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Near-infrared luminescent $\text{CaTiO}_3\text{:Nd}^{3+}$ nanofibers with tunable and trackable drug release kinetics†

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Abstract

750–850 nm (NIR I) and 1000–1400 nm (NIR II) in the near infrared (NIR) spectra are two windows of optical transparency for biological tissues with the latter capable of penetrating tissue deeper. Monitoring drug release from the drug carrier is still a daunting challenge in the field of nanomedicine. To overcome such a challenge, we propose to use porous Nd^{3+} -doped CaTiO_3 nanofibers, which can be excited by NIR I to emit NIR II light, to carry drugs to test the concept of monitoring drug release from the nanofibers by detecting the NIR II emission intensity. Towards this end, we first used electrospinning to prepare porous Nd^{3+} -doped CaTiO_3 nanofibers by adding micelle-forming surfactant Pluronic F127, followed by annealing to remove the organic component. After a model drug, ibuprofen, was loaded into the porous nanofibers, the drug release from the nanofibers into the phosphate buffered saline (PBS) solution was monitored by detecting the NIR II emission from the nanofibers. We found that the release of the drug molecules from the nanofibers into the PBS solution triggers the quenching of NIR II emission by the hydroxyl groups in the surrounding media. Consequently, more drug release corresponded to more reduction in the intensity of the NIR II emission, allowing us to monitor the drug release by simply detecting the intensity of NIR II from the nanofibers. In addition, we demonstrated that tuning the amount of micelle-forming surfactant Pluronic F127 enabled us to tune the porosity of the nanofibers and thus the drug release kinetics. This study suggests that Nd^{3+} doped CaTiO_3 nanostructures can serve as a promising drug delivery platform with the potential to monitor drug release kinetics by detecting the tissue-penetrating NIR emission.

†Electronic supplementary information (ESI) available: TG-DSC curves of the $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers; EDS and elemental mapping of the $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers; the EDS elemental quantification of $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers; SEM images of the as-formed $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ precursor nanofibers obtained at different PVP/CTO molar ratios; TEM and HRTEM images of $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers obtained at different F127/CTO molar ratios; TG curves of the $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers and pure IBU; PL emission intensity as a function of release time for IBU loaded $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers obtained at different F127/CTO molar ratios. See DOI: 10.1039/c5tb01158b

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1. Introduction

Over the past few decades, sustained drug delivery systems (SDDSs) have received increasing attention for biomedical and pharmaceutical applications in healthcare engineering. Conventional dosage administrations have shown considerable gastrointestinal or renal side effects.¹ In contrast, sustained drug delivery systems can deliver the therapeutic drugs to the targeted cells or tissues at the expected concentration over a desirable period of time,² thus improving the drug effectiveness and reducing the toxicity. Polymer-based pharmaceutical carrier systems have been utilized as such delivery systems for improving drug loading efficiency,³ but some of the synthetic polymers are non-biodegradable and potentially toxic.⁴ Recently, biocompatible porous inorganic materials, such as bioactive glass,⁵ silica,⁶ hydroxyapatite,⁷ calcium phosphate⁸ and calcium silicate,⁹ have been extensively investigated as efficient drug delivery systems owing to their sustained drug-release profiles, nontoxic nature and capability to deliver trace elements (*e.g.* Ca²⁺, Fe³⁺) essential for human health.

Calcium titanate (CaTiO₃, CTO) is an established bioceramic, which has been widely used as a coating material for orthopedic implant.¹⁰ It has been reported that coating of titanium (Ti) and its alloys with hydroxyapatite (HA) results in the formation of a CaTiO₃ interface layer.¹¹ Such an interface layer was found to increase the bonding strength between the Ti-based substrate and HA, and also suppress the progression of HA dissolution in an acidic environment induced by osteoclastic bone resorption in the body.^{10c,12} While CaTiO₃ is routinely used for bone implant devices, it has potential to serve as a drug carrier for building SDDSs. Apart from nontoxicity and biocompatibility, an ideal drug-delivery system is expected to transport the desired drug molecules to the targeted tissues and release them in a controlled manner. To achieve this, not only the drug release efficiency and the selection of disease therapy are of particular interest, but the monitoring and tracking of drug releasing kinetics in an external and *in situ* manner during the drug-release process is also equally critical.

Fluorescence materials have been established for bioimaging. The organic dyes and semiconductor quantum dots (QDs) have been synthesized and investigated extensively over the past few decades to monitor biological species and processes in living systems.¹³ Drug-carrying vehicles functionalized with fluorescence have been used to monitor and assess the drug release and disease therapy efficiency. In order to minimize high absorption and scattering as well as autofluorescence of light that occurs in biological tissues, near-infrared (NIR) light has played an eye-catching role for such a purpose in the biomedical field.¹⁴ The NIR light has shown the ability to penetrate ~10 cm of the breast tissue, ~4 cm of skull/brain tissue and deep muscle tissue under a microwatt spectral excitation. The NIR range of 750–850 nm, also called as the first near-infrared window (NIR I), has been considered as the ‘window of optical transparency’ for biological tissues,¹⁵ which allows for deep excitation light penetration, reduced photo damage effects, low autofluorescence and light scattering through tissue. Many organic dyes, including commonly used cyanine dyes, lie within this NIR I region and have been extensively investigated for drug delivery applications.¹⁶ Unfortunately, the rapid photobleaching rate of organic dyes limits the available detection time, and the short fluorescent lifetimes as well as broad emission are not beneficial for

reducing the background interference to increase the signal to noise ratio.¹⁷ These shortcomings have been successfully overcome by semiconductor QDs, which possess high quantum yields, good photostability and narrow emission.^{14,18} However, NIR-emitting QDs can only be efficiently excited using visible or UV light due to the low intensity of the first excitonic absorption band.¹⁹ In addition, there have been wide concerns on the inherent toxicity and chemical instability of QDs.²⁰

Recently, rare earth (RE) ion (typically trivalent) doped upconversion (UC) fluorescent nanomaterials, which can convert longer wavelength radiation (980 nm) to a shorter wavelength fluorescence (*e.g.* visible light) *via* a two-photon or multiphoton mechanism, have been proposed as an alternative candidate to quantum dots and dyes.²¹ The UC nanomaterials hold a range of advantages including narrow emission peaks, large Stokes shifts, good chemical stability, and low toxicity.²² However, a recent study has shown a temperature rise of 10 °C for samples (5 mg UCNPs per mL of deionized water) under the 980 nm excitation for 10 min at a power density of 35 W cm⁻², and observed the death of all cells (HEK 293T) at an excitation power density of 400 mW cm⁻² under 980 nm excitation.²³ In addition, the UC fluorescence (*e.g.* Er/Yb doped UC nanomaterials) lies in the visible region (red and green),^{21d,22} where soft tissues show strong absorption or scattering. In contrast, Nd³⁺ ions are considered as good candidates to achieve high down-conversion (DC) quantum efficiency and can also lower the thermal effects associated with the UC process under excitation with the 980 nm spectrum.²³ Besides, under excitation at 800 nm, Nd³⁺ ions induce a NIR DC emission within 850–1200 nm, which lies in the second near-infrared window (NIR II at 1000–1400 nm).²⁴ Compared to NIR I, the NIR II spectral window is much more appealing due to its deeper tissue penetration, minimal autofluorescence and negligible light scattering, which facilitates the monitoring of excited emission.^{24,25} The Nd³⁺ ion DC emission may be a facile and effective approach to tracking the drug delivery phenomenon in deep natural tissue. Furthermore, Nd atoms can be feasibly doped and ‘caged’ within the lattice of CaTiO₃ to avoid possible lanthanide element releasing due to its perovskite crystal structure and the dimensional matching of Nd and Ca atoms.²⁶ Meanwhile, CaTiO₃ is a transparent material with a high refractive index in visible and NIR wavelengths,²⁷ which makes it an efficient host material for Nd³⁺ doping. Therefore, Nd³⁺ doped CaTiO₃ nanomaterials are considered as a class of ‘smart’ carrier candidates for drug delivery applications.

In this study, a variety of Nd³⁺ doped CaTiO₃ nanofibers with controlled structural characteristics were successfully synthesized *via* electrospinning. Ibuprofen (IBU), an anti-inflammatory drug, was used initially as a test model. The drug loading quantity and releasing kinetics were systematically studied. More importantly, the NIR II emission of the nanofibers was found to be directly related to the amount of drug released from the nanofibers. The main mechanism for such a phenomenon was proposed. A promising local drug delivery platform with monitorable releasing kinetics *via* an external optical field has therefore been suggested to the community.

2. Results and discussion

2.1 Structure, morphology and the formation mechanism of $\text{CaTiO}_3\text{:}1\%\text{Nd}^{3+}$ nanofibers

Fig. 1a shows the typical photoluminescence (PL) emission spectra of $\text{CaTiO}_3\text{:Nd}^{3+}$ nanofibers excited at 800 nm. Pure CaTiO_3 nanofibers are not luminescent, as expected. The luminescence of CaTiO_3 nanofibers was observed when doped with different concentrations of Nd^{3+} ions. The characteristic peaks at 1072 nm correspond to $^4\text{F}_{3/2}\text{--}^4\text{I}_{11/2}$ transitions of Nd^{3+} , and the luminescence was quenched when the doping concentration was below or exceeds 1%. The maximum intensity at ~1072 nm was achieved on the nanofibers with the Nd^{3+} doping concentration of 1%, and thus this doping concentration was selected for further investigations.

The thermogravimetry-differential scanning calorimetry (TG-DSC) curves of the as-spun nanofibers (Fig. S1, ESI[†]) show ~14.82%, ~47.8% and ~10.88% weight losses with the increased temperature to ~600 °C, which are attributed to the evaporation of residual solvent, decomposition of nitrates and the degradation of PVP and surfactant Pluronic F127, and carbon oxidation released by the decomposition of tetrabutyl titanate and PVP, respectively.²⁸ No further significant weight loss is observed beyond 650 °C, indicating that the perovskite CaTiO_3 phase is formed. Therefore, the ultimate sintering temperature is set at 700 °C in the following experiments. Further, the crystallinity of 1% Nd^{3+} doped CaTiO_3 nanofibers annealed with this temperature was determined by the X-ray diffraction (XRD). As shown in Fig. 1b, well-defined diffraction peaks at (110), (111), (112), (210), (103), (022), (220), (204), (224) and (110) match with those of the orthorhombic CaTiO_3 . When compared with orthorhombic CaTiO_3 standard (JCPDS 82-0228), no impurity or secondary phases are present. The investigation using energy dispersive spectroscopy (EDS) shows the coexistence of the Ca, Ti, Nd and O elements with homogeneous distribution within $\text{CaTiO}_3\text{:}1\%\text{Nd}^{3+}$ nanofibers (ESI, [†] Fig. S2 and Table S1). The results above suggest that Nd^{3+} ions were efficiently doped into the CaTiO_3 crystal lattice.

The morphology of the $\text{CaTiO}_3\text{:}1\%\text{Nd}^{3+}$ nanofibers prepared with various F127/CTO molar ratios (denoted as χ) was examined (Fig. 2). The as-spun $\text{CaTiO}_3\text{:}1\%\text{Nd}^{3+}$ precursor fibers range from ~150 to ~180 nm in diameter with a smooth surface morphology (ESI, [†] Fig. S3). After annealing at 700 °C for 2 h, the nanofibers present a rough surface with a decreased diameter of ~80 to ~150 nm due to the decomposition of organic additives, inorganic salts and the formation of a perovskite CaTiO_3 phase. The porous structure of nanofibers after thermal treatment at 700 °C is mainly attributed to the removal of surfactant Pluronic F127 self-assembly micelles and PVP. The internal microstructural features of nanofibers were examined further using transmission electron microscopy (TEM), where the highly porous nature of all fibers can be clearly distinguished due to the degree of electron penetrability on different parts of the samples. The high-magnification TEM images confirm the porous microstructural characteristics, and show that the pore size tends to increase from ~5 to ~20 nm with an increased χ value (the insets of Fig. 2d–f). All nanofibers present a large scale of neatly arranged lattice fringes with no obvious defects, indicating high crystallinity of CaTiO_3 nanofibers. The spacing d -values are 0.382 nm, 0.270 nm and 0.342 nm, corresponding to the (110), (112) and (111) crystal facets of the orthorhombic CaTiO_3 .

phase, respectively (ESI,[†] Fig. S4). These results are in agreement with the findings from XRD.

The N₂ adsorption/desorption analysis was used to determine the effect of χ ratio on the porosity of CaTiO₃:1%Nd³⁺ nanofibers prepared, as illustrated in Fig. 3. All samples show representative IV-type isotherms, a typical sign for porous materials. The textural parameters of the corresponding samples are summarized in Table 1. CaTiO₃:1%Nd³⁺ nanofibers with a χ ratio of 5 m have a BET surface area of $\sim 16 \text{ m}^2 \text{ g}^{-1}$, a pore volume of $\sim 0.06 \text{ cm}^3 \text{ g}^{-1}$ and a narrow pore size distribution (insets of Fig. 3). The decrease of the χ ratio induces a significant increase of BET surface area and pore volume. The nanofibers synthesized without surfactant Pluronic F127 (χ ratio = 0) are found to have $\sim 34 \text{ m}^2 \text{ g}^{-1}$ surface area, $\sim 0.14 \text{ cm}^3 \text{ g}^{-1}$ pore volume and a relative broad pore size distribution (insets of Fig. 3). Both BET specific surface area and the BJH desorption cumulative pore volume have doubled and the pore dimension tends to be larger in comparison to the sample prepared with the highest χ ratio. The variation of surface area, pore volume and pore size is induced by the micelle aggregation of surfactant Pluronic F127, which was reported in previous studies.^{6a,29}

The formation process of porous structures in CaTiO₃:1%Nd³⁺ nanofibers is shown schematically in Fig. 4. Initially, PVP was added into the precursor of calcium titanate to prepare a suitable sol for a stable electrospinning process. When the surfactant Pluronic F127 was used, the F127 polymer chain self-assembled into micelles with hydrophobic groups as a core and hydrophilic groups as a shell in the fluid. When a large number of micelles aggregate, irregular round shaped pores formed and existed in the as-spun nanofibers. As χ increased, the micelles aggregate further, which promotes the formation of larger pores. When the F127/CTO molar ratio reaches 5m, the size of pores with irregular circular shape increases significantly. Therefore, CaTiO₃:1%Nd³⁺ nano-fibers with rather controllable porous structures were obtained by tuning the F127 concentrations after the annealing process.

2.2 Cytotoxicity

Cytocompatibility of a material is a crucial factor for its potential drug delivery applications. Hence, the *in vitro* cytotoxicity of CaTiO₃:1%Nd³⁺ nanofibers synthesized with different F127/CTO molar ratios and cultured with bone marrow-derived mesenchymal stem cells (BMSCs), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for the cytotoxicity assessment. As shown in Fig. 5, the viability of BMSCs with different concentrations of CaTiO₃:1%Nd³⁺ nanofibers varying from 100 to 4000 $\mu\text{g mL}^{-1}$ for 1, 3 and 5 days was investigated. It shows that the viability of BMSCs is not suppressed from the lowest concentration of 100 $\mu\text{g mL}^{-1}$ to the highest of 4000 $\mu\text{g mL}^{-1}$ for all time points (up to 5 days), indicating that the CaTiO₃:1%Nd³⁺ nanofibers are biocompatible and non-cytotoxic to live cells, which allows further investigation of drug loading and releasing behaviors.

2.3 Drug loading

The Fourier transform infrared (FTIR) spectra of CaTiO₃:1%Nd³⁺ nanofibers, pure IBU drug and IBU loaded CaTiO₃:1%Nd³⁺ nano-fibers are shown in Fig. 6. When

CaTiO₃:1%Nd³⁺ nanofibers were loaded with IBU drugs, typical characteristic peaks such as Ti–O bands at ~445 and ~556 cm⁻¹, C–C bands at ~1328, ~1460 and ~1512 cm⁻¹, COOH groups at ~1720 cm⁻¹ and C–H_x bonds at ~2862 and ~2953 cm⁻¹ were present. The characteristic peaks of curve b well matched the curve combination of pure CaTiO₃:1%Nd³⁺ nanofibers (curve a) and IBU drugs (curve c), confirming that IBU molecules were successfully loaded on the CaTiO₃:1%Nd³⁺ nanofibers. In addition, the thermal gravity (TG) analysis was pursued to investigate the drug loading capacity of different nanofibers. The most dramatic weight loss of IBU occurs at 220 °C (ESI,† Fig. S5). As shown in Fig. 7, no distinguishable weight loss is observed during the heating process of pure CaTiO₃:1%Nd³⁺ nanofibers. In contrast, all IBU loaded CaTiO₃:1%Nd³⁺ nanofibers displayed substantial weight loss at ~220 °C, as expected. When the F127/CTO molar ratio is increased from 0 to 5 m, the weight loss of IBU loaded CaTiO₃:1%Nd³⁺ nanofibers varies from ~50 wt% to ~30 wt%. Such a weight loss is mainly attributed to the decomposition of IBU molecules loaded on the nanofibers. Hence, the loading capacity of CaTiO₃:1%Nd³⁺ nanofibers with F127/CTO molar ratios of 0, 2.5 and 5 m is ~50 wt%, ~40 wt% and ~30 wt %, respectively. The decreased loading capacity is attributed to the enlarged pore dimensions, the decreased surface area and pore volume of the nanofibers with increased surfactant Pluronic F127 contents.

2.4 Drug release kinetics and photoluminescence (PL) intensities

During the drug releasing process in phosphate buffered saline (PBS) aqueous solution, accompanied by fluid diffusion into the pores of nanofibers, IBU molecules are liberated and diffuse into the fluid by a diffusion-controlled mechanism.^{2b,5a,30} The cumulative drug release profiles of all three IBU loaded CaTiO₃:1%Nd³⁺ systems in PBS are shown in Fig. 8a. IBU loaded CaTiO₃:1%Nd³⁺ nanofibers with different χ exhibit dramatically different releasing phenomena. When the χ ratio is 5 m, ~65% of total IBU loaded is released within the initial 5 h, and ~90% is released in ~20 h. With the decreased χ ratio, the drug releasing behavior shows a more sustained manner. The IBU loaded CaTiO₃:1%Nd³⁺ nanofibers, synthesized without surfactant Pluronic F127, present the slowest drug releasing behavior. Only ~25% of the IBU drug is liberated from the nanofibers within the initial 5 h, and as low as ~50% of the total drug load is released after 45 h. Therefore, it is confirmed that IBU loaded CaTiO₃:1%Nd³⁺ nanofibers synthesized without surfactant Pluronic F127 present the highest drug loading capacity and the most sustained release kinetics. This may be attributed to the strong interaction between IBU molecules and the fiber microstructures, such as the reduced pore dimensions and the increased surface area. Therefore, the drug loading and releasing behaviors have been successfully manipulated *via* the synthesis control of CaTiO₃:1%Nd³⁺ nanofibers.

More importantly, the photoluminescence phenomenon of CaTiO₃:1%Nd³⁺ nanofibers was found to respond effectively in tune with the drug releasing process. The PL intensity at ~1072 nm is dramatically decreased during the drug releasing for all types of nanofibers (ESI,† Fig. S6). The relationship between the relative PL intensity and the PBS immersion time is summarized in Fig. 8b. CaTiO₃:1%Nd³⁺ nanofibers with fast drug release behavior quench the PL most rapidly. ~25% of PL intensity is quenched within the initial 5 h, and ~50% is reduced after 10 h drug releasing. With the reduced χ ratio, the PL quenching effect

is weakened. In contrast, the nanofibers with most sustained drug releasing kinetics (χ ratio = 0) present a rather delayed PL intensity quenching effect; only ~10% of PL intensity is quenched within the initial 5 h, and as low as ~20% relative PL intensity is quenched after ~45 h immersion in PBS. The PL quenching effect is directly corresponding to the drug releasing process. In addition, one known fact is that the physiological environment is much more complex in comparison to the PBS solution. To verify the findings above, simulated body fluid (SBF), a well-known method for the bioactivity assessment of biomaterials,³¹ has been used to investigate the drug release behavior and PL variation in a simulated physiological environment. As shown in Fig. S7a (ESI[†]), the IBU release behavior in SBF has shown a quite similar phenomenon to that in PBS. More importantly, the relative PL emission intensity has been quenched in a similar manner to that for the samples immersed in PBS (Fig. S7b and c, ESI[†]). The relative PL intensity variation has thus been confirmed to effectively reflect the drug releasing phenomenon.

It is well-known that the PL emission of rare earth ions can be hindered by its environments that have a high phonon frequency.³² An FTIR study was carried out on the IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers with a χ value of 5 *m* to reveal the PL quenching mechanism associated with the drug release. As shown in Fig. 9, during the drug releasing process, the peaks at $\sim 2862\text{ cm}^{-1}$ and $\sim 2953\text{ cm}^{-1}$, assigned to C–H_x bonds of IBU molecules, gradually decrease with the drug liberation over time, as expected. In contrast, the intensity of the peak at $\sim 3430\text{ cm}^{-1}$, which belongs to hydroxyl groups, increases significantly. It has been reported that the hydroxyl group with a high frequency vibration at 3430 cm^{-1} is an effective quencher of fluorescence at 1072 nm for rare earth Nd^{3+} .^{32,33} Therefore, the IBU molecules play a shielding role in ‘protecting’ $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers from the hydroxyl groups in the PBS aqueous solution during drug releasing. Owing to the liberation of IBU molecules, the fluid invades the fiber surface and diffuses into the pores. The quenching effect of hydroxyl groups is enhanced significantly. Therefore, the sustained drug release kinetics induces a postponed PL quenching phenomenon. Such a matching correspondence between the PL emission intensity within the NIR spectral range and the drug release represents an effective approach to tracking and monitoring the drug release from $\text{CaTiO}_3:\text{Nd}^{3+}$ nanofibers.

3. Conclusions

In this study, a series of fine $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers, with the excitation in the first near-infrared window and emission spectra in the second near-infrared window, were synthesized for the first time *via* electrospinning. The *in vitro* study revealed that such photoluminescent nanofibers are cyto-compatible. The micro-structural characteristics of $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers can be successfully tuned *via* the control of surfactant Pluronic F127 concentration during the synthesis. Due to the high surface area, large pore volume and low pore dimensions, the nanofibers synthesized without surfactant Pluronic F127 present a highest drug loading capacity and most sustained release kinetics. More importantly, the relative intensity of PL emission with the NIR II range from the nanofibers responds effectively to the released quantity of IBU molecules. Furthermore, the PL quenching rate has shown a good correspondence with the drug release kinetics due to the quenching effect of hydroxyl groups, confirming that the drug release kinetics can be feasibly identified and

monitored *via* NIR spectra. Therefore, we anticipate that this study may provide another promising drug delivery platform with NIR-monitored releasing kinetics for biomedical applications.

4. Experimental section

4.1 Reagents and materials

All of the chemical reagents used as received without further purification, including acetic acid ($\text{C}_2\text{H}_4\text{O}_2$, A.R.), ethanol ($\text{C}_2\text{H}_6\text{O}$, A.R.), *N,N*-dimethylformamide ($\text{C}_3\text{H}_7\text{NO}$, A.R.), cyclohexane (C_6H_{12} , A.R.) and phosphate buffered saline (PBS, pH = 7.4) (Sinopharm Chemical Reagent Co., Ltd), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 99%), neodymium nitrate hexahydrate ($\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, >99.9%, tetrabutyl titanate ($\text{Ti}(\text{OC}_4\text{H}_9)_4$), >98.0% and polyvinylpyrrolidone (PVP, $M_W = 1\,300\,000$) (Aladdin), Pluronic F127 ($\text{EO}_{106}\text{PO}_{70}\text{EO}_{106}$, $M_W = 12\,600$, Sigma-Aldrich), and ibuprofen (IBU, 99 wt%, Nanjing Chemical Reagent Co., Ltd).

4.2 Synthesis of luminescence functionalized porous $\text{CaTiO}_3\text{:Nd}^{3+}$ nanofibers

In a typical process, $\text{Ti}(\text{OC}_4\text{H}_9)_4$ (~0.82 g) and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (~0.56 g) were dissolved in a mixture of acetic acid (2 mL) and ethanol (7 mL) to prepare solution A under constant stirring. Solution B was prepared by dissolving $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (~0.21 g) in 20 mL *N,N*-dimethylformamide. Subsequently, a series of mixture sols containing solution A and B were prepared with different Nd^{3+} ion concentrations (0, 0.5, 1, 2, and 3 mol%), and electrospun to synthesize $\text{CaTiO}_3\text{:Nd}^{3+}$ nanofibers with different doping concentrations. The sol, from which the nanofibers with highest photoluminescence properties were achieved, was added with surfactant Pluronic F127 with various F127/CTO molar ratios (denoted as χ) of 0, 2.5 and 5 m ($m = 10^{-3}$). Finally, a *N,N*-dimethylformamide solution of polyvinyl pyrrolidone (PVP, 3.25 wt%) was mixed with the above solution to prepare spinnable precursor sols. The electrospinning sol was fed into the conducting nozzle (2 mm ID) using an infusion pump (KDS-100, KD Scientific, USA) at a constant flow rate of 0.5 mL h^{-1} . The distance and voltage applied between the needle tip and the collector were set to be 15 cm and 10 kV (PS/FC30P04.0-22, Glassman High voltage Inc., USA), respectively. As-spun fibers were dried at 80 °C for 12 h and calcined in a furnace at 700 °C, with a heating rate of 2 °C min^{-1} for 2 h in air.

4.3 *In vitro* cytotoxicity assay

The *in vitro* cytotoxicity of Nd doped CaTiO_3 was determined using bone marrow-derived mesenchymal stem cells (BMSCs) *via* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, BMSCs were seeded in 96-well plates at a density of 6000–8000 cells per well and cultured in 5% CO_2 at 37 °C for 24 h to allow the cells to attach to the bottom of the plates. The $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers were sterilized using alcohol for 48 h, and then serial dilutions of the nanofibers at concentrations of 0, 100, 200, 500, 1000 and 4000 $\mu\text{g mL}^{-1}$ were incubated with cells for 1, 3 and 5 days in 5% CO_2 at 37 °C. After each incubation time point, MTT (20 μL , 10 mg mL^{-1}) solution was added to each well and incubated for another 4 h at 37 °C. Subsequently, the media containing the $\text{CaTiO}_3\text{:1\%Nd}^{3+}$ nanofibers were removed, Dimethyl sulfoxide (DMSO, 150 μL) was added

into each well, and the absorbance was measured at a wavelength of 490 nm using a microplate reader (Thermo Scientific Inc., USA). Tests were performed in six replicates.

4.4 Loading and release of drug

Ibuprofen (IBU, an anti-inflammatory drug) was selected as a model drug in the study.^{30a} Porous $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers (0.2 g) were added into IBU hexane solution (40 mg mL^{-1} , 50 mL) at ambient temperature. The mixture was subsequently stirred for 24 h to induce the drug diffusion into the pores. The IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ sample was collected by centrifugation and washed with hexane to remove the adsorbed IBU on the outer surface. After the samples were dried at 60 °C for 12 h, the IBU release behavior was investigated *in vitro*. IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers (~0.15 g) were equally divided into 12 portions, and immersed in PBS (10 mL) under gentle stirring at 37 °C. At each time interval, a 1 mL media sample was collected and diluted to measure the amount of IBU drug released by measuring the optical density at 222 nm using a UV/Vis spectrophotometer. Meanwhile, the nanofibers were filtered and air-dried for the examination of PL emission at each release time point (0 h, 4 h, 8 h, 12 h and 48 h). The nanofibers were pressed into a quartz plate with a cylindrical groove to ensure uniform thickness and distribution of the samples before PL measurement.

4.5 Characterization

An X-ray diffractometer (X'PertPRO MPD, Netherlands) with Cu $K\alpha$ radiation was used to investigate the crystal structure of nanofibers prepared. The morphological and microstructural characteristics were examined using field emission scanning electron microscopy (FESEM, Hitachi SU-70, Japan) and transmission electron microscopy (TEM, Philips TecnaiF20 S-TWIN, Netherlands), respectively. The specific surface area and pore size distribution were determined *via* N_2 adsorption/desorption analysis at liquid nitrogen temperature (77 K) using a coulter OMNISORP-100 apparatus. The FTIR spectra were recorded on a Perkin-Elmer 580B infrared spectrophotometer on KBr pellets (Tensor 27, Bruker, Germany). Thermogravimetry-differential scanning calorimeter measurement (TG-DSC, DSCQ1000, AT, USA) was used to measure the quantity of IBU drugs loaded onto the $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers. UV/Vis adsorption spectra were measured on a TU-1810 spectrophotometer. The photoluminescence (PL) measurements were performed on a FLS920 spectrophotometer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Optical and structural characterization of $\text{CaTiO}_3:\text{Nd}^{3+}$ nanofibers. (a) Emission spectra of $\text{CaTiO}_3:\text{Nd}^{3+}$ nanofibers excited at 800 nm with different doping concentrations, (b) XRD patterns of $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers annealed at 700 °C as well as the standard card of the orthorhombic CaTiO_3 phase (JCPDS 82-0228).

Fig. 2. SEM and TEM images of $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers obtained at different F127/CTO molar ratios: (a and d) 0, (b and e) $2.5m$, and (c and f) $5m$ ($m = 10^{-3}$). Insets: high-magnification TEM images of a single nanofiber.

Fig. 3.

N₂ adsorption/desorption isotherm of CaTiO₃:1%Nd³⁺ nanofibers obtained at different F127/CTO molar ratios: 0, 2.5*m*, and 5*m* (*m* = 10⁻³). Inset: pore size distribution plot.

Fig. 4.

Schematic diagram for the formation of $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers with different F127/CTO molar ratios.

Fig. 5.
The viability of BMSCs incubated with CaTiO₃:1%Nd³⁺ nanofibers as a function of fiber concentration and incubation time.

Fig. 6. FTIR spectra of $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers, IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers, and pure IBU.

Fig. 7. TG curves of (a) the $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers and (b–d) IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers obtained at different F127/CTO molar ratios: 0, 2.5m, and 5m ($m = 10^{-3}$).

Fig. 8.

(a) Cumulative drug release profiles and (b) relative PL emission intensity as a function of release time for IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers obtained at different F127/CTO molar ratios: 0, 2.5m, and 5m ($m = 10^{-3}$).

Fig. 9.

FTIR spectra of IBU loaded $\text{CaTiO}_3:1\%\text{Nd}^{3+}$ nanofibers ($\chi = 5m$) obtained at different release time and enlarged characteristic peaks of the marked area.

Table 1Structural parameters of CaTiO₃:1%Nd³⁺ nanofibers

F127/CTO molar ratio	BET Surface area (m ² g ⁻¹)	Pore volume (cm ³ g ⁻¹)
0	33.5	0.141
2.5 <i>m</i>	21.9	0.094
5 <i>m</i>	16.4	0.061