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Development of calcium fluoride thin film on Ti-6Al-4V material by a dip coating process with an intermediate shellac layer for biocompatible orthopedic applications

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Abstract

Calcium fluoride (CaF_2) is widely used for different bio applications ranging from biomedical imaging to cell labeling. The biocompatible properties of CaF_2 combined with superior mechanical properties of titanium alloy makes it a perfect choice for orthopedic and dental implants. A dip-coating process was employed to develop a thin film of CaF_2 coating on Ti6Al4V material with an intermediate thin layer of shellac (natural resin). The developed coating was subjected to X-ray powder diffraction method (XRD) and scanning electron microscopy (SEM) to evaluate the surface characteristics. The dip-coated implant material was also subjected to mechanical property evaluation, dissolution behavior study, and corrosion behavior study. In vitro study of the developed implant material was also carried out to assess the biocompatibility. The obtained results suggest use of CaF_2 coating developed by this method for producing biocompatible orthopedic implants.

Keywords: Calcium fluoride, Dip coating, Simulated body fluid, Cytotoxicity

Introduction

The use of metals as body implants is not a new concept. Superior mechanical properties of metals have made it the most suitable option for surgeries. Studies have been conducted on economic feasibility and viability of various metals and alloys for this purpose (Sovak et al. 2000). Stainless steel, cobalt-chrome alloy, commercially pure titanium (cpTi), and titanium alloys are commonly used metal implants (Søballe 1993). These metals are in general biocompatible (Manam et al. 2017). Studies have been carried out introducing new methodologies which promotes faster osseo-integration process. One such technique is to provide a coating on the metal implants with hydroxyapatite (Kuroda and Okido 2012). Different materials are investigated for developing coatings on the metal implants to promote the process of bone-implant interface bonding (Mishra et al. 2014). Calcium fluoride (CaF_2) nanopowders is extensively used for biomedical applications like imaging due to its high stability and brilliant luminescent properties (Cantarelli et al. 2014). For tumor therapies, cell-labeling concept is used to monitor the transplanted stem cells for a long period of time. Traditional cell-labeling agents like organic dyes have limited tissue penetration. CaF_2 can overcome this drawback (Li et al. 2016). CaF_2-based nanoparticles are also used as contrast agents in multimodal imaging techniques like magnetic resonance imaging (MRI) and photoluminescence (PL) microscopy (Straßer et al. 2017).

Coating methods can be broadly classified into pyro-processing and hydro-processing technique (Kuroda and Okido 2012). Coating methods like sputtering, plasma spray, and pulsed laser deposition give thin film coatings, whereas dip-coating and electrophoretic coatings gave comparatively denser coatings (Harun et al. 2017).

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Plasma spray is a commercially accepted coating technique (Khor and Cheang 1997). Studies have pointed out that high temperatures involved in this process, often alters the chemical composition of the material (Heimann 2018). Research is still underway to develop coatings at lower temperatures to get coatings with uniform chemical composition.

Another major application of CaF_2 is in the form of theranostic agents. These are materials which can be used as a combination for therapy and diagnosis (Li et al. 2016). CaF_2 is also showing promising results in the application of nano-medicine. The CaF_2 nanoparticles are capable of carrying a functional protein (like GCAP1) on its surface and can also dissociate it at appropriate cell locations to locally treat the related disease (Marino et al. 2017). A study has reported the preparation of a new type of bioactive glass in which the composition is altered by the inclusion of CaF_2 and when this newly prepared bioactive glasses are immersed in static simulated body fluid, a complete biocompatible hydroxyapatite layer formed on the glass surface within a period of 3 weeks (Tulyaganov et al. 2010).

Many methods have been adopted for developing biocompatible coatings on metal surfaces. Dip-coating process has been used to deposit CaF_2 sols onto borosilicate glasses as an anti-reflective coating (Rehmer et al. 2015). The scope of CaF_2 as a biocompatible coating material for dental applications has been studied extensively (Al-Noaman et al. 2012). These coatings reported to have a drawback of poor adhesion characteristics (Mohseni et al. 2014). To overcome this, researchers have adopted different coating methodologies. This paper reports development of a thin film of CaF_2 on titanium alloy (Ti6Al4V) using a dip-coating technique with the help of an intermediate shellac (a natural resin) layer for better adhesion. The characterization, dissolution rate, cell viability, and corrosion behavior of the developed coating has been studied in a detailed manner.

Materials and methodology

The CaF_2 solution for dip coating was prepared using ethanol (99.99% purity), glycerin, and CaF_2 nanopowders (Nice Chemicals (P) Ltd.). Magnetic stirring was performed at room temperature for 4h to obtain a homogenous solution. Ti6Al4V samples (10mm × 20mm × 2mm thickness) were first abraded using the SiC emery papers with grit size ranging from from 80 to 400. For cleaning the samples, they were immersed in acetone solution and sonication was provided for 45 min. The samples were then air-dried before the coating procedure. A dip-coating apparatus (HOLMARC HO-TH-01B) was used for this purpose. The samples were first dipped in shellac solution and subsequently in the CaF_2 solution.

The crystallinity of the developed thin film was analyzed using XRD (Bruker AXS D8) machine. The surface morphology was studied using SEM (JEOL JSM-6390LV) analysis. The bonding strength (adhesion) between CaF_2 thin film and the base substrate was measured according to ASTM D7234 standard, and the coating thickness was measured in accordance with standard IS - 3203 – 2001. The average surface roughness was measured using Mitutoyo SJ-210 series portable profiler.

The dissolution of CaF_2 in the simulated body fluid (SBF) prepared using Kokubo method with pH value maintained at 7.4 was measured (Kokubo and Takadama 2006). The ion concentration of the as prepared SBF is found similar to that of the human blood plasma. Inductively, the coupled plasma – mass spectrometry (ICP-MS) analysis was used to quantify the ion concentration (in ppm) of SBF. The coated samples were immersed in 30mL of SBF in static position with temperature maintained at 37 ± 0.5°C. Dissolution rate was measured at different time intervals of 7, 14, 21, and 28 days. The SBF-related studies were done as per ISO standard 10993-14.

The samples were sterilized using ethylene oxide (ETO) for performing the cell viability study. Procedures as specified in ISO 10993-12 standard was adopted for the study. The MG-63 cell line was used for the study. The cells were cultured with Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine solution (FBS), and incubated at 37°C in 5% CO_2 for 24 h. For extract preparation, the sterilized samples were immersed in the medium without shaking for 72 h and maintained at 37°C. The extracted medium was then diluted to 50%, 25%, and 12.5% in the same culture medium. MTT assay was used to determine the in vitro cytotoxicity. 10000 cells were seeded in each well of a 96-well plate. After the cells were attached, the medium was treated with 100μL of serially diluted extract solution.

The plates were then incubated at 37°C and 5% CO_2 for 24h. For MTT assay, the medium from wells were removed carefully after incubation. Then, it is further incubated with 180μL of complete medium and 20μL of MTT reagent. The plates were further incubated for 4 h in 5% CO_2 incubator. After the incubation 100μL of solubilizing agent dimethyl sulfoxide (DMSO) and 100μL of pure ethanol were added to each well and mixed well by micro-pipet. The plates were then incubated for 20 min in order to dissolve formazan crystals. The absorbance was measured using TECAN microplate reader at 595nm by using medium in the blank.

The corrosion behavior of the samples was investigated using the potentiodynamic polarization tests. The

electrochemical behavior was measured as per ASTM standard G5-94. A three electrode electrochemical cell of a 250-ml capacity was used for in vitro potentiodynamic corrosion tests. Platinum was used as the counter electrode, saturated calomel electrode (SCE) as the reference electrode, and the samples were kept as the work electrode. Prepared SBF is the electrolyte, and the temperature was maintained at 37°C to simulate the body conditions. 1 cm^2 area of samples was exposed to the electrolyte. The samples were kept in contact with the solution till a steady open circuit potential (OCP) was obtained. The stabilization period of 3600 s was given, and the electrode potential was raised from −1000mV to 1000mV. The scanning rate was $0.005mVs^{-1}$. The potentiodynamic polarization curve was obtained for the coated and uncoated samples, and corrosion current densities were determined by Tafel extrapolation. The corrosion potential (E_{corr}), polarization resistance (R_p), anodic tafel slope (β_a), and cathodic tafel slope (β_c) were recorded.

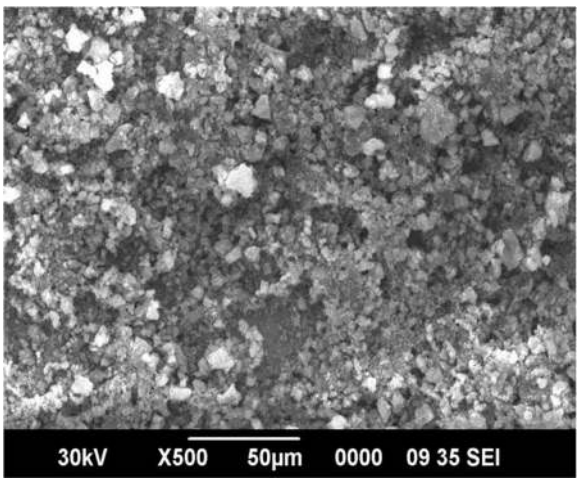

Fig. 2 SEM photograph of a coated sample

Results and discussion

XRD profile of the developed coating is shown in Fig. 1. The crystalline structure of CaF_2 thin films is observed at 2θ angles of 28.28°, 47.02°, and 55.76°. These peaks fall in line with the database of JCPDS (card No. 87 – 0971) as reported in other-related research works (Tahvildari et al. 2012). Figure 2 shows the surface morphology of the substrate coated by a dip-coating process. A thin coating of CaF_2 is seen to be deposited on implant surface. The coating formed is porous in nature, but the thin and uniform layer of shellac applied underneath reduces the effect of porous structure. The average surface roughness parameters have been measured as 0.94µm (R_a), 1.28µm (R_q), and 6.73µm (R_z). The roughness factor would most likely promote osseointegration (Novaes Jr et al. 2010).

The adhesion strength between the coated film and the substrate was measured to be 3MPa. Studies have revealed that post heat treatment process could improve the adhesion strength, but the possibility of crack formation on the coated surface is also very high (Mohseni et al. 2014). Another parameter leading to the crack formation is the thickness of the coated film. Thicker films tend to initiate crack formation faster than thin films. The thickness of the coating developed here was measured to be 6µm.

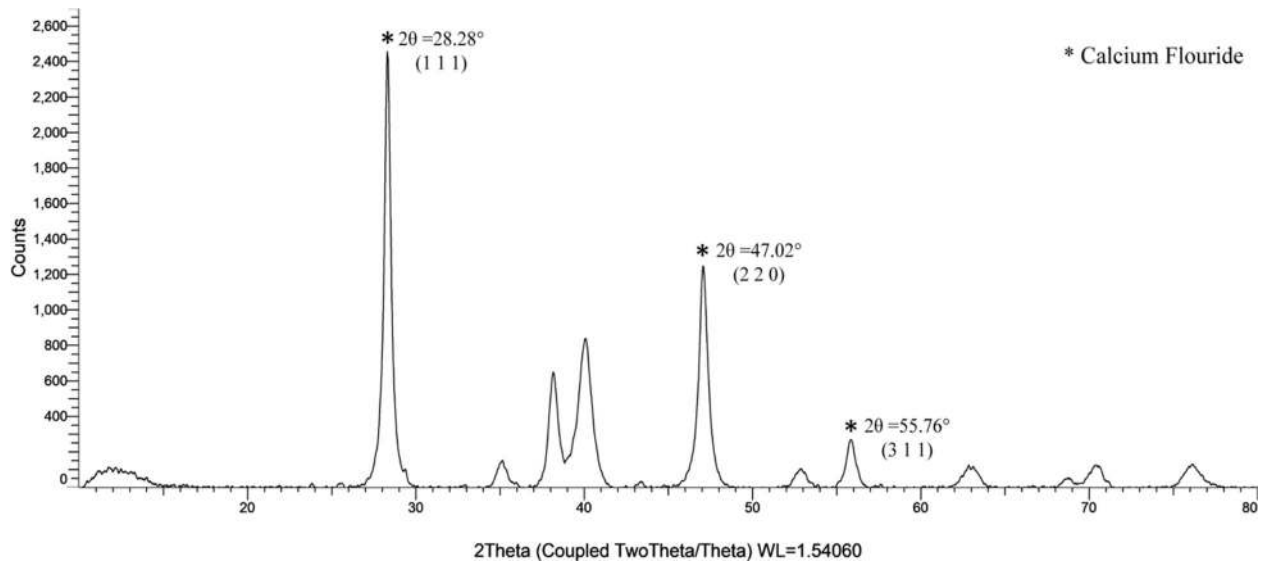

Fig. 1 XRD of a coated sample showing CaF_2 peaks

The stability of the thin film was further analyzed by dissolving the coated samples in the prepared SBF for different time periods. Ionic concentration of the prepared SBF was measured using inductively coupled plasma mass spectrometry (ICP-MS), and the results are shown in Table 1. The dissolution rate of calcium and fluorine ions from the coated samples when immersed in the SBF was analyzed using ICP-MS and is plotted in Fig. 3. The dissolution rate of both Ca and F ions is almost constant over a period of 4 weeks. The dissolution of Ca ions tends to slightly increase in the 2nd and 3rd week of immersion. This is possibly because of apatite formation. Ca^{2+} ion from the coated surface makes bonding with the constituents of SBF. The F ion dissolution in the SBF is almost constant.

The cell viability percentage of the samples was calculated using the following equation, where A (sample) is the absorbance value of the extract solution seeded with cells and A (blank) and A (control) are the absorbance values of medium alone and medium seeded with cells respectively.

$$\%\text{Cell Viability} = \frac{\text{A(Sample)} - \text{A(Blank)}}{\text{A(Control)} - \text{A(blank)}}$$

The cell viability percentage is plotted for both uncoated and CaF_2-coated samples and is shown in Fig. 4. The cell viability is calculated for different dilution proportions. The extracted medium was serially diluted to 50% (50% extract solution mixed with 50% DMEM), 25% (25% extract solution mixed with 75% DMEM), and 12.5% (12.5% of extract solution mixed with 87.5% of DMEM). A decreasing trend of cell viability percentage reduction in dilution proportion is noticed. At the same time, it is also seen that with 50% extract dilution when mixed with 50% DMEM, the cell viability is ~80% which is acceptable by ISO 10993-5 standards. The results indicate that the coatings have no toxic behavior.

The cells were seen attached to the surface after a 72-h duration. Spherical MG-63 cells can be seen attached to the substrate material as shown in Fig. 5. During the study, it was observed that the cell attachment started after a 24-h duration itself. The cells size ranged from 1.63μm to 2.87μm.

The corrosion behavior was verified by electrochemical corrosion studies in simulated body fluid. The potentiodynamic polarization curves of the coated and uncoated samples are shown in Fig. 6. The measured values of parameters like E_{corr}, I_{corr}, β_a, and β_c for coated and uncoated samples are given in Table 2.

The I_{corr} value is directly proportional to the corrosion resistance. So the lower the I_{corr} value, the better the corrosion resistance. From Fig. 6, it is clear that a CaF_2-coated sample have better resistance to the accelerated corrosion. In orthopedic application, degradation of surfaces takes place because of corrosion and it affects the performance of implants. Even a partial corrosion will lead to release of solutes to an implant bone interface (Eliaz 2019). These corrosion products will cause severe histological changes like local irritation, inflammation, and tissue damage (Munir et al. 2016).

The thickness of the CaF_2-coated film has a considerable effect on the corrosion rate. Studies have shown that thin films exhibit better corrosion resistance as compared to thick films (Li et al. 2019). Coating film thickness of more than 40μm will result in producing surface cracks. And these surface cracks will expose the implant surface with the body fluid, and it further reduced the corrosion resistance. Also studies have shown that with increasing film thickness, porosities also increase. These porosities will be the key areas of corrosion. The body fluid will occupy the porosities and it blocks the free oxygen transfer. As we increase the potential, these porosities are filled with corrosion products (Aksakal et al. 2010). No surface cracks were observed on implant surface. The corrosion studies also indicate that the coating developed is stable as the release of metallic ions are very less. Corrosion resistance also augments cell metabolism (Hamanaka and Yoneyama 1989).

Table 1 Composition of human blood plasma and prepared SBF

Ions	Human blood plasma (ppm)	Prepared SBF (ppm)
Na^+	142.0	136.0
Cl^-	103.0	107.0
HCO_3^-	27.0	21.0
K^+	5.0	8.0
Mg^{2+}	1.5	2.0
Ca^{2+}	2.5	3.0
HPO_4^{2-}	1.0	0.5
SO_4^{2-}	0.5	0.3

Conclusion

A crystalline thin film of CaF_2 was dip coated with good adhesion strength on Ti6Al4V implants using shellac as an intermediate layer. The film deposited is observed to be highly stable as the spallation of Ca and F ions in the prepared SBF was negligible. A better corrosion resistance of the metal samples has been observed after coating with CaF_2. A strong bonding has been observed between the implant material and the coating. The thin layer of shellac applied in between the coating material and metal implant can be attributed for higher adhesion strength. Severe cracks were not observed on the coated

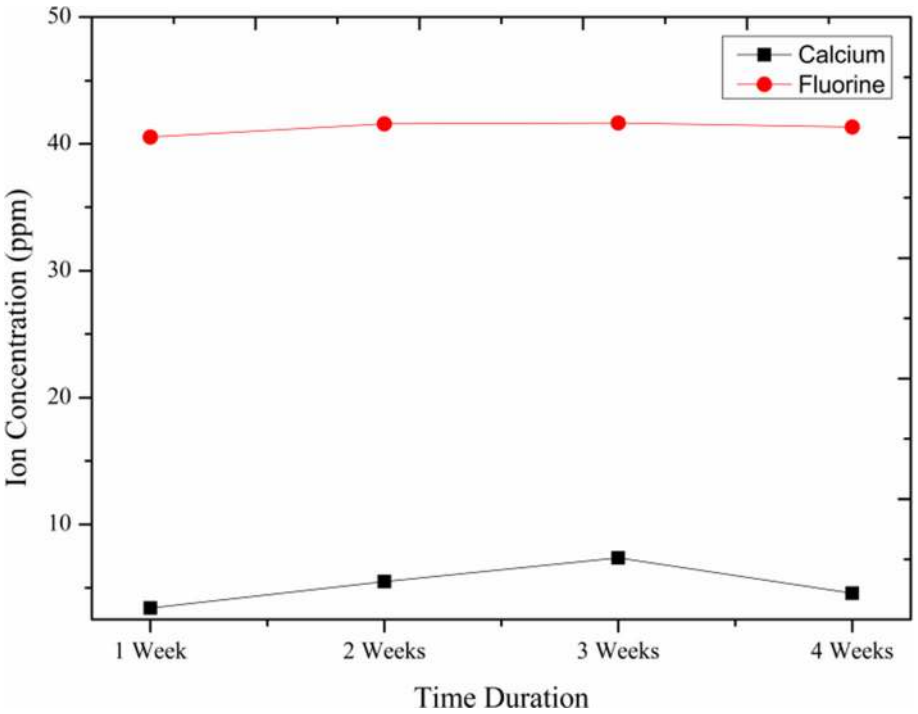

Fig. 3 Change in a concentration of Ca ions and F ions with respect to immersion time

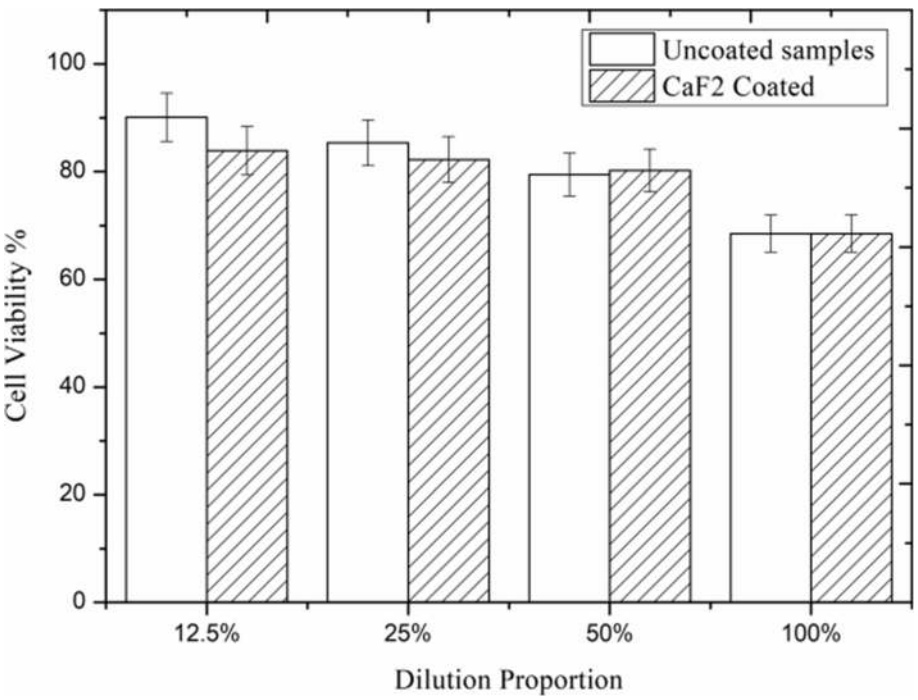

Fig. 4 Graph showing cell viability for different dilution ratios

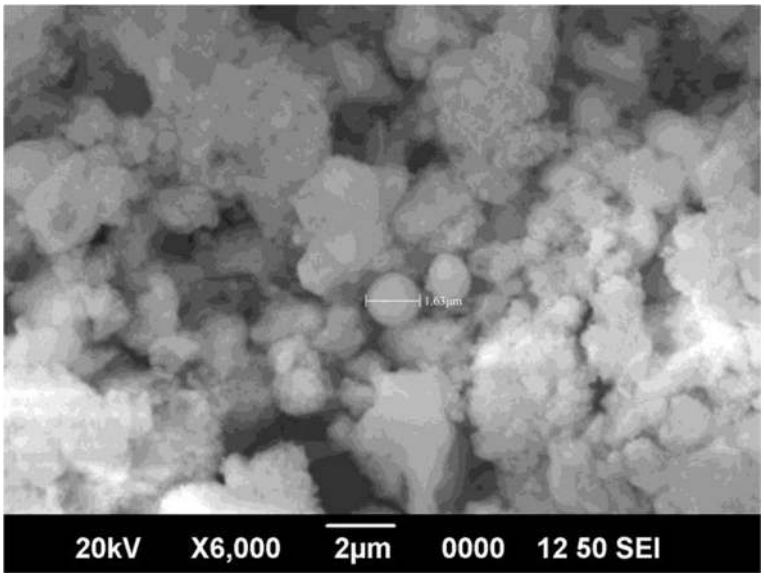

Fig. 5 SEM image showing a cell attachment on the implant surface

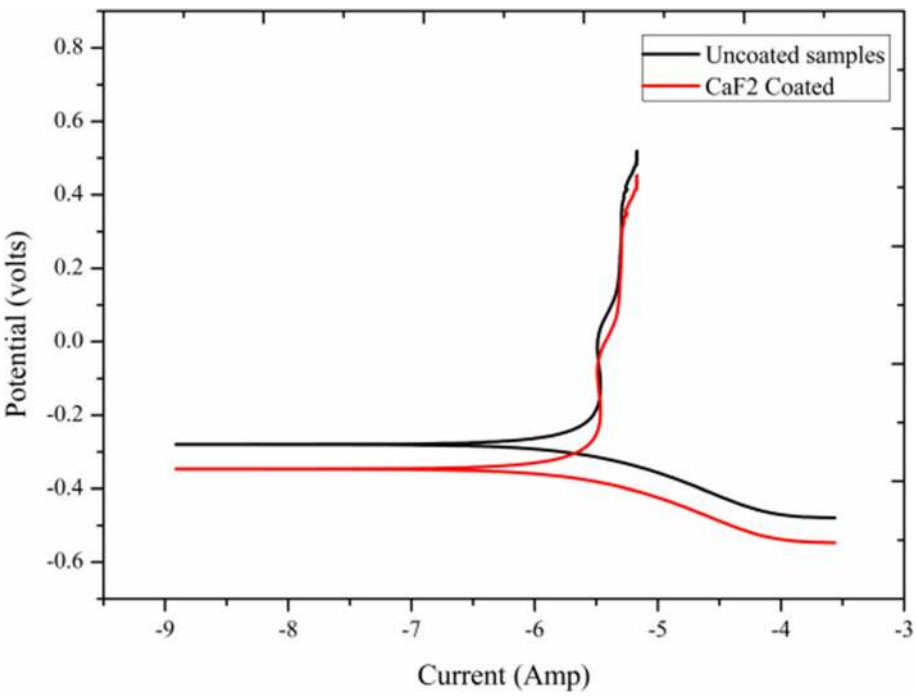

Fig. 6 Potentiodynamic polarization curve of an uncoated and CaF_2-coated sample

Table 2 Electrochemical parameter values from the polarization tests

Sample details		E_{corr} (V)	J_{corr} (A/cm^2)	I_{corr} (A)	β_a V.dec^{-1}	β_c V.dec^{-1}	R_p Ώ. Cm^2	Corrosion rate
Uncoated Samples	Trial 1	−0.28263	1.10×10^{-6}	1.10×10^{-6}	0.092278	0.06007	14382	0.012767
	Trial 2	−0.27971	7.78×10^{-6}	7.78×10^{-6}	−0.64099	0.18418	14434	0.090361
Coated Samples	Trial 1	−0.29542	2.1×10^{-7}	2.1×10^{-7}	0.11634	0.0697	90192	0.0024837
	Trial 2	−0.38292	1.06×10^{-6}	1.06×10^{-6}	1.3465	0.15317	56209	0.012347

surface. The film obtained though porous in structure promoted the cell growth as evidenced from the cytotoxicity tests and cell attachments. The mechanical parameters and in vitro performance of the CaF_2-coated samples suggest its use for biomedical application.

Abbreviations

XRD: X-ray powder diffraction method; SEM: Scanning electron microscopy; MRI: Magnetic resonance imaging; PL: Photoluminescence; SBF: Simulated body fluid; ICP-MS: Inductively coupled plasma – mass spectrometry; ETO: Ethylene oxide; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dimethyl sulfoxide; SCE: Saturated calomel electrode; OCP: Open circuit potential

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Authors' contributions

Ritwik. A has contributed to the experimental and analytical work of the research, and K K. Saju has involved himself in the validation and evaluation of the findings reported in the paper. Both authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that data supporting the findings of this study are available within the article.

Declarations

Competing interests

The authors declare that they have no competing interests.

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References

Aksakal, B., Gavgali, M., & Dikici, B. (2010). https://doi.org/10.1007/s11665-009-9559-7). The effect of coating thickness on corrosion resistance of hydroxyapatite coated Ti6Al4V and 316L SS implants. *Journal of Materials Engineering and Performance*, *19*(6), 894–899.

Al-Noaman, A., Rawlinson, S. C. F., & Hill, R. G. (2012). The influence of CaF_2 content on the physical properties and apatite formation of bioactive glass coatings for dental implants. *Journal of Non-Crystalline Solids*, *358*(15), 1850–1858 https://doi.org/10.1016/j.jnoncrysol.2012.05.039.

Cantarelli, I. X., Pedroni, M., Piccinelli, F., Marzola, P., Boschi, F., Conti, G., & Speghini, A. (2014). Multifunctional nanoprobes based on upconverting lanthanide doped CaF2: towards biocompatible materials for biomedical imaging. *Biomaterials Science*, *2*(9), 1158–1171 https://doi.org/10.1039/C4BM00119B.

Eliaz, N. (2019). Corrosion of metallic biomaterials: a review. *Materials (Basel, Switzerland)*, *12*(3), 407 https://doi.org/10.3390/ma12030407.

Hamanaka, H., & Yoneyama, T. (1989). Corrosion of metallic biomaterials: a review. *Corrosion Engineering*, *38*(6), 333–339 https://doi.org/10.3323/jcorr1974.38.6_333.

Harun, W. S. W., Mohd Asri, R., Alias, J., Zulkifli, F., Kadirgama, K., Bin Che Ghani, S., & Shariffuddin, J. (2017). A comprehensive review of hydroxyapatite-based coatings adhesion on metallic biomaterials. *Ceramics International*, *44* https://doi.org/10.1016/j.ceramint.2017.10.162.

Heimann, R. B. (2018). https://doi.org/10.1007/s11666-018-0737-8). Plasma-sprayed hydroxylapatite coatings as biocompatible intermediaries between inorganic implant surfaces and living tissue. *Journal of Thermal Spray Technology*, *27*(8), 1212–1237.

Khor, K. A., & Cheang, P. (1997). Plasma sprayed hydroxyapatite(HA) coatings produced with flame spheroidised powders. *Journal of Materials Processing Technology*, *63*(1-3), 271–276 https://doi.org/10.1016/S0924-0136(96)02634-9.

Kokubo, T., & Takadama, H. (2006). How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials*, *27*(15), 2907–2915 https://doi.org/10.1016/j.biomaterials.2006.01.017.

Kuroda, K., & Okido, M. (2012). Hydroxyapatite coating of titanium implants using hydroprocessing and evaluation of their osteoconductivity. *Bioinorganic Chemistry and Applications*, *2012*, 1–7 https://doi.org/10.1155/2012/730693.

Li, M., Jin, Z.-X., Zhang, W., Bai, Y.-H., Cao, Y.-Q., Li, W.-M., & Li, A.-D. (2019). Comparison of chemical stability and corrosion resistance of group IV metal oxide films formed by thermal and plasma-enhanced atomic layer deposition. *Scientific Reports*, *9*(1) https://doi.org/10.1038/s41598-019-47049-z, 10438.

Li, Z., Zhang, Y., Huang, L., Yang, Y., Zhao, Y., El-Banna, G., & Han, G. (2016). Nanoscale "fluorescent stone": luminescent calcium fluoride nanoparticles as theranostic platforms. *Theranostics*, *6*(13), 2380–2393 https://doi.org/10.7150/thno.15914.

Manam, N. S., Harun, W. S. W., Shri, D. N. A., Ghani, S. A. C., Kurniawan, T., Ismail, M. H., & Ibrahim, M. H. I. (2017). Study of corrosion in biocompatible metals for implants: a review. *Journal of Alloys and Compounds*, *701*, 698–715 https://doi.org/10.1016/j.jallcom.2017.01.196.

Marino, V., Borsatto, A., Vocke, F., Koch, K.-W., & Dell'Orco, D. (2017). CaF_2 nanoparticles as surface carriers of GCAP1, a calcium sensor protein involved in retinal dystrophies. *Nanoscale*, *9*(32), 11773–11784 https://doi.org/10.1039/C7NR03288A.

Mishra, V. K., Bhattacharjee, B. N., Parkash, O., Kumar, D., & Rai, S. B. (2014). Mg-doped hydroxyapatite nanoplates for biomedical applications: a surfactant assisted microwave synthesis and spectroscopic investigations. *Journal of Alloys and Compounds*, *614*, 283–288 https://doi.org/10.1016/j.jallcom.2014.06.082.

Mohseni, E., Zalnezhad, E., & Bushroa, A. R. (2014). Comparative investigation on the adhesion of hydroxyapatite coating on Ti–6Al–4V implant: a review paper. *International Journal of Adhesion and Adhesives*, *48*, 238–257 https://doi.org/10.1016/j.ijadhadh.2013.09.030.

Munir, S., Oliver, R. A., Zicat, B., Walter, W. L., Walter, W. K., & Walsh, W. R. (2016). The histological and elemental characterisation of corrosion particles from taper junctions. *Bone & Joint Research*, *5*(9), 370–378 https://doi.org/10.1302/2046-3758.59.2000507.

Novaes Jr., A. B., de Souza, S. L. S., de Barros, R. R. M., Pereira, K. K. Y., Iezzi, G., & Piattelli, A. (2010). Influence of implant surfaces on osseointegration. *Brazilian Dental Journal*, *21*(6), 471–481 https://doi.org/10.1590/s0103-64402010000600001.

Rehmer, A., Scheurell, K., & Kemnitz, E. (2015). Formation of nanoscopic CaF_2via a fluorolytic sol–gel process for antireflective coatings. *Journal of Materials Chemistry C*, *3*(8), 1716–1723 https://doi.org/10.1039/C4TC02510E.

Søballe, K. (1993). Hydroxyapatite ceramic coating for bone implant fixation. *Acta Orthopaedica Scandinavica*, *64*(sup255), 1–58 https://doi.org/10.3109/17453679309155636.

Sovak, G., Weiss, A., & Gotman, I. (2000). Osseointegration of Ti6Al4V alloy implants coated with titanium nitride by a new method. *The Journal of Bone and Joint Surgery. British Volume*, *82-B*(2), 290–296 https://doi.org/10.1302/0301-620X.82B2.0820290.

Straßer, M., Schrauth, J. H. X., Dembski, S., Haddad, D., Ahrens, B., Schweizer, S., & Sextl, G. (2017). Calcium fluoride based multifunctional nanoparticles for multimodal imaging. *Beilstein Journal of Nanotechnology*, *8*, 1484–1493 https://doi.org/10.3762/bjnano.8.148.

Tahvildari, K., Esmaeilipour, M., Ghammamy, S., & Nabipour, H. (2012). CaF_2 nanoparticles: synthesis and characterization. *International Journal of Nano Dimension*, *2*, 269–273 https://doi.org/10.7508/ijnd.2011.04.008.

Tulyaganov, D. U., Agathopoulos, S., Valerio, P., Balamurugan, A., Saranti, A., Karakassides, M. A., & Ferreira, J. M. F. (2010). Synthesis, bioactivity and preliminary biocompatibility studies of glasses in the system CaO–MgO–SiO_2–Na_2O–P_2O_5–CaF_2. *Journal of Materials Science: Materials in Medicine*, *22*(2), 217–227 https://doi.org/10.1007/s10856-010-4203-5.

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