Multifunctional bioactive Nd-Ca-Si glasses for fluorescence thermometry, photothermal therapy, and burn tissue repair

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Photothermal therapy (PTT), an emerging tumor treatment technology, has attracted tremendous interest, but excessive heat will cause damage to surrounding healthy tissues. Therefore, in situ temperature monitoring during PTT is of great importance to determine optimal treatment temperature and repair heat-damaged normal tissues. Here, we report the preparation of multifunctional Nd-Ca-Si silicate glasses and glass/alginate composite hydrogels that not only have photothermal property but also emit fluorescence under 808-nm laser irradiation, and its fluorescence intensity is linearly correlated with in situ temperature. With this feature, optimal PTT temperature for effective tumor treatment with minimal normal tissue damage can be obtained. In addition, because of the bioactive silicate components, the composite hydrogel has bioactivity to repair heat damage caused by PTT. This implantable multifunctional material with unique temperature monitoring, photothermal function, and wound healing bioactivity can be used for localized thermal therapy.

RESULTS AND DISCUSSION

The Nd-Ca-Si (Nd-BG) and CS-Si (CS-BG) bioglasses were prepared by the containerless processing (CP) technique (Fig. 1) and characterized by x-ray diffraction (XRD) analysis, which showed amorphous phase for all the prepared glass materials (fig. S1A). The particle size
of the glass powders was 33.52 μm (Nd-BG1), 29.70 μm (Nd-BG2), 31.31 μm (Nd-BG3), and 32.78 μm (CS-BG), respectively (fig. S2, A to D). The scanning electron microscopy (SEM) images revealed micrometer-sized particle morphology (fig. S2, E to H). We analyzed the photothermal and fluorescence properties of the glasses and found that the obtained Nd-BG glasses had excellent photothermal and fluorescence characteristics as compared with CS-BG glasses without Nd doping. The temperature of the Nd-BG glasses significantly increased under continuous irradiation of 808-nm laser, and the temperature increased with the increase in Nd doping amount and laser power density (fig. S3). The final temperature of Nd-BG powders with different Nd doping reached 112.4 °C (Nd-BG1; molar ratio, Nd:Ca:Si = 1:9:20), 132.9 °C (Nd-BG2; molar ratio, Nd:Ca:Si = 2:8:20), and 144.2 °C (Nd-BG3; molar ratio: Nd:Ca:Si = 3:7:20), respectively, at the power density of 0.6 W cm⁻² (fig. 2A). Furthermore, the emission intensity was dependent on Nd doping amount, and a decrease in the emission intensity was observed with the increase in the Nd doping amount (Fig. 2C). It is clear to see that the maximal fluorescence emission intensity at 1062 nm for Nd-BG powders was linearly correlated with temperature in the biological thermal range, which is critical for the in vivo temperature-monitoring performance applications (Fig. 2D).

It is known that the Nd³⁺ ([Xe] 4f³) ions have particular electron configuration, and it has a half-filled 4f orbit. The electrons are optically excited with 808-nm irradiation up to the excited states (⁴F₅/₂), and then they fast relax back to the metastable ground states ⁴F₃/₂ by the nonradiative process. Once in the ⁴F₃/₂ state, Nd³⁺ ions undergo a radiative decay to the lower energy states (such as ⁴F₃/₂→⁴F₁₁/₂, and 1336 nm (⁴F₃/₂→⁴F₁₃/₂)). The electron transition imparts photothermal and fluorescence properties to the Nd-BG powders. We also prepared Nd-Ca-Si bioceramic (Nd-BC) powders by heat treatment of glasses at 800°C, which showed a multiphase composition (fig. S1B). Similarly, the Nd-BC powders also had photothermal and fluorescence properties as Nd-BG powders under 808-nm laser irradiation (fig. S4, A to D). Some studies have shown that the crystallinity of glass and ceramics affected the fluorescence properties of the materials, and quenched glasses have higher fluorescence intensity than crystalized ceramic phase (27, 28). In our study, we also found that as the crystallinity of the material increased, the photothermal and fluorescence properties of the powder gradually decreased, and the Nd-BG glasses had higher photothermal and fluorescence performance than Nd-BC ceramics (fig. S4, E to F). Considering the higher photothermal and fluorescence performance,
Nd-BG2 with specific molar ratio Nd:Ca:Si = 2:8:20 was selected for further experiments.

In the process of PTT treatment, high temperature may cause damage to normal tissues around the tumor. Therefore, it is important to detect the temperature in the tumor site during the PTT process to obtain the optimal treatment temperature at which the tumor can be completely eliminated while surrounding normal tissues are not damaged or only minimally damaged. Previous studies have shown that rare earth elements Yb3+ and Er3+ doped materials had fluorescence emission at 525 and 545 nm under 980-nm laser irradiation, which was linear correlated with temperature and might be used for temperature sensing (29–32). However, these Yb3+ and Er3+ doped materials do not have photothermal property, and the light absorption under 980-nm irradiation is high in vivo by body fluids. In contrast, Nd materials may have both photothermal and fluorescence properties under 808-nm laser irradiation and lower light absorption in vivo by body fluids (33), and the 1062-nm NIR-II window has a higher spectral penetration depth. Therefore, Nd doped LaF3 and NaYF3 nanoparticles were synthesized (13, 34), but which could only be used as nanosuspension for injection and had no biological activity to repair heat-damaged tissues after PTT. In the present study, we successfully synthesized Nd-BG, which not only has both fluorescence temperature-monitoring and photothermal functions but also has bioactivity and could repair the damaged tissue caused by local overheating (21, 22).

Encouraged by the excellent photothermal and fluorescence properties of Nd-BG materials and considering the in vivo application, we further designed and prepared Nd-BG2/alginate injectable composite hydrogel by incorporating Nd-BG2 particles into alginate matrix (Fig. 3A). The injectable hydrogels could be easily injected into the tumor site, which avoids surgical damage during implantation (35, 36). It could be seen from the SEM image (fig. S5) that the Nd-BG2 particles were randomly distributed in the alginate hydrogel matrix, and the energy-dispersive x-ray spectroscopy (EDS) elemental mapping analysis of Ca, Nd, and Si also confirmed uniform distribution of glass particles within the hydrogel matrix. The prepared Nd-BG2/alginate composite hydrogel showed good photothermal and fluorescence performances same as pure Nd-BG glasses, in addition to good fluidity and injectability. Under 808-nm laser irradiation (1.5 W cm−2), the temperature of the composite hydrogel quickly rose to more than 50°C, which is enough for PTT.

Fig. 3. Photothermal/fluorescence properties of the Nd-BG2 composite hydrogel in vitro. (A) Images of the formation of Nd-BG2 composite hydrogels. (B) Heating curves of the Nd-BG2 and CS-BG composite hydrogels (under 808-nm laser irradiation at the power density of 1.5 W cm−2). (C) Linear fitting relationship between 1062-nm fluorescence intensity and temperature for Nd-BG2 composite hydrogels. (D) Room temperature emission spectra of the hydrogel through the mouse tissues with different thickness (1.12 W cm−2). (E) Linear relationship between fluorescence intensity through mouse tissues with different thicknesses and the temperature. (F) Relationship between temperature difference (∆T) and thickness of mouse skin tissue. (G) Room temperature emission spectra of the hydrogel through different animal tissues with same thickness (1.64 mm; under 808-nm laser irradiation at the power density of 1.12 W cm−2). (H) Linear relationship between fluorescence intensity through different animal tissues with same thickness and temperature. (Photo credit: Lingling Ma, State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, P. R. China; Center of Materials Science and Optoelectronics Engineering, University of Chinese Academy of Sciences, Beijing 100049, P. R. China.)
increased, finally reached 60°C, and remained stable within 5 min (fig. S6). In contrast, the temperature of the CS-BG composite hydrogel without Nd was substantially unchanged under the same laser irradiation. Figure 3B shows photothermal stability of the composite hydrogel under 808-nm laser irradiation (1.5 W cm\(^{-2}\)), and after four laser on/off cycles, the final temperature of the hydrogel still reached the same value as the first irradiation. In addition, the Nd-BG2 composite hydrogel showed similar linear fluorescence intensity/temperature correlation at 1062 nm in the biological thermal range as the Nd-BG2 glass (Fig. 3C). These results indicate that the alginate (SA) polymer component did not affect the property of the Nd-BG2 glasses.

Although the composite hydrogel showed good fluorescence thermometry property with good fluorescence intensity–temperature correlation, for in vivo application of the composite hydrogel, another important issue is whether the temperature monitoring will be affected by tissue type and thickness, because tumors at different locations are often surrounded by tissues with different density, composition, and thickness, which will affect the intensity of the fluorescence penetrating through them. Therefore, we investigated the effects of different types of animal tissues and tissue thickness on the fluorescence properties and temperature dependence of the composite hydrogel. Figure 3D shows that the fluorescence emission intensity through mouse tissue decreased when the tissue thickness increased (808 nm, 1.12 W cm\(^{-2}\)). However, although different thickness of mouse skin tissue affected fluorescence intensity, a good linear correlation between fluorescence intensity and hydrogel temperature still remained (Fig. 3E), indicating that tissue thickness did not affect the fluorescence-temperature correlation. The temperature difference (\(\Delta T\)) and thickness of mouse skin tissue showed perfect linear relationship (Fig. 3F), which guaranteed the in vivo fluorescence thermometry application. By measuring the temperature on both sides of the skin, the formula \(y = 4.858x - 0.1723\) (x, the thickness; \(y\), the \(\Delta T\)) was obtained, and with this linear correlation, the temperature under the skin tissue with different thickness can be accurately determined. Furthermore, tissue type also affected the fluorescence intensity. Figure 3G shows the intensity of the fluorescence emitted from the composite hydrogel through different animal tissues with same thickness (1.64 mm) under 808-nm continuous irradiation at the powder density of 1.12 W cm\(^{-2}\). It is clear to see that the fluorescence intensity through different animal tissues was different. The intensity of the chicken tissue group was higher than that of the mouse tissue group, and the pig tissue group showed the highest intensity among the three groups. An important finding was that, although different tissues affected fluorescence intensity differently, the linear correlation between fluorescence intensity and the temperature of composite hydrogels through different animal tissues still remained (Fig. 3H), which indicates that the Nd-BG composite hydrogel can be applied to different animal species for temperature monitoring.

It is known that calcium-silicate–based biomaterials have excellent bioactivity to stimulate tissue regeneration. However, it is unknown whether Nd-Ca-Si materials also have similar bioactivity, and whether Nd will affect the activity of the material. To determine the bioactive role of Nd, we further studied the effects of composite hydrogel on cell viability, cell migration, in vitro angiogenesis, and angiogenic gene expression of human umbilical vein endothelial cells (HUVECs). The results showed that both CS-BG and Nd-BG2 hydrogel extracts significantly promoted HUVEC proliferation in certain concentration range as compared with the control (fig. S7A). As compared with the CS-BG hydrogel extracts, the Nd-BG2 extracts showed higher stimulatory activity than the CS-BG extracts. The cell migration and in vitro angiogenesis assays showed similar trends. Both CS-BG and Nd-BG2 hydrogel extracts revealed bioactivity to stimulate cell migration and in vitro angiogenesis at the 1/4 dilution (fig. S7, B to E). The gene expression analysis further confirmed that the Nd-BG2 hydrogel extracts further up-regulated the angiogenic gene expression of HUVECs including vascular endothelial growth factor (VEGFD), kinase insert domain-containing receptor (KDR), hypoxia inducible factor–1\(\alpha\) (HIF–1\(\alpha\)), and endothelial nitric oxides (eNOS) as compared with CS-BG extracts (fig. S7F). Although no bioactivity of Nd-containing biomaterials has been reported before, some previous studies have shown that rare earth ions over the concentration range of 1 to 100 \(\mu\)M are able to stimulate human dermal fibroblast proliferation (37). Our results suggest that Nd-BG2 had higher bioactivity in stimulating angiogenesis than the CS biomaterial, and the introduction of Nd ions in the CS material system not only endows the material with photothermal and fluorescence thermometry functions but also enhances bioactivity for stimulating tissue regeneration. However, further studies are required to explore the mechanisms of the bioactivity of Nd-containing biomaterials in regulating cellular activities.

Considering diversity of tumor type, location, and surrounding tissue environment, it is critical to determine optimal PTT treatment temperature to obtain the best therapeutic effect with minimal heat damage to normal tissue. To the best of our knowledge, the lack of such study is due to the difficulty in measuring the in situ temperature during the PTT treatment. In this study, we determined optimal temperature for tumor ablation by injecting the composite hydrogel around the tumor site and applied power to control the treatment temperature (Fig. 4A). We found that tumor growth was obviously inhibited when it was treated at 48°C or above (Fig. 4B and fig. S8). However, the tumor recurred at day 7 and grew 52-fold in volume at day 14 in the Gel 48°C group (Fig. 4C). Similarly, the tumors treated at lower than 48°C and the control groups all showed tumor regrowth, such as the relative tumor volume of the Gel 43°C group grew to 86 times, the tumors of the Gel-no–L (the Nd-BG2 composite hydrogel without Laser), CS + L (the CS–BG2 composite hydrogel with Laser), CS-no–L (the CS–BG2 composite hydrogel without Laser), and blank groups grew to 73, 80, 179, and 87 times after 12 days, respectively. In contrast, the tumors of the Gel 53°C and Gel 60°C groups completely disappeared and did not reappear within 14 days. When we compared the results of the Gel 53°C and Gel 60°C groups, it was observed that the photothermal temperatures above 53°C obviously resulted in burning skin tissues (Fig. 4D). The skin scar area treated at 60°C was four times larger than that treated at 53°C. Furthermore, hematoxylin and eosin (H&E) staining (Fig. 4E) not only confirmed complete ablation of tumors in the Gel 60°C and Gel 53°C groups but also revealed that the skin epidermis, dermis, and subcutaneous tissues in the treatment area of the Gel 60°C group showed a large area of necrosis. In contrast, for the Gel 53°C group, although necrosis in the epidermis, dermis, and subcutaneous area was visible, the size of the necrosis was much smaller than that in the Gel 60°C group, indicating minimal tissue damage. In the Gel 48°C group, although a large number of tumor cells were killed and the size of the tumor significantly reduced, a small tumor cell mass was still visible (blue arrow). In all other groups including the Gel 43°C and CS + L groups, tumor tissues still existed, and the
tumor cells revealed high nuclear-cytoplasmic ratio and large cell volume and were multinucleated (blue arrow) in the cytoplasm. Many granulation tissues in the dermis and subcutaneous area were observed (red arrow, fibroblasts; yellow arrow, inflammatory cells; green arrow, new blood capillary), which indicates the start of the tissue repair process (38, 39) and suggests that the bioactive composite hydrogel has activity to enhance burn wound healing during PTT therapy.

We further examined skin damage on top of the tumor tissues, and the results showed that the skin of the Gel 60°C, Gel 53°C, and Gel 48°C groups suffered certain photothermal heating damage as compared with that of the Gel 43°C and CS + L groups, and the degree of damage was Gel 60°C > Gel 53°C > Gel 48°C (fig. S9). The treatment at 60°C significantly damaged the skin tissue, causing irreversible burn damage, and the epidermis, dermis, and subcutaneous tissues were all destroyed. In addition, the scar area was
significantly larger than that of the other groups, and the tissue reached third-degree burn damage (40, 41). In contrast, the treatment of the Gel 53°C group caused minor burn damage on the epidermis, dermis, and subcutaneous tissues, which reached deep second-degree burn. These results suggested that a treatment temperature for completely eliminating tumors without any damage on normal tissue might not be possible, and therefore, it is important for the PTT materials with bioactivity to repair minor heat-damaged tissues during and after the PTT therapy.

To confirm the regenerative bioactivity of our composite hydrogel, we further evaluated burn wound healing after treatment with the composite hydrogel and confirmed that the bioactive hydrogel could effectively promote healing of skin burn wounds. As compared with the blank and SA groups covered by necrotic tissues, the burned area of the Nd-BG2/SA composite hydrogel group was scarred at day 3, the scald wound area was significantly reduced at day 7, and the burned scar disappeared at day 14 (Fig. 5A). The wound closure rate of Nd-BG2/SA group was significantly higher than that of the blank and SA groups (Fig. 5B). Comparing the three groups after 7 days, the blank group revealed a severe skin burn state, and the SA group showed large scar, while the Nd-BG2/SA group showed reduction in scar area and the scarring part of the skin began to degenerate and the epithelium started to regrow (Fig. 5C). After 14 days, scars in the blank and SA groups were still visible, and the SA group started forming new dermis. In contrast, the scar in the Nd-BG2/SA group almost disappeared, new epidermis and dermis tissues were visible, and the initial hair follicles appeared. These results further confirmed that the Nd-BG2/SA composite hydrogel not only had photothermal and temperature measuring functions but also had regenerative bioactivity to enhance burn wound healing because of the multifunction of Nd-BG2 glasses. These results together with the tumor ablation observation suggest that 53°C is the best treatment temperature for the PTT therapy with best tumor ablation and minimal tissue burn damage in the mouse subcutaneous tumor model. It is noteworthy to indicate that the optimal PTT temperature for different tumor types and locations in different animal species might be different because of the different tumor/surrounding tissue environment, and further studies are required to identify specific treatment temperature.

For in vivo application of a new biodegradable material, it is important to investigate its degradation profile and the distribution of the degradation products inside the body. Therefore, we further studied the degradation of the composite hydrogel and investigated the in vitro and in vivo hydrogel degradation and the elemental...
Our results demonstrated that this type of multifunctional biomaterials may not only have potential application as implantable medical devices but may also be used as a temperature-monitoring material for measuring in situ temperature in other types of thermal therapies.

**MATERIALS AND METHODS**

**Synthesis and characterization of Nd-Ca-Si glasses and ceramics**

In this experiment, Nd-Ca-Si precursors with different amounts of Nd were prepared by the coprecipitation process to obtain samples with different Nd contents (molar ratio, Nd:Ca:Si = 1:9:20, Nd/CS1; Nd:Ca:Si = 2:8:20, Nd/CS2; Nd:Ca:Si = 3:7:20, Nd/CS3). Briefly, for Nd/CS1 powder, 21.2535 g of calcium nitrate tetrahydrate [Ca(NO$_3$)$_2$·4H$_2$O; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China] and 4.3835 g of nitrate hexahydrate [Nd(NO$_3$)$_3$·6H$_2$O; Aladdin, Shanghai, China] were dissolved in deionized water (200 ml) and stirred in a magnetic mixer. Then, aqueous ammonia (NH$_3$·H$_2$O; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was added dropwise while the solution was stirred vigorously until the pH value of the solution reached 13. Then, 56.84 g of sodium silicate nonahydrate (Na$_2$SiO$_3$·9H$_2$O; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was dissolved in deionized water (400 ml) and added into the solution described above dropwise. The mixed solution was stirred in a magnetic stirrer for 12 hours at room temperature and then filtered. The obtained powders were washed three times with deionized water and anhydrous ethanol, respectively, and then dried at 60°C for 48 hours. Ca-Si precursor material (CS; molar ratio, Ca:Si = 1:2) without Nd was also prepared through the same coprecipitation method.

The dried powders were sintered at 800°C for 2 hours and then ground to obtain micrometer-grade Nd-Ca-Si bioceramics [Nd-BG with different Nd contents (Nd-BG1, Nd-BG2, and Nd-BG3)] and the Ca-Si bioceramic (CS-BG) powders through a 200-mesh sieve. At the same time, Nd-Ca-Si bioglasses [Nd-BG with different Nd contents (Nd-BG1, Nd-BG2, Nd-BG3)] and the Ca-Si bioglass (CS-BG) were prepared using the above obtained bioceramic powders by the CP processing technique. The specific schematic diagram is shown in Fig. 1. First, the ceramic powders were pressed into a block shape (diameter: $d = 6$ mm; thickness: $h = 1$ to 2 mm) placed in a homemade aerodynamic levitation furnace, floating in the furnace with the oxygen, melted using a carbon dioxide (CO$_2$) laser with power of 100 W, and quenched by deep undercooling to form BG spheres at a rate of 300 (°C·s$^{-1}$). Then, the BG spheres were heated rapidly to 500°C in a fast-heating annealing furnace and immediately poured into deionized water. After that, the spheres were crushed into pieces, grounded into powders, and sieved through a 200-mesh to obtain micrometer-sized BG powders.

The phase composition of samples with different Nd contents was examined by XRD (Rigaku D/Max-2550 V, Geiger-flex, Japan). The XRD patterns were scanned from 20 angles from 10° to 65° with a scan speed of 6° min$^{-1}$. The size distribution of the Nd-Ca-Si and Ca-Si bioglass powders was analyzed using a laser particle size analyzer (Bettersize2600, Bettersize Instruments Ltd., China). The morphology of the Nd-Ca-Si and Ca-Si bioglass powders was observed by SEM (S-4800, Hitachi, Japan). The photothermal property of the powders was determined by an infrared thermal imaging system (PM100D, Thorlabs GmbH, Munich, Germany). The low-temperature absorption spectrometer (FLS-980, Edinburgh, UK) was used to explore the fluorescence performance of bioglasses and bioceramics powders.
Preparation of composite hydrogels

Alginic acid sodium salt from brown algae (SA, medium viscosity, UK) was dissolved in deionized water to produce 3% (w/v) solution. The Nd-BG2 powders and d- (+)-gluconic acid δ-lactone (Aladdin, Shanghai, China) were dispersed in the SA solution through syringes to get homogeneous composite hydrogel with different contents of Nd. The distribution of Nd-BG2 powders in the composite hydrogel was studied by SEM (S-4800, Hitachi, Japan). The elemental distribution

Fig. 6. Fate of composite hydrogel in mice. (A) Ca ion concentrations in liver, heat, spleen, lung, and kidney. (B to D) Ca, Si, and Nd ion concentrations in stool, respectively. *P < 0.05 and **P < 0.01. (E and F) Ca and Si ion concentrations in blood, respectively. *P < 0.05. (G and H) Ca and Si ion concentrations in urine, respectively. ***P < 0.001.
in the composite hydrogel was characterized by using an SEM accessory EDS system. The photothermal effect of the Nd-BG2/alginate composite hydrogels (Nd-BG2/SA) was investigated by the infrared thermal imaging system (PM100D, Thorlabs GmbH, Munich, Germany). The fluorescence performance of the Nd-BG2 composite hydrogel was tested by the low-temperature absorption spectrometer (FLS-980, Edinburgh, UK). The CS-BG/SA composite hydrogel was prepared through the same process and used as control.

The degradation of the Nd-Ca-Si bioglass powder and the composite hydrogel in vitro

To investigate the degradation of the Nd-BG2 powder and composite hydrogel, the Nd-BG2 powder and composite hydrogel samples were soaked in tris-HCl buffer solution (pH 7.4) at 37°C in a shaking water bath for 1, 3, 7, and 14 days, respectively. The ratio of the tris-HCl buffer volume to the Nd-BG2 powder and composite hydrogel was 200 ml g⁻¹. After different time periods (1, 3, 7, and 14 days), the supernatant fluid was collected, and the samples were weighed followed by addition of fresh tris-HCl buffer. The Nd, Ca, and Si ion concentrations were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Vista AX, Varian, USA). Three samples were used for each group for reproducibility.

The viability, migration, tube formation, and angiogenic gene expression of HUVECs in the presence of hydrogel extracts

Preparation of hydrogel extracts

The composite hydrogels were soaked in extracellular matrix (ECM) basal medium (Cyagen US Inc., USA) at a solid/liquid ratio of 0.1 g ml⁻¹ and incubated at 37°C for 24 hours. Then, the mixture was centrifuged at a speed of 6000 rpm for 10 min. The supernatant fluid was filtered through a membrane (Millipore; 0.22 mm) to obtain sterile hydrogel extracts. The filtered extracts were then diluted using ECM basal medium to different concentrations (1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 dilution) for further cell culture experiments.

Cell viability

The ECM basal medium containing 5% fetal bovine serum (Cyagen US Inc., USA), 1% penicillin (Cyagen US Inc., USA), and 1% streptomycin and glutamine (Cyagen US Inc., USA) was used to culture the HUVECs. The HUVECs (four passages) were seeded in a 24-well plate (600 cells per well) and cultured at 37°C with 5% CO₂ for 24 hours. Then, the culture medium was replaced by the medium containing extracts with different concentrations. The extract-containing medium was refreshed every 2 days. At selected time points (1, 3, and 5 days), cell viability was evaluated by CCK-8 assay (Cell Counting Kit-8, Dojindo Molecular Technologies, Japan) according to the manufacturer’s instruction. The culture medium was replaced by mixture medium (CCK-8:medium = 1:9). After 1.5 hours, the absorbance of the reaction product was measured under 450 nm using an enzyme-linked assay microplate reader (Epoch, BioTek, Winooski, VT, USA).

Cell migration

The effect of the hydrogel extract on cell migration was evaluated by a scratch test. Briefly, the HUVECs were seeded in a six-well plate and incubated at 37°C with 5% CO₂. At 80% cell confluency, cells were scraped with a plastic tip (200 μl), washed with phosphate-buffered saline (PBS), and cultured in medium containing hydrogel extract at 1/4 dilution. After 12 hours, the HUVECs were stained with crystal violet and washed with PBS. Photographs were taken using an optical microscope (Leica) at 0- and 12-hour time points. Last, the migration rate (M) was calculated: \( M = (S_0 − S)/S_0 \times 100\% \) (S₀, initial scratch area; S, final scratch area).

The in vitro angiogenesis assay

To evaluate the tube formation of HUVECs in vitro, 50 μl of Matrigel (ECMatrix gel, Millipore) was added into a 96-well plate and placed in a 37°C incubator with 5% CO₂ for 1 hour. Then, HUVECs were seeded onto the Matrigel surface at a density of 2 × 10⁴ per well, and 100 μl of culture medium containing hydrogel extracts was added with four parallel samples per group. After 6 hours of incubation, optical images were taken using a microscope (DMi8, Leica, Germany), and the tubules formed were counted in the field of view by software ImageJ 1.52a (National Institutes of Health, USA).

The expression of angiogenic genes

The effect of the composite hydrogel on the angiogenic gene expression of the HUVECs was further investigated by using real-time quantitative polymerase chain reaction. HUVECs were seeded on culture plate and cultured in the medium containing the hydrogel extract. After 3 days, the culture medium was removed from the plate and then washed twice with PBS, and RNA was extracted from the cells using TRIzol reagent (Invitrogen). The concentration of RNA was measured through a NanoDrop 2000 reader (Thermo Fisher Scientific). Complementary DNA was synthesized using a PrimeScript RT reagent kit (TaKaRa) according to the manufacturer’s instructions. Primers of VEGF, HIF-1α, VEGF receptor 2 (VEGF-2) (KDR), eNOS, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were synthesized [all from BioSune Biotech (Shanghai) Co. Ltd.] according to a previous study (20). The primer sequences are listed below.

- **VEGF**: forward 5'-TGCGGATCAAACCTCACA, reverse 5'-CAGGGATTTTTCTGTCTTGCT.
- **HIF-1α**: forward 5'-CCATGTGACCATGAGGAAAT, reverse 5'-CGGCTAGTTAGGTACACCTT.
- **KDR**: forward 5'-GGTATCGGAAATGCACTGGAG, reverse 5'-CATGTTGTCCTACAAAGGAGCCA.
- **eNOS**: forward 5'-GTCCACATCTGCTGAAATTTG, reverse 5'-AGGAGGTCTTCTGTGATGCC.
- **GAPDH**: forward 5'-TCACACTGAAATTGTCACAGA, reverse 5'-GTGATTCATAGGGCAGGTT.

Data were normalized according to the gene GAPDH mRNA expression in each condition and were quantified relative to the corresponding gene expression from the control sample (cells cultured with pure endothelial cell medium with 10% fetal bovine serum), which were standardized to 1.

The effect of tissue type and thickness on fluorescence thermometry

To investigate the effect of biological tissues on the fluorescence temperature measurement properties of the Nd-BG2 composite hydrogels, animal tissues with different types and thickness were placed on the Nd-BG2 composite hydrogels, and the changes in fluorescence and temperature through the tissues were measured. First, 1 ml of hydrogels was injected into each well of a 24-well cell culture plate. Then, three different animal tissues such as pig tissue, chicken tissue, and mouse tissue with same thickness (1.64 mm) were placed on the top of the hydrogels, respectively. Then, the tissues with hydrogels underneath were treated under 808-nm laser irradiation, and the fluorescence intensity through different tissues
was measured using the low-temperature absorption spectrometer, and the temperature ($T_1$) of the hydrogels and the skin tissue surface temperature ($T_2$) were measured by infrared thermal imaging system.

In addition, the effect of different thicknesses of mouse tissue (0.84, 1.64, and 2.79 mm) on the fluorescence intensity and temperature properties of the Nd-BG2 composite hydrogel was also evaluated. First, the mouse tissues with different thicknesses were placed on the hydrogels. Under 808-nm NIR irradiation, the temperature of the hydrogel and the skin tissue surface temperature were measured, and the temperature difference between the tissue surface and the Nd-BG2 composite hydrogel was recorded as $\Delta T$ ($T_1 - T_2$). The fluorescence intensity through the tissues was also measured. Last, the Nd-BG2 composite hydrogel temperature and fluorescence intensity through the different tissue types with same thickness and the mice tissues of different thicknesses were recorded, and the relationship between $\Delta T$ and tissue thickness was analyzed.

In vivo evaluation of the photothermal function of composite hydrogels on tumors and the measurement of in situ treatment temperatures

LM8 cells ($2 \times 10^5$) were subcutaneously injected into nude mice (4 to 6 weeks old). All the experimental protocols were carried out in accordance with the guideline approved by the Shanghai Key Laboratory of Regulatory Biology and the rules of the National Ministry of health. When the tumor grew to 6 to 8 mm in diameter, the nude mice with tumor were randomly divided into eight groups ($n = 5$): Gel 60°C, Gel 53°C, Gel 48°C, Gel 43°C, Gel-no-L, CS + L, CS-no-L, and blank. Then, the Nd-BG2 and CS-BG composite hydrogels were injected into the tumor site. For the laser treatment groups (Gel 60°C, Gel 53°C, Gel 48°C, Gel 43°C, and CS + L), each nude mouse was exposed to the laser for 15 min, and the tumor surface temperature was controlled by adjusting the laser power. Meanwhile, the day of the first NIR irradiation time was defined as day 0. The tumor volume (length and width) was measured every 2 days by caliper. The shortest length direction of the tumor was defined as the width of the tumor, and the longest one was the length. The tumor sizes were calculated by the formula $V = (\text{tumor length}) \times (\text{tumor width})^2 / 2$ – hydrogel volume. The relative tumor volume was expressed as $V/V_0$, in which $V_0$ was the volume on day 0. Whole-body fluorescence imaging of the nude mice was recorded on day 0 and day 14. The mice were euthanized, and the tumor and surrounding tissues were collected and photographed. Then, the tissues were fixed in 4% paraformaldehyde (Shanghai, China). In addition, after 3 days of continuous NIR irradiation on the injected hydrogels, the skin tissues at the tumor site were taken and placed in the paraformaldehyde solution. All of the fixed tissues were embedded in paraffin (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) and stained with H&E (Aladdin, Shanghai, China) and then observed using a light microscope (Leica, DM400B).

The degradation of the composite hydrogel in vivo

Eight-week-old male mice were used to investigate the degradation of the composite hydrogel and elemental accumulation inside the body. The dorsal hairs of mice were shaved, and 0.5 ml of hydrogel was injected into the subcutaneous area of the mouse through a syringe. At different time points (0, 7, and 14 days), stool, urine, blood, and important organs including heart, liver, pancreas, spleen, and kidney were collected. The organs were digested with concentrated nitric acid (HNO$_3$; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) and hydrogen peroxide (H$_2$O$_2$; Aladdin, Shanghai, China), and the stool and blood were digested in nitrohydrochloric acid before the measurement of the Ca, Si, and Nd contents by ICP-AES. In addition, the remaining hydrogel matrix in the body was also collected and weighed. It was found that the hydrogels removed on days 7 and 14 contained tissues. Then, each hydrogel sample was dissolved in 1.5 ml of 10% ethylenediamine disodium tetra-acetate (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), and the dissolved hydrogel was centrifuged (6000 rpm, 5 min). The remaining tissue was collected, and the remaining liquid on the tissue surface was wiped up with filter paper, and the tissue weight was weighed.

In vivo evaluation of the bioactive effect of composite hydrogels on burn wound healing

Eight-week-old female mice (BALB/c) were used to establish the second-degree skin burn model. First, the dorsal hairs of mice were shaved and removed. Then, a boiled iron block (constant temperature of 100°C) was placed on the back of the clean hairless mice skin for 5 s to create an 8-mm-diameter burned wound. The burned mice were optionally divided into three groups ($n = 5$): blank group, SA group, and Nd-BG2/SA group. The blank group did not receive any treatment, the SA group was treated with alginate hydrogel, and the Nd-BG2/SA group was treated with the Nd-BG2 composite hydrogel on burned wound. Photographs were taken to record the healing state of the burned area at days 0, 3, 7, and 14, and the wound closure was calculated as follows ($S_0$, initial wound size; $S$, wound size): wound closure (%) = ($S_0 - S$)/$S_0 \times 100\%$. The wound tissue samples were collected at days 7 and 14, fixed with 4%, and 5% paraformaldehyde, embedded in paraffin, sectioned and stained with H&E, and observed using a light microscope.

**REFERENCES AND NOTES**

1. Global Burden of Disease Cancer Collaboration, The global burden of cancer 2013. *JAMA Oncol.* 1, 505–527 (2015).
2. X. Huang, W. Zhang, G. Guan, G. Song, R. Zou, J. Hu. Design and functionalization of the NIR-responsive photothermal semiconductor nanomaterials for cancer theranostics. *Acc. Chem. Res.* 50, 2529–2538 (2017).
3. Q. Tian, J. Hu, Y. Zhu, R. Zou, Z. Chen, S. Yang, R. Li, Q. Su, Y. Han, X. Liu, Sub-10 nm Fe$_3$O$_4$@C$_x$N$_y$ core–shell nanoparticles for dual-modal imaging and photothermal therapy. *J. Am. Chem. Soc.* 135, 8571–8577 (2013).
4. Z. Chen, L. Zhang, Y. Sun, J. Hu, D. Wang, 980-nm laser-driven photovoltaic cells based on rare-Earth up-converting phosphors for biomedical applications. *Adv. Funct. Mater.* 19, 3815–3820 (2009).
5. Z. Zhou, T. Fan, Y. Yan, S. Zhang, Y. Zhou, H. Deng, X. Cai, J. Xiao, D. Song, Q. Zhang, Y. Cheng. One stone with two birds: Phytic acid-capped platinum nanoparticles for targeted combination therapy of bone tumors. *Biomaterials* 194, 130–138 (2019).
6. Y. Liu, T. Li, H. Ma, D. Zhai, C. Deng, J. Wang, S. Zhou, J. Chang, C. Wu. 3D-printed scaffolds with bioactive elements-induced photothermal effect for bone tumor therapy. *Acta Biomater.* 73, 531–546 (2018).
7. Y. Wang, Q. Huang, X. He, H. Chen, Y. Zou, Y. Li, K. Lin, X. Cai, J. Xiao, Q. Zhang, Y. Cheng. Multifunctional melanin-like nanoparticles for bone-targeted chemo-photothermal therapy of malignant bone tumors and osteolysis. *Biomaterials* 183, 10–19 (2018).
8. H. Kang, S. Hu, M. H. Cho, S. H. Hong, Y. Choi, H. S. Choi. Theranostic nanosystems for targeted cancer therapy. *Nano Today* 23, 59–72 (2018).
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25.
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691
9.
31
21.
19.
27.
446
12.
9.
2.
11.
248
239
upconverted fluorescence. Adv. Mater. 25, 615–626 (2013).
14. D. Jaque, C. Jacinto, Luminesprobe nanoparticles for thermal bio-sensing: Towards controlled photo-thermal therapies. J. Lumin. 169, 394–399 (2016).
15. O. A. Savchuk, J. J. Carvajal, M. C. Pujol, E. W. Barrera, J. Massons, M. Aguilo, F. Diaz, H0;Yb3+;2;W9;O41; nanoparticles: A versatile material for multiple thermal sensing purposes by luminescence thermometry. J. Phys. Chem. C 119, 18546–18558 (2015).
16. Z. Cao, X. Wei, L. Zhao, Y. Chen, M. Yin, Investigation of Sr6;2;O35;Sm3+ as a multimode temperature sensor with high sensitivity. ACS Appl. Mater. Interfaces 8, 34546–34551 (2016).
17. M. Ding, C. Lu, L. Chen, Z. Ji, Ce3+;Yb3+ co-doped NaYF4:Yb3+ europium-doping phosphors for self-referencing optical thermometry. J. Alloys Compd. 763, 85–93 (2015).
18. C. Wang, K. Lin, J. Chang, J. Sun, Osteogenesis and angiogenesis induced by porous Na2SiO3/3PDGAl composite scaffold via activation of AIPK/ERK1/2 and PI3K/Akt pathways. Biomaterials 34, 64–77 (2013).
19. H. Li, J. Chang, Stimulation of proangiogenesis by calcium silicate bioactive ceramic. Acta Biomater. 9, 5379–5398 (2013).
20. W. Zhai, H. Lu, L. Chen, X. Yin, H. Huang, K. Dai, K. Naoki, G. Chen, J. Chang, Silicat bioceramics induce angiogenesis during bone regeneration. Acta Biomater. 8, 341–349 (2012).
21. C. Wu, W. Fan, Y. Zhou, Y. Luo, M. Gelinsky, J. Chang, Y. Xiao, 3D-printing of highly uniform CaSiO3 ceramic scaffolds: Preparation, characterization and in vivo osteogenesis. J. Mater. Chem. 22, 12288–12295 (2012).
22. L. Lu, J. Wang, P. Xu, Y. Han, H. Ma, H. Xu, S. Chen, J. Chang, Q. Ke, M. Liu, Z. Yi, C. Wu, A conductive bioceramic/polymer composite biomaterial for diabetic wound healing. Acta Biomater. 9, 128–143 (2017).
23. D. Jaque, L. M. Maestro, B. del Rosal, P. Haro-Gonzalez, A. Benayas, J. L. Plaza, E. Martin Rodriguez, J. Garcia Solé, Nanoparticles for photothermal theranostics. Nanoscale 6, 9494–9530 (2014).
24. J. W. Stouwdam, F. C. J. M. van Veggel, Near-infrared emission of dispersible Er3+, Nd3+, and Ho3+ doped LaF3 nanoparticles. Nano Lett. 2, 733–737 (2002).
25. X. Wang, Q. Liu, Y. Bu, C.-S. Liu, T. Liu, X. Yan, Optical temperature sensing of rare-earth ion doped phosphors. RSC Adv. 5, 86219–86236 (2015).
26. E. Hemmer, P. Acosta-Mora, J. Méndez-Ramos, S. Fischer, Optical nanoprobes for biomedical applications: Shining a light on upconverting and near-infrared emitting nanoparticles for imaging, thermal sensing, and photodynamic therapy. J. Mater. Chem. B 5, 4365–4392 (2017).
27. B. Ahrens, C. Einsenschmidt, J. A. Johnson, P. T. Miclea, S. Schweizer, Structural and optical investigations of Nd-doped fluorozirconate-based glass ceramics for enhanced upconverted fluorescence. Appl. Phys. Lett. 92, 061905 (2008).
28. J. Zhong, D. Chen, Y. Peng, Y. Lu, X. Chen, X. Li, Z. Jia, An review on nanostructured glass ceramics for promising application in optical thermometry. J. Alloys Compd. 763, 34–48 (2018).
29. X. Sun, J. Sun, B. Dong, G. Huang, L. Zhang, W. Zhou, J. Lv, X. Zhang, M. Liu, L. Xu, X. Bai, W. Xu, Y. Yang, X. Song, H. Song, Noninvasive temperature monitoring for dual-modal tumor therapy based on lanthanide-doped up-conversion nanocomposites. Biomaterials 201, 42–52 (2019).
30. G. Ramirez-Garcia, M. A. Honrado-Colín, E. De la Rosa, T. López-Luke, S. S. Panikar, J. de Jesus Ibáñez-Sánchez, V. Piazza, Theragnostic nanocompounds of gold-decorated upconversion nanoparticles for optical imaging and temperature-controlled photothermal therapy. J. Photochem. Photobiol. A Chem. 384, 112053 (2019).
31. H. Li, X. Sun, M. K. Shazhad, L. Liu, Facile preparation of upconversion microfibers for efficient luminescence and distributed temperature measurement. J. Mater. Chem. C 7, 7984–7992 (2019).
32. S. Liu, H. Ming, J. Cui, S. Liu, W. You, X. Ye, Y. Yang, H. Nie, R. Wang, Color-tunable upconversion luminescence and multiple temperature sensing and optical heating properties of Ba3Y2O6:Er3+/Yb3+ phosphors. J. Phys. Chem. C 132, 16289–16303 (2018).
33. Q. Shao, Z. Yang, G. Zhang, Y. Hu, Y. Dong, J. Jiang, Multifunctional lanthanide-doped core/shell nanoparticles: Integration of upconversion luminescence, temperature sensing, and photothermal conversion properties. ACS Omega 3, 188–197 (2018).
34. B. del Rosal, A. Pérez-Delgado, M. Misiak, A. Bednarkiewicz, A. S. Vanetsev, Y. Orlovskii, D. J. Jovanović, M. D. Dramacanin, U. Rocha, K. Upeanda Kumar, C. Jacinto, E. Navarro, E. Martin Rodriguez, M. Pedroni, A. Spiegeli, G. A. Hirata, I. R. Martin, D. Jaque, Neodymium-doped nanoparticles for infrared fluorescence bioimaging: The role of the host. J. Appl. Phys. 118, 143104 (2015).
35. L. Yu, J. Ding, Injectable hydrogels as unique biomedical materials. Chem. Soc. Rev. 37, 1473–1481 (2008).
36. R. Xing, K. Liu, T. Jiao, N. Zhang, K. Ma, R. Zhang, Q. Zou, G. Ma, X. Yan, An injectable self-assembling collagen-gold hybrid hydrogel for combinatorial antitumor photothermal/photodynamic therapy. Adv. Mater. 28, 3669–3676 (2016).
37. W. Jenkins, P. Perone, K. Walker, N. Bhagavathula, M. N. Adlam, M. DaSilva, M. K. Darne, J. Varani, Fibroblast response to lanthamoid metal ion stimulation: Potential contribution to fibrotic tissue injury. Biol. Trace Elem. Res. 144, 621–635 (2011).
38. C. Wu, Y. Zhou, M. Xu, P. Han, L. Chen, J. Chang, Y. Xiao, Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity. Biomaterials 34, 422–433 (2013).
39. X. Wang, J. Chang, C. Wu, Bioactive inorganic/organic nanocomposites for wound healing. Appl. Mater. Today 11, 308–319 (2018).
40. B. J. Duffy, P. M. McLaughlin, M. R. Eichelberger, Assessment, triage, and early management of burns in children. Clin. Pediatr. Emerg. Med. 7, 82–93 (2006).
41. D. M. Jackson, The diagnosis of the depth burning. Br. J. Surg. 40, 588–596 (1953).

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