Erbium-doped barium titanate/hydroxyapatite composites with enhanced piezoelectric and biological properties

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Shortening the period of bone repair based on $BaTiO_3$/hydroxyapatite(HA) composites with osteoconductivity and osteoinductivity is important. However, when the piezoelectric properties of composites are matched with natural bone, the content of non-degradable $BaTiO_3$ needs to reach 90%, which may not conducive to clinical application. The barium titanate content in the composite was reduced by doping Er^{3+} into the $BaTiO_3$ of $BaTiO_3$/HA composites with enhanced piezoelectric properties. The piezoelectric constant and compressive strength of composites increased with the increase of Er^{3+}-doped barium titanate (BTEr) content. Appropriate piezoelectric stimulation can promote the growth of osteoblasts. 70% BTEr/30% HA is more beneficial to the proliferation of osteoblasts than 70% $BaTiO_3$/30% HA.

1. Introduction: Hydroxyapatite (HA) is widely used in manufacturing bone repair materials [1–3]. Since HA is only conductive and not inductive, HA-induced new bone growth cycles are usually long, which is not conducive to early postoperative patients [4, 5]. Compared with the induction of growth factor, protein and drug, electrical stimulation can effectively affect many biochemical reactions in the body [6–9]. As a kind of electrical stimulation, the piezoelectric effect has received widespread attention [10]. $BaTiO_3$(BT), as a well-known ferroelectric material [11], has been reported as a hard tissue replacement material [12]. Although BT is osteoinductive, its non-degradability makes it unsuitable as scaffolds alone. In addition, HA/BT composites have both the conductivity of HA and the inducibility of barium titanate, which has potential application value in dental repair and hard tissue replacement. When $BaTiO_3$/HA were influenced by cycle loading, the piezoelectric effect of $BaTiO_3$ promoted the growth of osteoblasts and interaction with HA [13]. Studies have shown that when the BT content in the BT/HA composite is 90%, the composite has the same piezoelectric coefficient as human bone to make its effect on osteoblasts greater than that of HA alone [14, 15]. However, barium titanate is non-degradable, and the higher content of barium titanate in BT/HA composites is not conducive to clinical application. Furthermore, the high non-degradable BT content makes the degradation rate of BT/HA composite not match the growth rate of new bone. Reducing the content of $BaTiO_3$ in $BaTiO_3$/HA piezoelectric biomaterials can considerably decrease the composite material's piezoelectric properties and lead to loss of inductivity. Some scholars have established the dielectric and thermal modelling of multiphase composites to predict the relationship between the overall properties of the material and the properties of each component [16, 17]. If the piezoelectric phase in the composite can be improved, the necessary piezoelectric phase content in the composite can be reduced. In recent years, many studies have shown that Re^{3+}-doped $BaTiO_3$ can improve the piezoelectric and dielectric properties of barium titanate [18]. Er^{3+}-doped BNT not only possesses good photoluminescence properties but also show favourable dielectric properties [19–21], whereas the 5 mol.% Er^{3+}-doped $BaTiO_3$ exhibits superior up-conversion luminescence properties [22]. However, the effect of rare earth erbium ions on osteoblasts urgently needs to be solved.

In this work, 5 mol.% Er^{3+} doped $BaTiO_3$ (BTEr) was prepared. The effect of BTEr content on the compressive strength and piezoelectric constant of BTEr/HA composites was studied. The cytotoxicity, cell viability, and morphology of the composites were also characterised. The research results are of great value for improving the bone repair and clinical safety of piezoelectric biomaterials. In addition, it has high potential application prospects in the rapid repair of bone defects and rapid recovery after dental implants.

2. Materials and methods

2.1. Preparation of BTEr powder and BT powder: BTEr powder was prepared by sol–gel method. First, butyltitanate ($C_{16}H_{36}O_4Ti$, Kelong, China, AR) was dissolved in a solution of acetylacetone ($C_5H_8O_2$, Fuchen, China, AR) and then added dropwise to an aqueous solution of barium acetate ($C_4H_6BaO_4$, Guangfu, China, AR). In the above solution, the molar ratio of Ba^{2+} and Ti^{4+} was 1.01, and the solution was kept at 90°C for 1 h. An aqueous solution of 5 mol% erbium nitrate ($Er(NO_3)_3{\cdot}6H_2O$, aladdin, China, AR) was added dropwise to the above solution and diluted to 0.3 M with anhydrous ethanol. Whlie the pH of the solution was adjusted with glacial acetic acid (CH_3COOH, Tianli, China, AR) to a value of 4 to obtain a clear, transparent, light yellow sol. Second, the prepared xerogel was aged for 24 h and then dried at 90°C to obtain a xerogel. The xerogel was annealed at 750°C for 1 h. Finally, the product obtained was ground in an agate mortar. The prepared powder is washed in dilute hydrochloric acid and then dried to obtain BTEr powder. All of the synthesis processes were carried out in the air.

The preparation method of barium titanate powder is basically the same as that of BTEr powder, the only difference is that no aqueous solution of Erbium Nitrate is added.

2.2. Preparation of BTEr/HA or BT/HA piezoelectric biomaterials: HA (Sigma-Aldrich Chemical Co. Inc., USA) with a size range from 0.5 to 1.0 µm was used as a biological phase, and 5 mol% BTEr powder (or BT powder) was used as a piezoelectric phase. Polyvinyl alcohol (Sigma-Aldrich Chemical Co., Inc.) was used as a binder. The raw materials were weighed in accordance with the mass ratio (the BTEr or BT contents of the composite were 90, 80, 70 and 60 wt.%) and ball milled for 12 h. The milled slurry mixture was combined with 3–5% binder in deionised water and then pressed into circular plates at 10 MPa for 60 s. Calcination was performed at 1300°C in air for 2 h, where the heating rate was kept at 2 °C/min. The polarisation field intensity

was 1.5 kv/mm; The polarisation time was 30 min, and BTEr/HA or BT/HA piezoelectric biomaterials were polarised in silicone oil at 55°C.

2.3. Characterisation: The morphology of the sample was examined by high-resolution transmission electron microscopy (HRTEM) (JEM-3010, Japan Electronics Co. Ltd., Japan). The piezoelectric constant d_{33} of the sample was tested using a quasi-static d_{33} tester (ZJ-3AN, Institute of Acoustics, Chinese Academy of Sciences). The sample size for d_{33} testing was $\Phi 10 \times 2$ mm. The compressive strength was measured on cylindrical samples of $\Phi 10 \times 10$ mm by using a computer server to control the material testing machine (HT-2402-100KN, Hungta, Taiwan) with a pressure speed of 0.5 mm/min. Five samples were tested to obtain an average value. X-ray diffraction (XRD) analysis was carried out on an X-ray diffractometer (Model 7000, Shimadzu Limited, Japan) with Cu Kα radiation.

After sterilised with gamma rays emitted from the isotope source (Cobalt-60), the polarised BTEr/HA piezoelectric biomaterials were placed in a 24-well plate. Third-generation neonatal Sprague–Dowley neonatal rat osteoblasts were digested into monoplast suspension, and $\sim 2 \times 10^4$ osteoblasts were inculated evenly onto the samples in each well and incubated at 37°C. The fresh medium was used to change the culture medium every 2 days. After culturing for 1, 4 and 7 days, the MTT solution was added to each well and incubated at 37°C for 4 h. Dimethyl sulfoxide was added to each well, and the solution was transferred to 96-well plates. The absorbance of solutions at 492 nm was measured with an MK-2 microplate reader after culturing for 1, 4 and 7 days, and the relative growth rate (RGR) was calculated by equation, i.e. RGR = (OD value of experimental group/OD value of negative control proup) × 100%. The morphologies of the osteoblasts seeded on the samples were observed with a scanning electron microscope. Experimental results were analysed using SPSS19.0 software, and $p < 0.05$ was considered to indicate the statistical difference.

3. Results and discussion

3.1. BTEr powder phase composition: The XRD pattern revealed the characteristic peaks of tetragonal BT phase (Fig. 1). The average grain size of the nanomaterial was determined using the Scherrer's equation and analysis, using three independent diffraction peaks which gave an average grain size of the materials was determined to be 16.9 ± 0.20 nm.

The BTEr nanoparticle size was in the range of 10–50 nm (Fig. 2*a*). The interplanar spacing of 0.28 nm in the HRTEM image represents the (110) of the $BaTiO_3$ phase, and the interplanar spacing of 0.40 nm corresponds to the (100) crystal plane of $BaTiO_3$ (Fig. 2*c*). The BTEr interplanar spacing increases relative

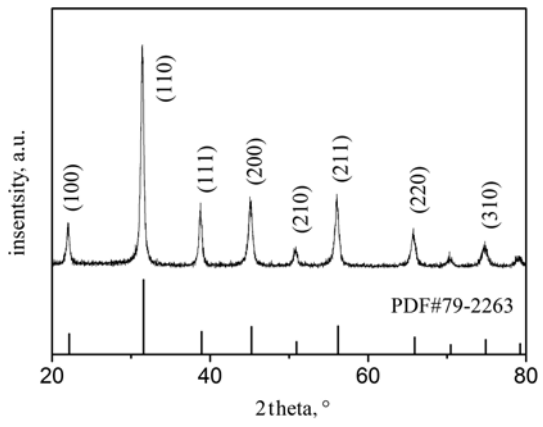

Fig. 1 *XRD pattern of BTEr powder*

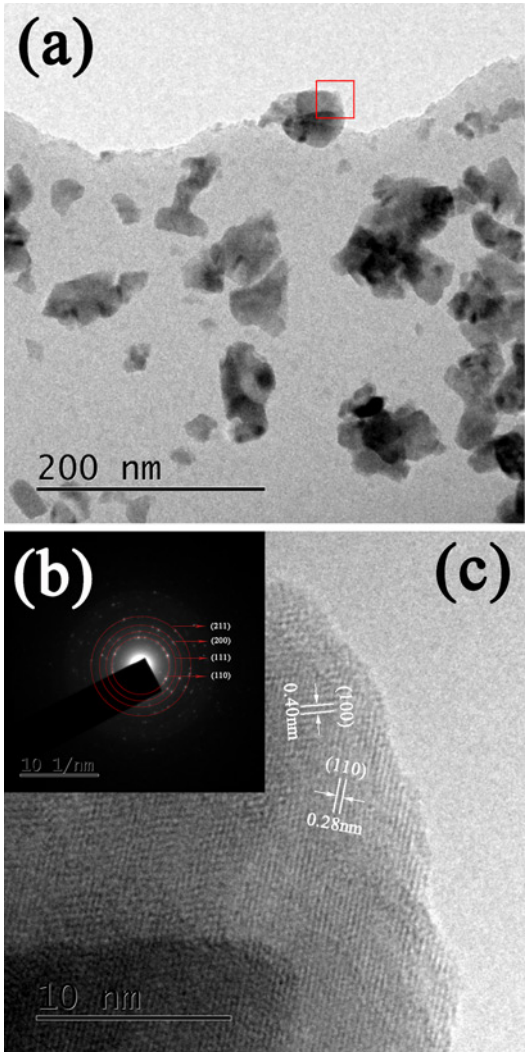

Fig. 2 *TEM/HRTEM images and SAED pattern of BTEr nanoparticle*
a TEM image of the BTEr
b SAED pattern
c Corresponding HRTEM image

to that of pure $BaTiO_3$ [23]. It suggests that the BTEr crystal lattice was distorted, and the rare earth Er^{3+} cation substitutes for both Ti^{4+} and Ba^{2+} [24]. The SAED pattern of the BTEr sample was composed of discrete diffraction points, indicating the polycrystalline nature of the sample with good crystallinity (Fig. 2*b*).

3.2. Effect of BTEr content on the compressive strength and piezoelectric constant d_{33} of BTEr/HA and BT/HA piezoelectric biomaterials: The compressive strength and piezoelectric coefficient of BTEr/HA are higher than BT/HA (Fig. 3). According to Fig. 2, BTEr is distorted compared to the lattice of $BaTiO_3$, and the interplanar spacing is increased, which is beneficial to improve the compressive strength and piezoelectric coefficient of the composites. The compressive strength and piezoelectric constant of BTEr/HA composites rose with increasing BTEr content. The main reason for this result is that the BTEr/HA composite is composed of BTEr and HA. There is no chemical reaction between them. With the increase of BTEr content in the composite, the content of HA in the composite decreased. BTEr particles in the composite gradually formed a network, and HA was gradually embedded in the BTEr network in isolation. Ultimately, the piezoelectric constant and compressive strength of the composites increase. When the BTEr content of BTEr/HA composites was 70%,

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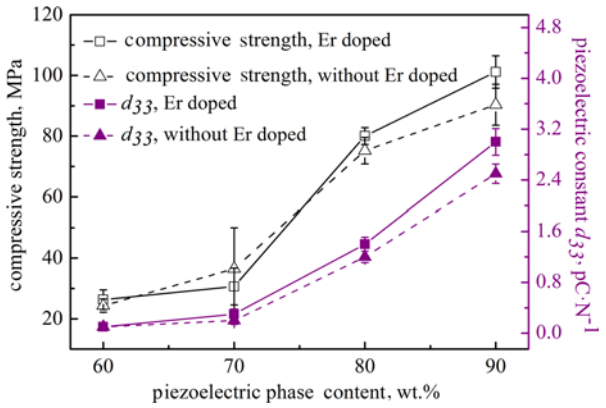

Fig. 3 *Compressive strength and piezoelectric constant of BTEr/HA or BT/HA biomaterials with different piezoelectric phase contents*

the compressive strength was 30.63 MPa, and the piezoelectric constant was 0.3 pC/N.

3.3. Cell compatibility of the BTEr/HA piezoelectric biomaterials: The composites showed good biocompatibility when the BTEr content was 70%, and the difference was statistically significant, with 90% BTEr content at days 1 and 4 (Fig. 4). Notably, the composites with piezoelectric phase content of 90% exhibited poor cell compatibility. Studies have shown that a persistent rise in electrical stimulation instead inhibits the growth of cells when the electrical stimulation reaches a certain limit [25]. Compared with 70% BTEr/30% HA composites, the composites with 90% BTEr content have a much higher piezoelectric coefficient. 90% of the composites have excessive electrical stimulation, which exceeds the tolerance of osteoblasts and causes some cells to die [26]. In addition, the 70% BTEr/30% HA composite has a higher HA content, and the cell compatibility of HA is better than that of BT. With the increase of co-culture time, the electrical stimulation of composites progressively decreased from too high to a suitable range which can promote the proliferation of osteoblasts. As a result, the differences between groups were insignificant after co-cultivation for seven days.

Fig. 5 show that the osteoblasts were in close contact with the surface of the composites and spread on the surface of the negative electrode of the composites. The morphology of the osteoblasts was flat, and the filopodia were attached to the surface of the composites or connected with nearby cells.

3.4. Cell viability of piezoelectric biomaterials: Fig. 6 shows the results of the MTT test. These data are expressed as cell viability

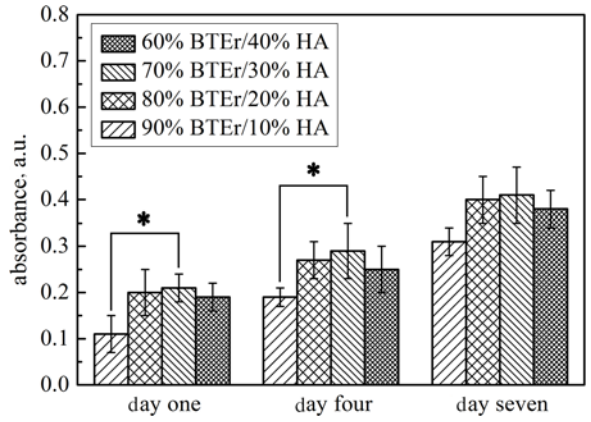

Fig. 4 *MTT assay of BTEr/HA piezoelectric biomaterials after co-culture with osteoblast cells. Significant differences are reported when $p < 0.05$ (*); * refers to the same time points for different groups*

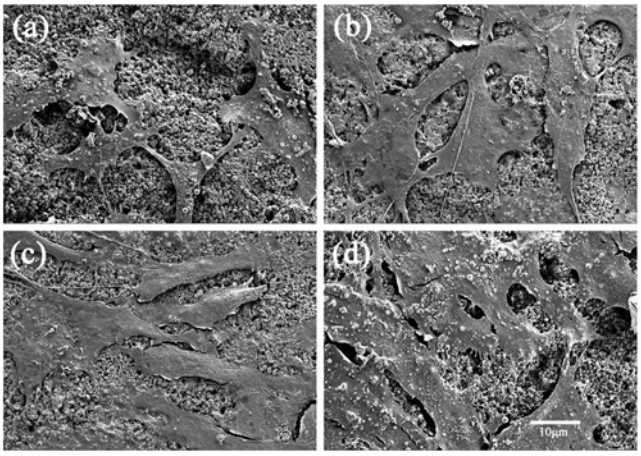

Fig. 5 *Morphologies of BTEr/HA piezoelectric biomaterials after co-culture with osteoblast cells for 4 days with different BTEr contents*
a 90% BTEr
b 80% BTEr
c 70% BTEr
d 60% BTEr

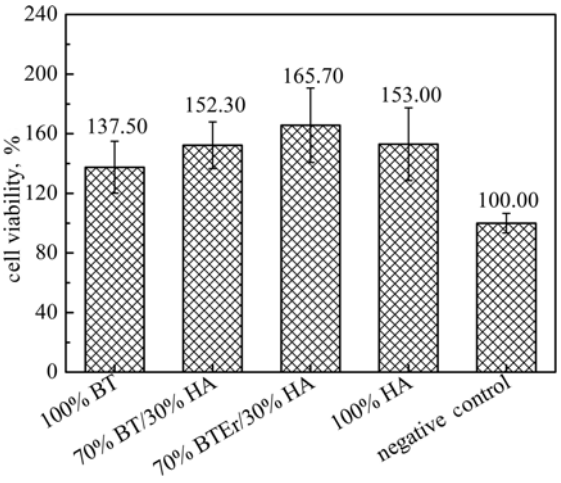

Fig. 6 *MTT test for the 70% BT/30% HA composites, 70% BTEr/30% HA composites, 100% BT, 100% HA on osteoblasts after four-day culture (each group of samples is unpolarised)*

and were related to the negative control, which was considered to correspond to 100% viable cells. It is worth noting that 70% BTEr/30% HA composite with the highest cell viability up to 165.70% was achieved. It is indicated that the addition of erbium ions to HA/BT is more conducive to osteoblast proliferation than the absence of erbium ions. On the one hand, lower concentrations of rare earth ions have the effect of promoting cell protein synthesis and increased mitochondrial succinate dehydrogenase activity, which accelerates cell proliferation. On the other hand, since Er^{3+} and Ca^{2+} have many similarities in properties and structures, Er^{3+} can not only occupy the Ca^{2+} position but also replace the bound calcium to increase the intracellular Ca^{2+}. It is known that an increase in the concentration of Ca^{2+} in the cytosol is required during cell proliferation. Therefore, Er^{3+} can promote cell proliferation by promoting extracellular Ca^{2+} into the cell [27].

4. Conclusion: In summary, A polycrystalline BTEr powder with an average grain size of 16.9 nm was prepared by a sol–gel method. Er^{3+} doping increases the interplanar spacing of barium titanate, resulting in BTEr/HA composites with higher compressive strength and piezoelectric coefficients than undoped BT/HA

composites. The 70% BTEr/30% HA composite has good biocompatibility due to suitable piezoelectric coefficient, large HA content, and a small amount of Er^{3+}, and has the highest cell activity up to 165.70%. In the future, A physiological load will be applied to the 70% BTEr/30% HA composite to characterise changes in the properties of the composite and its effect on osteoblasts.

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