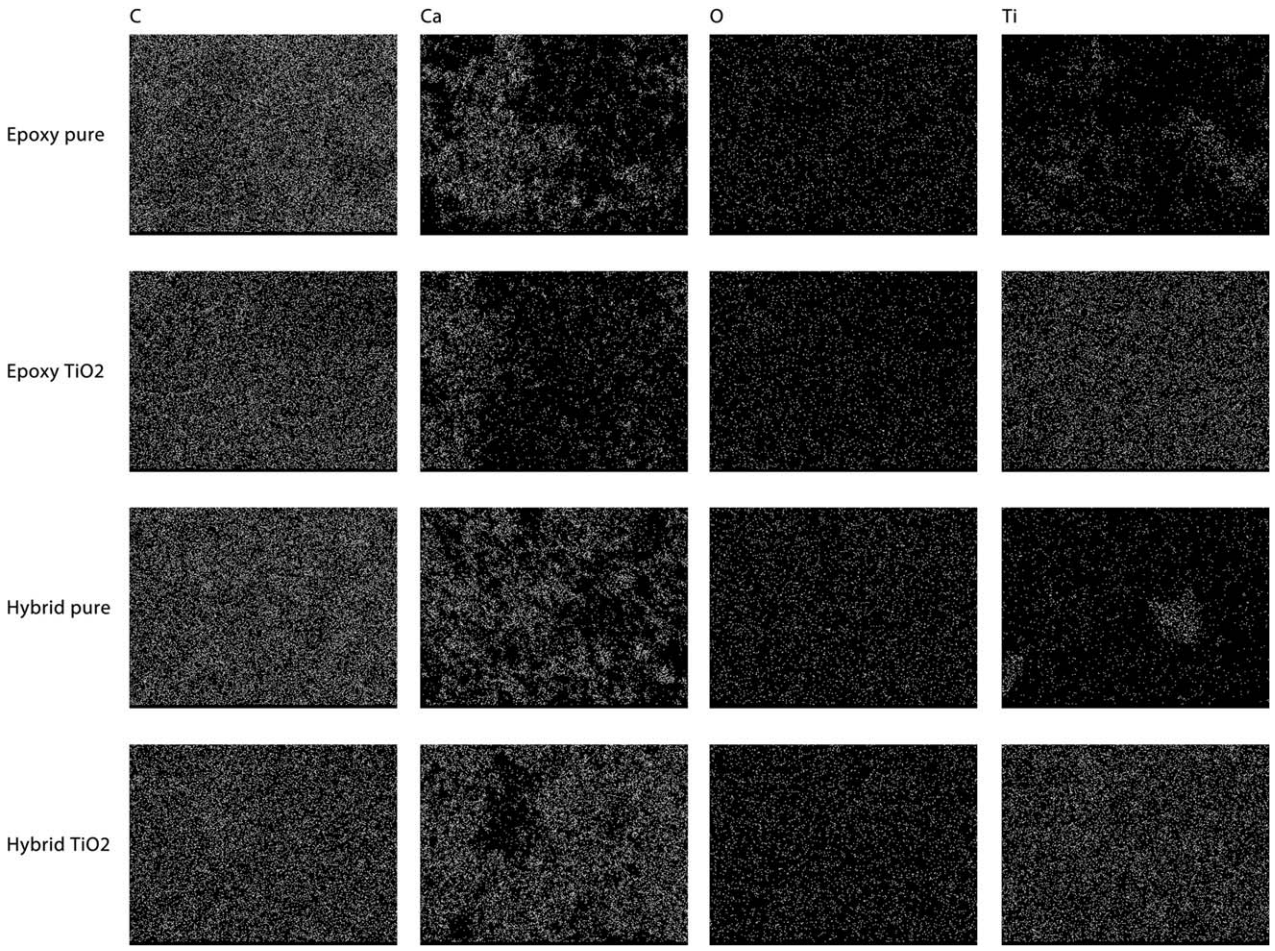

Figure 4. The coated surfaces were analyzed by elemental mapping.

into largely analogous ultrafine powder particles. These formulations of the ultrafine base polymers (Table I) were mixed with CaO (5% w/w) as the bioactive agent, and nanoparticles of TiO_2 (0.5% w/w) as the flow modifier (Table II), and they were all ground and sieved through the same process (Figure 1). The resulting coating particles were found to exhibit similar properties and dimensions, despite the differences in their chemical composition. Particle size analyses of these mixtures showed that 50% of the particles (D 0.5) had a volume diameter that was <35 μm, confirming the ultrafine characteristic of the powders (Figure 2). The Epoxy and epoxy/polyester Hybrid particles had an almost identical particle size distribution, whereas the Epoxy-TiO_2 were clearly smaller (D 0.5 = 18.71), and the Hybrid-TiO_2 were even smaller (D 0.5 = 16.34).

TiO_2/Polyester Enrichment Reduced Powder Flow Minimally

All of the ultrafine particles exhibited excellent flow properties that were largely similar for all of the formulations. All of the powders were assessed for their flowability by analyzing their angles of repose, avalanche angles, and resting angles. The angles of repose [Figure 3(A)] showed that there was a small but statistically significant ($P < 0.05$) reduction in flowability when epoxy resin was enriched with TiO_2 and/or polyester. Similarly, the avalanche [Figure 3(B)] and resting angles [Figure 3(C)] showed that there were slight reductions in flowability for TiO_2 and/or polyester containing formulae, although these differences were statistically insignificant.

Surface Calcium was Increased on Polyester Coatings

When these ultrafine powders were electrostatically sprayed onto grounded sheets of titanium and then cured in an oven, their constituents were incorporated into surface coatings. Elemental mapping of their surfaces showed that the predominant elements were carbon (C) and oxygen (O), which originated from the epoxy and polyester resin polymers (Figure 4). There were also high levels of titanium (Ti) on the coatings that had been enriched with micron-sized TiO_2, and much lower levels on the remaining surfaces, which only contained nTiO_2 that served as a flow additive in all of the formulae. Similarly, CaO (5%) had been added to all of the formulae as a bioactive additive. However, surface calcium levels varied widely from being high on the epoxy/polyester Hybrid-TiO_2 and epoxy/polyester Hybrid coatings, and lower on the Epoxy-TiO_2 and Epoxy pure surfaces.

Epoxy/Polyester/TiO_2 Coatings Supported Cell Attachment and Spreading

All of the ultrafine dry powder coatings supported cell attachment and spreading (Figure 5). Careful optical microscopic examination under lower power (100×) showed that there were

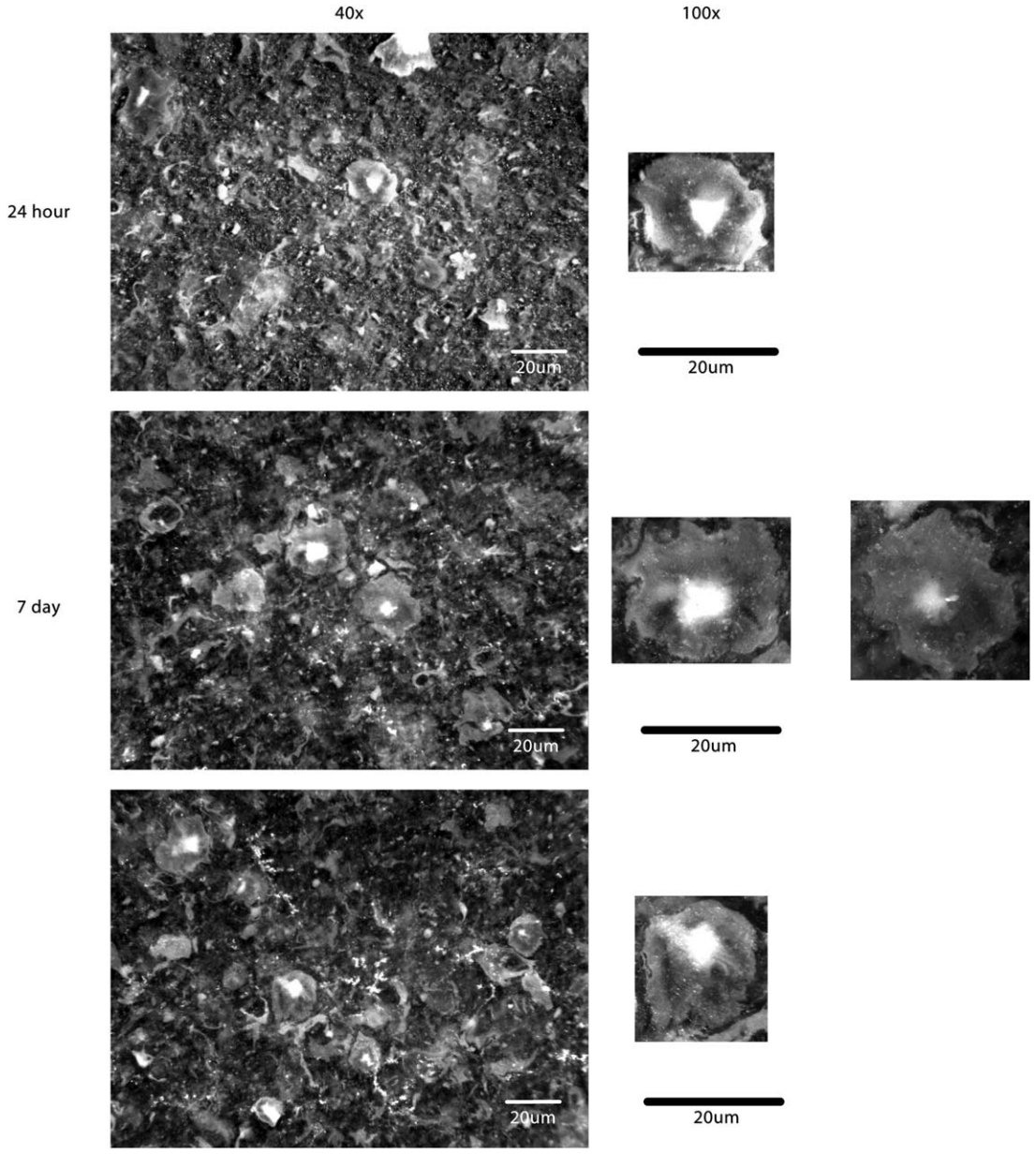

Figure 5. Optical microscopy images taken at low power (100x), and at high power (400x).

clusters of human mesenchymal cells that had attached to the coating surfaces. Further examination under high power (400×) showed that the attached cells exhibited a "spreading" morphology with extended filopodia, which suggested intimate interactions with the underlying coating surfaces.

Biomineralization Increased on Polyester Coatings

The human mesenchymal cell cultures that were grown on epoxy/polyester Hybrid and Hybrid-TiO_2 coatings showed the *in vitro* initiation of biomineralization. The Alizarin Red staining showed that there were moderate amounts of mineral deposits on the epoxy/polyester Hybrid and Hybrid-TiO_2 surfaces, and also on the Epoxy-TiO_2, within 2 weeks of growth and differentiation [Figure 6(A)]. These increased in the cultures grown on epoxy/polyester Hybrid and Hybrid-TiO_2 surfaces, so that there were numerous calcified mineral deposits after 4 weeks of growth and differentiation [Figure 6(B)]. There was only background staining on all of the coatings that were without cells (DMEM Control), and no calcified deposits were observed on the titanium surfaces.

DISCUSSION

This study showed that either epoxy resin alone, or epoxy/polyester mixtures could be utilized in the application of ultrafine dry powder coating technology[19] to create polymeric powder coatings that are biocompatible. The epoxy resin and hybrid formulations served as effective substitutes for polyester resin, which was previously utilized as the base polymer in dry powder coatings.[12] In addition, micron-sized TiO_2 (25% w/w), nanoparticles of TiO_2 (0.5% w/w) and CaO (5% w/w) could adequately be incorporated as enrichments within the coatings.

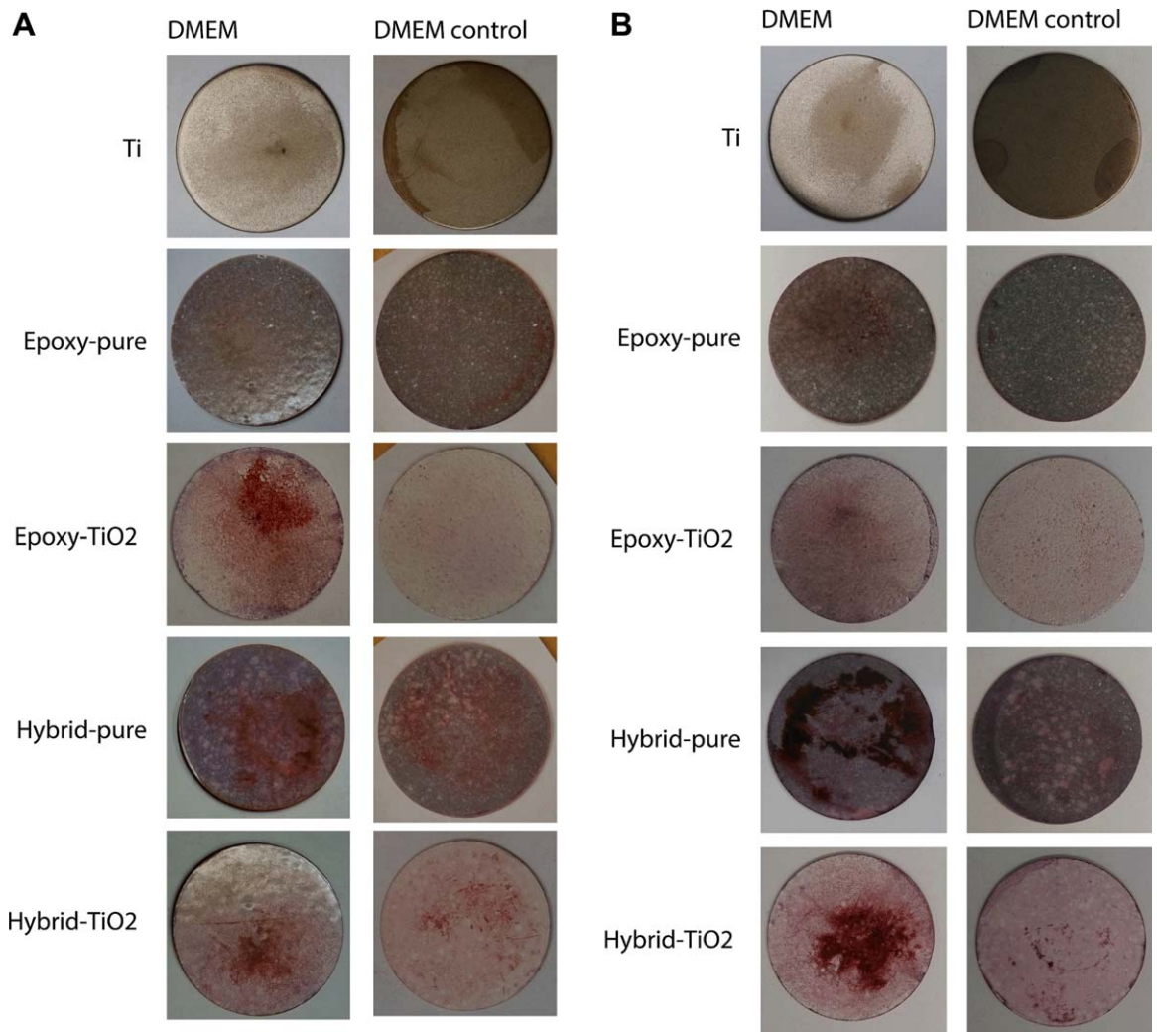

Figure 6. Human mesenchymal cell cultures were stained with Alizarin Red-S after 2 weeks (A), and 4 weeks (B) of growth and differentiation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

These dry powder coatings were created through the size-reduction of larger polymer chips into ultrafine particles, and their enrichment with flow additives and bioactive agents. The original procedure utilized polyester resin alone as the base polymer, and created highly biocompatible polymeric powder coatings, as previously reported.[12]

For the novel coatings in this study, the base polymers consisted of either epoxy resin, or a mixture of epoxy and polyester in equal amounts (1:1). By using either epoxy resin, or the epoxy/polyester mixture, TGIC was no longer required in their preparation. Since exposures to TGIC during the manufacturing process are potentially a health hazard, its effective exclusion from the ultrafine dry powder coating process is a marked enhancement in the coating process. Furthermore, the toxic solvents and volatile organic compounds (VOCs) that are usually associated with liquid coating procedures, were also eliminated by utilizing the dry powder coating process.

The most important benefit of this dry powder coating technology is the enhanced flowability of ultrafine powder particles. A decrease in particle size leads to an increase in inter-particular forces, which decreases the flowability of ultrafine particles.[20] Their poor flowability then creates uneven coating surfaces, and these powders can clog up the spraying apparatus. Yet, prior studies have shown that ultrafine dry powders can create nano-scale surface topographies and roughness on titanium substrates.[10,13–15] These coated surfaces can then enhance cellular responses and the osteointegration of implants.[21–27] Indeed, the particle-size analyses showed that the powders were ultrafine particles, since their D0.5 were measured at under 32 μm (Table II). Furthermore, the powder formulations that contained micron-sized TiO_2 (25% w/w) had even smaller D0.5 than those without the enrichment. Therefore, the inclusion of $nTiO_2$ (0.5% w/w) nanoparticles as flow modifiers was critical to ensure adequate flowability in all of the formulations. The nanoparticles act as spacers between particles, to minimize inter-particular attraction and improve flowability. Analyses of angles of repose, avalanche and resting angles confirmed that the ultrafine powders were sufficiently flowable for the Corona-gun spray.

These ultrafine powder constituents were fully incorporated into coatings, as confirmed by the subsequent analyses of their surfaces. Energy dispersive X-ray (EDX) spectroscopy showed that the coatings were largely composed of carbon and small amounts of oxygen, which account for the polyester and/or epoxy resins that were in the base polymers. As expected, the levels of titanium were higher in the coatings that had been enriched with micron-sized TiO_2 (25% w/w), and lower in those that only had the $nTiO_2$ (0.5% w/w) as a flow modifier. In addition, elemental mapping showed that carbon, oxygen and titanium were evenly distributed across the coating surfaces, which confirmed their homogeneity and continuity. Likewise, prior analyses of polyester-based coatings showed similar results.[12,15]

However, in thus study all of the epoxy and epoxy/polyester hybrid formulations had been enriched with CaO (5% w/w) as a bioactive agent to enhance the biocompatibility and osteo-inductivity of the coatings.[12] This functional additive was limited to 5% (w/w) of the formulations, so as to maintain the integrity, continuity and homogeneity of the coating surfaces. However, unlike the other constituents, elemental mapping showed that the calcium was distributed in more of a cluster fashion, a phenomenon that has also been seen in other studies.[10,12]

Ultimately the coatings were assessed for their biocompatibility and osteo-inductivity with human mesenchymal cell cultures *in vitro*. Optical microscopy showed that cells readily attached and spread out onto the coating surfaces. They attached and interacted favorably with the underlying coatings through cellular extensions and projections onto their surfaces. In addition, the cell cultures that were carefully maintained for 2–4 weeks of growth and osteogenic differentiation, showed the initiation of biomineralization on the coating surfaces. Alizarin Red-S detected calcified minerals in the cultures. Its sulfonic acid and hydroxyl groups binds specifically to mineral deposits, and has long been used to detect mineralization.[28] There was little staining of the controls, and unmodified titanium surfaces. But mineralization could be detected within 2 weeks, and increased after 4 weeks for the cultures that were grown on coating surfaces. Furthermore, these mineral deposits were more plentiful on the epoxy/polyester Hybrid and Hybrid-TiO_2 surfaces, suggesting that TiO_2 and polyester may have promoted biocompatibility and osteo-inductivity. Similarly, prior studies found that TiO_2 incorporation appeared to enhance the cellular response.[10–15] Polyester has been widely studied for medical applications,[29–31] its polarity makes it hydrophilic and highly biocompatible.[32–34]

CONCLUSIONS

Resin base polymer mixtures that are devoid and independent of TGIC as the curing agent, can be suitable for ultrafine dry powder coating. The epoxy resin or epoxy/polyester hybrid formulations can be enriched with TiO_2 and CaO to create biocompatible surface coatings that support human mesenchymal cell cultures *in vitro*.

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