Electroplating of HAp-brushite coating on metallic bioimplants with advanced hemocompatibility and osteocompatibility properties

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Abstract
In cases of severe bone tissue injuries, the use of metallic bioimplants is quite widespread due to their high strength, high fracture toughness, hardness, and corrosion resistance. However, they lack adequate biocompatibility and show poor metal-tissue integration during the post-operative phase. To mitigate this drawback, it is beneficial to add a biocompatible polymer layer to ensure a quick growth of cell or tissue over the surface of metallic bioimplant material. Furthermore, this additional layer should possess good adherence with the underlying material and also accompany a rapid bonding between the tissue and the implant material, in order to reduce the recovery time for the patient. Therefore, in this work, we report a novel green electroplating route for growing porous hydroxyapatite-brushite coatings on a stainless steel surface. The malic acid used for the production of hydroxyapatite-brushite coatings has been obtained from an extract of locally available apple fruit (Malus domestica). We demonstrate the effect of electroplating parameters on the structural morphology of the electroplated composite layer via XRD, SEM with EDS, and FTIR characterization techniques and report an optimized set of electroplating parameters that will yield the best composite coating in terms of thickness, adherence to substrate and speed. The hemocompatibility and osteocompatibility studies on the electroplated composites coating show this technology’s effectiveness and potential applicability in biomedical applications. Compared to other routes reported in the literature, this electroplating route is quicker and yields better composite coatings with faster bone tissue growth potential.

Keywords
Malic acid, brushite-hydroxyapatite, electrodeposition, osteocompatibility

Date received: 17 March 2022; revised: 5 May 2022; accepted: 8 May 2022

Introduction
Metallic materials have been extensively used for biomedical applications can be tracked down to the past three decades. Researchers have been successfully developed various bio-compatible materials and technologies for human body. The materials that are used are inert to body fluids. These body fluids flow over the bone and between the tissues and help in the circulation of oxygen and various other nutrients that is required by the human body.1 The most commonly used materials that are used in the human body are stainless steel (316L), Ti-based alloys, and Co-based alloys. These metallic materials and alloys are typically used in orthopedic, dental, and cardiovascular applications in the human body.2 Due to lower costs,
stainless steel is the most widely used material. Apart from being cost-effective, they have good resistance to corrosion by body fluids, superior mechanical properties, and good biocompatibility. These properties present in the metallic materials make them a perfect candidate for use in the fabrication of biomedical implants. The movement of human body joints is the final act of a coordinated action of the muscles and the bones where they are inserted. Thus, any undesired interruption of the chronological action may lead to an impairment in the joint. In this light, good biometallic implants used to replace injured bone tissue play a crucial role in the maintenance of the whole physiological and biomechanical scenario. It is also crucial for all the tissue around the joint to have a good adherence to the surface of the implant.

The use of metallic materials as an implant material has several limitations. They have a weak bone-bonding ability. Long-term usage of these metallic materials causes the release of toxic ions into the body fluid. The aggressive body fluids cause a quick degradation of these materials inside the human body. Various osteoconductive coatings like hydroxyapatite (HAp) or other calcium phosphates (CaPs) are applied over the implant material to enhance their biocompatibility. After the implant is fixed in the body, the bone tends to form a bonding with the bioactive layer. As a result, no other fibrous interface layers forms. CaPs can exist in various phases, depending on the Ca:P ratio. The crystallinity and the bioactive behavior can be controlled by changing the Ca:P ratio. Hydroxyapatite (HAp) is the most stable phase of calcium phosphate in the physiological environment (pH \( \approx 7 \)). There are many other phosphates like brushite [BS, \( \text{CaHPO}_4 \)], monetite (\( \text{CaHPO}_4 \cdot 2\text{H}_2\text{O} \)), dicalcium phosphate dihydrate (\( \text{CaHPO}_4 \cdot 2\text{H}_2\text{O} \)), and octacalcium phosphate (\( \text{Ca}_8(\text{PO}_4)_2(\text{OH})_4 \cdot 5\text{H}_2\text{O} \)), which are stable in an acidic environment. In body fluids, brushite has a higher solubility than that of HAp. However, HAp has a greater amount of similarity to natural human bone. Therefore, HAp exhibits an excellent re-mineralization ability and bioactive behavior. Likewise, brushite also can be used as an alternative since it acts as an HAp precursor by slowly transforming into HAp while coming in contact with suitable body fluids that are alkaline in nature.

Metallic surfaces can be coated with these bioactive coatings by using various techniques like pulsed laser deposition, sputtering, plasma spraying, electrophoretic deposition (EPD), sol-gel, and electrodeposition (ED). Electrodeposited coatings have a uniform thickness can be produced at a relatively low cost. The phase purity of the coatings, degree of crystallinity, and morphology can be tailored by varying the deposition temperature, pH, Ca:P ratio, and the concentration of additives. However, the above mentioned methods employ organic solvents or organic templating agents that are a potential threat to the environment as well as the biological systems. Therefore, this work focuses on the synthesis of HAp-brushite composite coatings by using abundant environmental-free compounds because of their renewability and cost-effectiveness compared with other synthetic organic templates. There are various environmentally compatible alternatives to traditional reagents used for the production of HAp-brushite composite coatings. Primarily they are based on plant extracts. Locally available apple (\( \text{Malus domestica} \)) contains malic acid. The extraction of malic acid from apples is an easy technique, and it does not require any costly equipment. The malic acid extracted from apples are used as reducing agents and they also serve as antioxidant. Therefore, they can be employed to control the morphology and achieve a green technique for obtaining electrodeposited HAp-brushite composite coating.

Pioneering work carried out by Gopi et al. demonstrate the use of “green templates” that is, naturally extracted organic compounds for the synthesis of HAp such as pectin derived from banana peels, extracts of banana, grape, and tamarind, and sucrose derived from natural as well as commercial sources. They also reported the use of apple-derived malic acid for the preparation of HAp nanoparticles via a sol-gel route. They compared the effectiveness of naturally extracted malic acid from apples apple (\( \text{Malus domestica} \)) with the commercially available malic acid in the resulting HAp samples and investigated the antibacterial activity against the two pathogen bacteria strains viz. Escherichia coli and Klebsiella. Their observations suggest that the naturally extracted malic acid derived HAp samples showed improved antimicrobial activity compared to the commercial malic acid derived HAp samples. The HAp samples derived from the apple extract showed reduced crystallinity and crystallite size compared to that obtained from the commercial malic acid which helped in exhibiting a strong antibacterial activity against both the Gram-negative bacteria of E. coli and Klebsiella. However, for biomedical application, the technology is not quite ready as it lacks scalability and requires additional processes like spray-coating for coating the prepared HAp particles over biological implants. Furthermore, investigating the hemocompatibility and osteocompatibility properties of the HAp samples is also equally vital.

Hence, this present study focuses on developing a consistently adherent composite layer of HAp (Ca to P ratio 1.67) and brushite (Ca to P ratio of 1) over a surface of stainless steel via electrodeposition technique in the presence of naturally extracted malic acid. The use of electrodeposition allows the direct deposition of the bioactive HAp-brushite composite coating on the metallic biomaterials with good adhesion. The deposited coatings have the required amount of a crystallinity and adequate surface area for initiation of tissue growth. The bioactive behavior of the HAp-brushite composite coatings is also investigated.
Experimental procedure

Substrate preparation

The substrates for the electrodeposition of HAp-brushite composite were prepared from the stainless steel (316L) sheet having a thickness of 180 µm procured from Jiangsu Taigang Puxin Stainless Steel Co., Ltd., China. The elemental composition of the substrate, as obtained from energy dispersive spectroscopy (EDS) analysis, is shown in Table 1. The sample surface was polished with different grits of silicon carbide emery papers (600, 800, 1500, and 2000 grits). After polishing, the sample surface was cleaned thoroughly with distilled water and rinsed with ethanol. An exposed surface area of 2 cm² was maintained by masking the extra surface with a layer of non-conductive epoxy resin.

Materials used for malic acid assisted HAp-brushite composite coating fabrication

Ultrapure forms of phosphoric acid (H₃PO₄), calcium chloride dihydrate (CaCl₂.2H₂O) and aqueous ammonia solution were procured from Merck. All the chemicals were of laboratory use grade, and they were used directly. Locally available apple (Malus domestica) was used as a natural source for the extraction of malic acid. The extraction protocol used by Gopi et al.34 was followed for sourcing the malic acid extract.

Electrodeposition of HAp-brushite composite coating

The composite layer was electrodeposited over the stainless steel (SS) substrate via pulse electroplating route. A square-wave pulse was applied with 50% duty cycle (T_on=T_off=10 ms). A typical two-electrode system with SS plates as working as well as the counter electrode was used. The working electrode had an exposed area of 2 cm², and the counter electrode had an exposed area of 4 cm². The electroplating bath was prepared by a consistent mixing 0.1 M of CaCl₂.2H₂O (Merck, >98% purity), 0.15 M of NH₄H₂PO₄ (Merck, >98% purity) along with 0.1 M of malic acid assisted HAp particles in MiliQ water. 2.0 M of NaCl (Merck, 99.8% purity) was added to the solution in order to enhance the conductivity of the electroplating bath. The deposition was carried out at a current density of 50, 100, and 200 mA/cm² for 30 min. All coatings were deposited at room temperature. Excessive hydrogen evolution at the cathode occurs if the deposition voltage exceeds 1.23 V. This may lead to over-porosity in the coatings, which may create adhesion issues of the coating. Therefore, the electrodeposition voltage was maintained below 1.23 V.

Material characterization of the HAp-brushite composite coatings

The phase purity of the composite coatings was analyzed by X-ray diffraction (XRD) (Rigaku SmartLab diffractometer). A Cu target was used with CuKα radiation (with a wavelength of 0.15406 nm). Two theta range between 10° and 50° was scanned with a step size of 0.02° and scanning rate of 0.05° per second. The crystallinity of the coating surface was measured from the obtained XRD patterns, using the modified Scherrer equation:

\[
\beta = \frac{K \lambda}{D \cos \theta + \varepsilon \tan \theta}
\]

Where β is the full width half maximum (FWHM in radian), K is the Scherrer constant (taken as 0.94 here), λ is the wavelength of the X-ray radiation, θ (radian) is the angle of diffraction, D is the interplanar spacing, and ε is the rms strain present in the sample. Because of peak broadening due to the instrumental errors, the accurate value of β is taken per the following formula:

\[
\beta = \sqrt{B^2 - b^2}
\]

Where B is the actual measured value from XRD pattern of the peak and b is the full width at half maximum for the Si single crystal from the same 2θ range.

The microstructure of the composite coatings at different current densities was characterized by field emission scanning electron microscope (FE-SEM) (Hitachi SU9000). Fourier transform infrared spectroscopy (FTIR) was carried out to confirm the presence of phosphate group in the coated layer. FTIR was carried out using Nicolet 6700 spectrometer in the range of 4000–400 cm⁻¹, with a resolution of 8 cm⁻¹ using the KBr pellet method. The hardness of the coatings was measured using nanoindentation method. A constant load of 2.2 mN was applied with a dwelling time of 10 s. All the experimental measurements were carried out at around 25°C.

Hemocompatibility assessment

As per the guidelines laid down by ASTM F 756-00, the hemolytic assay of the samples was conducted in sterilized physiological saline. Coated samples were immersed in saline solution and incubated for 12 h at body temperature that yielded test extract. In order to remove any particulate matter from the same and to ensure only dissolved

| Table 1. Elemental analysis of the SS 316L substrate (weight %). |
|-----------------------------|
| S  | P  | Si | Mn | Mo | Ni | Cr | Fe |
| SS 316L | <0.1 | <0.1 | 0.8 | 2.0 | 2.2 | 9.6 | 17.6 | 67.8 |

components from the coating remain in in-vivo conditions, the solution was subjected to centrifugation, and the resulting supernatant was used as a test extract. Fresh human blood was collected using liquid EDTA as an anticoagulant from a volunteer and diluted in a 1:5 ratio with physiological saline itself. The diluted blood was added to the prepared test extract and set for incubation at body temperature for an hour. Positive and negative controls were taken as Distilled water and Saline, respectively, and the incubated Blood-Test extract was subjected to absorbance measurement in a UV-Vis scanning spectrophotometer (Shimadzu UV-1800) at 545 nm wavelength. The acquired data was tabulated, and Hemolysis ratio (Z) was calculated using the following formula:

\[ Z \text{(Haemolytic ratio)} = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}} \times 100\% \]

where \( D_t \) is the absorbance value of the test samples, and \( D_{pc} \) and \( D_{nc} \) are the positive (water) and negative (saline) control, respectively.

**Cytotoxicity assessment**

Osteosarcoma cell lines MG63 collected from The School of Life Sciences and Biotechnology (SLSB) of Shanghai Jiao Tong University (SJTU) were cultured in DMEM with antibiotic and antimycotic solutions and fetal bovine serum procured from Servicebio Ltd. China. Test extracts as prepared for Hemolytic studies were used to assess the cytotoxicity levels, wherein different volumes (25, 50, 100 µg/ml) were used to determine the optimized release concentration of the samples based on weight measurements of metal samples in coated and uncoated states. Weight measurements of respective coated samples as bare and coated states were used to determine the volume of media to meet weight/volume concentration of test extracts mentioned above. Cultured cells were washed with PBS and incubated with test extracts for 24 h in a serum-free media at 1 × 10^4 cells/well with cells treated with sterilized physiological saline serving as medium for test extracts as control and undergoing similar treatments as test extracts throughout. The medium was aspirated after the incubation period and treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) prepared in PBS and further incubated for 4 h, followed by washing of the cells with PBS. The as-formed crystals that turned purple were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm. Collected data was put to the following equation, and relative cell viability was measured.

\[ \text{Percent Cell Viability} = \frac{\text{Recorded Intensity of Sample}}{\text{Recorded Intensity of Control}} \times 100 \]

**Results and discussion**

**Phase analysis of coating**

Figure 1(a) represents the X-ray diffraction patterns of the composite coatings deposited at various current densities. The XRD of the electrodeposited surface is in good agreement with the standard data of brushite and HAp. Peaks of brushite and HAp can be clearly seen. Therefore, the formation of a HAp-brushite composite layer is confirmed. The coatings deposited at 200 mA/cm² show high-intensity HAp peaks indicating the more amount of HAp presence in the coating compared to the coatings deposited at 100 and 50 mA/cm². The average crystallinity of the coatings deposited was found around 71%. The proposed electroplating protocol delivers a mixture of hydroxyapatite and brushite in the resulting coatings instead of a pure hydroxyapatite coating. According to literature, pure hydroxyapatite coatings obtained via electroplating route show relatively inferior adhesion to the underlying metallic substrates. In contrast, we observed that the presence of brushite phase along with the hydroxyapatite phase improved the adhesion of the coatings significantly. This also does not affect the purpose of this technology as the brushite phase slowly transforms into hydroxyapatite when it comes in contact with body fluids. Similar observations are already studied and documented in literature. Therefore, we allowed some amount of brushite phase to be present along with the hydroxyapatite phase to ensure that the adherence of the coating to the underlying metallic substrate is maintained.

**Hardness and coating adhesion**

The hardness of the coatings was measured using nanoindentation method. All the coatings were exposed to a constant load of 2.2 mN and a dwelling time of 10 s. This was repeated three times on each sample to get the average values. The samples deposited at higher current density showed higher resistance to the load and had higher hardness. Figure 1(b) shows the load versus penetration depth curve for the three samples. The adhesion strength of the samples was checked by a cello-tape pull-out test. The sample deposited at 200 mA/cm² had good adhesion strength, and the coating remained intact with the substrate upon pulling out the cello tape. The samples deposited at 100 and 50 mA/cm² failed to pass the test. Therefore, the structural and biological characterization of the sample deposited at 200 mA/cm² was only carried out.

**Morphology of coating**

The SEM micrographs of the HAp-brushite composite coating deposited at 200 mA/cm² are shown in Figure 2. The observed composite layer has been found to be less crowded and has a porous, spongy structure. The porous
structure is clearly witnessed at higher magnification (Figure 2(b)). Figure 2(c) shows the corresponding EDS spectrum. The presence of Ca, P, and O are evident, and they are present in major quantities. Some traces of Ca$^{2+}$ and PO$_4^{3-}$ is also found.

FTIR and Raman spectroscopy

The FTIR spectra of coating deposited at 200 mA/cm$^2$ are shown in Figure 3(a). It shows the various absorption regions. The bands at ((i) in insert) 3143 and (ii) 668 cm$^{-1}$ are originated from OH$^-$ stretching. The band at (iii) 1554 cm$^{-1}$ is present due to HOH$^-$ bending. The bands for PO$_4$ asymmetric stretching are found at (iv) 1045 and (v) 987 cm$^{-1}$. The band of PO$_4$ asymmetric bending is found at (vi) 571 cm$^{-1}$. The presence of both HPO$_4^{2-}$ and PO$_4^{3-}$ ions in the coating is confirmed from the data obtained. The Raman spectra of the coating deposited at 200 mA/cm$^2$ are shown in Figure 3(b). Bands are observed at positions of 145, 268, 318, 458, 605, and 646 cm$^{-1}$. These bands correspond to HAp-brushite. No other major bands corresponding to any other phases were witnessed. These results obtained are in good agreement with the results attained from the X-ray diffraction analysis, which confirm the presence of HAp and brushite.

Hemolytic assay

Hemolysis, an indication of the destruction of red blood cells (RBCs), can lead to Anemia or Jaundice that makes its assessment against the prepared coatings necessary. The absorbance values dependent on the changes in color intensity show negligible hemolysis as a virtue of the excellent biocompatibility of HAp coatings. The additional layer of coatings forms a physical barrier for the corrosive free ions such as Cl$^-$ to come in contact with the implant surface, thereby preventing physiological leech out of toxic components from the alloy. However, a slight but still biocompatible increase was found when coated alloys were subjected to prolonged immersion in physiological saline-based test extract (Table 2). Such long-term exposure enables fluids to seep through porous coatings given time, and hence the additional components from the alloy affect overall favorable levels of hemolytic activity. Optical micrographs confirm the claims made over absorbance-based measurements and show proper structural integrity of around 80% visible RBCs (Figure 4). A few damaged cells were also observed that could be justified given less than ideal in-vitro working conditions as compared with in-vivo environments, which has also led to agglomeration of cells at preferable areas over slides. Porosity is an essential feature of bioactivity as it increases the available surface area not just for fluids such as saline but rather a cocktail of other components making up the body fluid which is almost impossible to replicate in vitro. Hence it can be stated successfully that even with prolonged exposure, the coatings have hemocompatible features that are necessary for an implant as blood is the most important connective tissue, which not only transports necessary enzymes to bone regeneration sites but also flushes out metabolic by-products.

Cytotoxicity evaluation

Quantifying the cell viability using MTT assay has been elaborated in Figure 5. Human osteosarcoma cell lines were used to assess the toxic nature of coated implants over healthy cells. The assay gives an idea about how the
material will interact with healthy cells under given circumstances in-vitro. Assessment of percent viable cells is performed over the change in color intensity of purple formazan from blue colored MTT. The change in color is by virtue of treatment of the cells, but the sustenance is dependent on live and healthy cells as only live cells can uptake and maintain formazan; hence the color intensity can be established as a parameter of healthy

**Figure 2.** (a) BSE SEM micrographs of the coating deposited at 200 mA/cm² showing a porous structure, (b) coating morphology at higher magnification, and (c) EDX spectrum of the coating.

**Figure 3.** (a) FTIR spectra of the coating deposited at 200 mA/cm² showing various absorption regions and (b) Raman spectrum of the coating deposited at 200 mA/cm².
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The absorbance and the cytotoxicity of the coated sample after a different time frame of treatment at different concentrations are presented in Table 3. It can be seen that most of the samples show favorable cell proliferation rates, but the high mass to solution ratio favors it the most. Within the first 12 h of treatment, the cells proliferate at the highest rate ever with an increase in release concentration as HAp is readily available to cells to stimulate their proliferation levels. An increase in treatment time further elevates the proliferation levels, which faces a slight setback with solutions containing low mass to volume ratio wherein it is speculated that ionic components from the alloy are leached out in the test extract thereby affecting cell growth. Even with such a slight setback at higher release rates, the overall cytotoxicity levels are well within admissible levels, confirming positive biocompatibility of the coatings in-vitro.

A qualitative assessment of the above results was performed to check morphological features of cells by cells measured against a UV spectrophotometer. The absorbance and the cytotoxicity of the coated sample after a different time frame of treatment at different concentrations are presented in Table 3. It can be seen that most of the samples show favorable cell proliferation rates, but the high mass to solution ratio favors it the most. Within the first 12 h of treatment, the cells proliferate at the highest rate ever with an increase in release concentration as HAp is readily available to cells to stimulate their proliferation levels. An increase in treatment time further elevates the proliferation levels, which faces a slight setback with solutions containing low mass to volume ratio wherein it is speculated that ionic components from the alloy are leached out in the test extract thereby affecting cell growth. Even with such a slight setback at higher release rates, the overall cytotoxicity levels are well within admissible levels, confirming positive biocompatibility of the coatings in-vitro.

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Table 2. Absorbance and hemolytic ratio of coated sample calculated with respect to OD values.

| Sample   | Absorbance (545 nm) | Hemolysis ratio | Mean   | Std. Dev. |
|----------|---------------------|-----------------|--------|-----------|
| DD water | 1.059               | 1.058           | 1.059  |           |
| Saline   | 0.053               | 0.053           | 0.053  |           |
| 6H       | 0.054               | 0.055           | 0.054  | 0.0999001 | 0.1332  | 0.06 |
| 12H      | 0.055               | 0.055           | 0.054  | 0.1998002 | 0.0999001 | 0.1665 | 0.07 |
| 24H      | 0.056               | 0.055           | 0.056  | 0.2997003 | 0.1998002 | 0.2997003 | 0.2664 | 0.06 |
| 48H      | 0.058               | 0.056           | 0.059  | 0.4995005 | 0.2997003 | 0.5994006 | 0.4662 | 0.15 |

Figure 4. Micrographs of blood samples treated with test extracts after (a) 6 h, (b) 12 h, (c) 24 h, and (d) 48 h.

Figure 5. Hemolysis % of blood samples treated with test extracts after 6, 12, 24, and 48 h.
observing the same under a digital microscope. The presence of interfering mediums reduced proper visibility that called for the assistance of color filters to view structural features of the cells (Figure 6). All the samples showed intact structural morphology of MG-63 cell lines, which can be attributed to metabolic stimulation by Hydroxyapatite residues released in the test extract. The micrographs were used only to confirm any kind of structural damage to cells. The methodology used to place cells over the observable platform is such inconsistent that cell density observed in the images cannot be taken into account of the proliferation levels. With an increase in treatment time, as discussed earlier, the porous nature of coatings allows the introduction of toxic components from the alloy to come in contact with the cells that cause reversible damage to the same as observed in Figure 7(h) and (i). These changes are brought by the compositional change in environment by those free ions in the form of pH fluctuation or deactivation of a cell wall structure and morphology by change in protein motive force or alteration over binding sites.43 However, the changes do not seem apoptotic and can be recovered in the due process hence supporting the quantitative claims of biocompatibility against MG-63 cells.

### Conclusion

A composite coating of HAp and brushite is deposited successfully on stainless steel surface via pulsed electro-deposition route. The electroplating of the HAp-brushite composite coating is carried out in the presence of malic acid extracted from commercially available apple (*Malus domestica*) via a green route, with no use of any harmful organic templates or solvents. The average crystallinity of the composite layer is around 72%. This is the ideal crystallinity range to balance the growth rate of the tissue on the surface of the implant material and adherence of the tissue with the implants.44 It is observed that the coating deposited at a higher current density (200 mA/cm²) has the highest crystallinity and hence is found to be the most adherent coating among all. The coating at deposited 200 mA/cm² has a porous structure which is best desirable for implant coating. The purity of the composite layer is established from the XRD studies and is further confirmed by Raman spectroscopy. FTIR studies provide the various bonding regions present in the coating deposited at the current density of 200 mA/cm². Cytotoxicity and biocompatibility assessment against RBCs and human osteosarcoma cell lines showed favorable results for an extended period of time. In-vitro assessments were favorable enough to be incorporated within in-vivo environments. Apart from being almost neutral to RBCs, Osteosarcoma cell lines did proliferate in a given environment denying the presence of any kind of negative feedback. The structural integrity of the target cells supported the quantitative data and associated results, which establishes the favorable biocompatibility of coated implants. Hence, the authors can state that HAp Brushite composite coatings over surgical grade steel is compatible with “first-contact” cells in-vitro and can be further studied for commercial application as the entire fabrication was free from any toxic pathway that could

### Table 3. Absorbance and percent cytotoxicity of the coated sample after a different time frame of treatment at different concentrations.

| Time | Sample concentration | Recorded OD | Percent cell viability | Mean   | Std. Dev. |
|------|----------------------|-------------|------------------------|--------|-----------|
|      | Control              | 0.342       | 0.344                  | 0.342  | –         | –         | –        |
| 12 H | 25 µg/ml             | 0.334       | 0.336                  | 0.336  | 97.66     | 98.23     | 98.22    | 98.04    | 0.32     |
|      | 50 µg/ml             | 0.338       | 0.337                  | 0.339  | 98.83     | 98.53     | 99.12    | 98.82    | 0.29     |
|      | 100 µg/ml            | 0.339       | 0.339                  | 0.34   | 99.12     | 99.12     | 99.41    | 99.21    | 0.16     |
| 24 H | 25 µg/ml             | 0.332       | 0.33                   | 0.331  | 97.08     | 96.49     | 96.78    | 96.78    | 0.29     |
|      | 50 µg/ml             | 0.338       | 0.337                  | 0.338  | 98.83     | 98.53     | 98.83    | 98.73    | 0.17     |
|      | 100 µg/ml            | 0.333       | 0.333                  | 0.332  | 97.36     | 97.36     | 97.08    | 97.26    | 0.16     |
| 48 H | 25 µg/ml             | 0.33         | 0.33                   | 0.331  | 96.49     | 96.49     | 96.78    | 96.59    | 0.16     |
|      | 50 µg/ml             | 0.291       | 0.293                  | 0.298  | 85.08     | 85.67     | 87.13    | 85.96    | 1.05     |
|      | 100 µg/ml            | 0.283       | 0.285                  | 0.28   | 82.74     | 83.33     | 81.87    | 82.64    | 0.73     |

*Figure 6. Cytotoxicity of the coated sample after a different time frame of treatment at different concentrations.*
otherwise interfere with bone regeneration metabolism. In the overall scheme of things, the electroplated HAp-brushite composite coatings show good adherence to the underlying metallic substrates and demonstrate excellent osteocompatibility properties. The optimized electroplating process itself can be commercially scaled for coating various metallic implant surfaces of different sizes and shapes. However, a minor limitation of this technology is that it requires a pulse-plating attachment to be added to existing direct electroplating machines in order to assimilate this protocol commercially.

**Data availability statement**
The raw data used in this work may be obtained from the corresponding author upon request.

**Declaration of conflicting interests**
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This
study was supported by the Fundamental Research Funds for the Central Universities (grant 22120210569), the National Scientific Foundation of China (82171993), the Clinical Research Plan of SHDC (SHDC2020CR3083B), the Technology Project of Shanghai Science and Technology Commission (19441902700), the Clinical Research Program of Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine (JYLJ202122), the Project of Biobank from Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine (YBKB202116).

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