Enhancing Bone Regeneration and Biocompatibility through Erbium-Doped Hydroxyapatite

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ABSTRACT

Introduction:

Human bones are naturally occurring composite materials, with approximately 65% of their structure comprising inorganic nanocrystalline ceramic with an apatite structure. Hydroxyapatite (HA), a key member of the calcium phosphate family, is widely recognized for its superior osteoconductive properties, making it a valuable bioceramic for applications such as hard tissue repair, dental treatments, and bioceramic coatings. Erbium (Er), a rare-earth element naturally present in bone, has demonstrated potential for biomedical applications due to its non-toxic nature. This study aims to evaluate the biocompatibility of erbium-doped hydroxyapatite (Er-HA) for potential applications in bone regeneration.

Materials and Methods:

A mixture was prepared by adding 2.1g of Cetyltrimethylammoniumphosphate (CTAP) to 0.099 mol of Calcium Nitrate and 0.01g of Erbium Nitrate. Diammonium Hydrogen Phosphate (0.67g) was then added, and the mixture was heated at 90°C overnight, followed by an acetone wash. The sample was sintered at 400°C for 3 hours. Afterward, it was subjected to calcium staining, alkaline phosphatase, and collagen estimation.

Results:

The findings indicated that Er-HA exhibited slightly lower cell viability than pure HA at all tested concentrations. However, cell viability remained stable as the concentration increased. Additionally, Er-HA demonstrated higher alkaline phosphatase activity and calcium content compared to the control, suggesting enhanced biomineralization potential. Collagen content remained comparable between the experimental and control groups.

Conclusion:

Er-HA exhibited slightly reduced cell viability compared to pure HA, its increased alkaline phosphatase activity and calcium content highlight its potential for bone regeneration. These findings suggest that Er-HA could serve as a promising material for bone tissue engineering applications.

INTRODUCTION

Human bones are naturally occurring composite materials, with approximately 65% of their structure consisting of inorganic nanocrystalline ceramic in an apatite form. Biological apatite, present in bones, is a nonstoichiometric form of calcium phosphate (CP) that incorporates trace elements such as Mg^{2+}, CO_3^{2-}, and Na^+. In contrast, synthetic hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is stoichiometric, maintaining a Ca/P ratio of 1.67, whereas biological CP exists at the nanoscale and exhibits lower crystallinity [1]. Hydroxyapatite is widely recognized for its exceptional osteoconductive properties, making it a key material within the calcium phosphate family. Due to these properties, HA is extensively used as a bioceramic in various clinical applications, including hard tissue repair, bioceramic coatings, and dental treatments. As the demand for advanced biomaterials grows, researchers continue to explore novel materials for medical applications [2,3].

Bone defects, often caused by periodontal diseases, lead to excessive bone resorption and require effective treatment strategies. Bone grafting remains one of the most common approaches for addressing severe bone defects, utilizing xenografts, allografts, or autografts. Hydroxyapatite is one of the most frequently used graft materials due to its suitability as both an inert scaffold and a graft material in bone healing. Additionally, HA offers several advantageous properties, including biocompatibility, bioaffinity, bioactivity, osteoinduction, osteoconduction, and osseointegration [4,5].

Rare earth (RE) elements, such as erbium (Er), have been shown to influence bone mineral composition and cellular activity, either directly or indirectly. These effects include the regulation of bone circulation homeostasis, management of bone mineral density disorders, modulation of bone remodeling, and impact on both bone formation and resorption. Moreover, the antimicrobial, antibacterial, anti-inflammatory, and biocompatible properties of rare earth elements have sparked interest in their potential applications in tissue engineering [6]. A previous study by Huang et al. demonstrated that rare earth-based bone repair materials support osteoblast proliferation and differentiation [7]. Biocompatibility refers to the ability of a material to perform its intended function without causing adverse effects in living organisms[8]. Assessing the

biocompatibility of materials such as erbium-doped hydroxyapatite is essential for determining their suitability in biomedical applications, particularly in bone implants and tissue engineering. This study aims to evaluate the biocompatibility of erbium-doped hydroxyapatite to explore its potential for use in regenerative medicine.

MATERIALS AND METHODS

Sample Preparation:

A solution was prepared by adding 2.1 grams of CetylTrimethylAmmoniumPhosphate (CTAP) to 0.099 mol of Calcium Nitrate and 0.01 grams of Erbium Nitrate. Subsequently, 0.67 grams of Diammonium Hydrogen Phosphate was introduced into the mixture, which was then maintained at 90°C overnight. The resulting product was washed with acetone and sintered at 400°C for 3 hours.

Calcium Staining:

After one week, calcium production in both the control (HA) and experimental (Er-HA) groups was evaluated using Alizarin Red S (ARS), a dye with a strong affinity for calcium salts. Cells treated with ARS were incubated at room temperature for 20 to 30 minutes with 1 milliliter of 40 mM ARS per well. Following a three-day incubation period, the cells were rinsed with PBS and fixed with 4% formaldehyde at room temperature for 15 minutes. The samples were then examined under a fluorescent microscope.

After agitation and a 30-minute incubation, 10% (v/v) acetic acid was added to the ARS-treated cells. The mixture was transferred into tubes, vigorously shaken for 30 seconds, and incubated at 85°C for 10 minutes. It was then centrifuged for 15 minutes, and the absorbance of 200 µl of supernatant mixed with 22.5 µl of 10% NH_4OH (v/v) was measured at 405 nm.

Calcium, primarily present in bone tissue as calcium phosphates, plays a critical role in bone matrix composition. It promotes bone growth and development through calcification and regulates cellular signaling essential for bone repair. Additionally, calcium-generated nitric oxide stimulates mature bone cells, encouraging precursor cell development for bone regeneration. Calcium ions also activate the ERK1/2 and PI3K/Akt pathways, which support osteoblastic bone formation and extend osteoblast lifespan. Furthermore, calcium ions play a key role in regulating osteoclast synthesis and bone resorption.

Alkaline Phosphatase:

Once the cells reached confluence, the samples were introduced and incubated for three days. The cells were then lysed using Triton X-100 and incubated for one minute. To evaluate protein production and alkaline phosphatase (AP) activity, an AP buffer was applied. Following the addition of BCIP/NBT and a 30-minute incubation, cell density was measured at 405 nm using an ELISA plate reader, and fluorescence microscope images were captured.

For protein content analysis, a modified Bradford assay was performed. Samples were transferred to a fresh 96-well plate and treated with BCIP and NBT, followed by the addition of Bradford reagent and AP buffer. Optical density was measured at 595 nm. To normalize the optical density, the cell count at each culture time point was divided by the measured optical density.

Collagen Estimation:

Cells were initially cultured in a 96-well plate for 24 hours to facilitate adherence and achieve confluence. The test compound was then introduced by replacing the culture medium with one containing 2% fetal calf serum. The cells were incubated for an additional 48 hours at 37°C, with the medium renewed every 24 hours for both the treated and control groups.

Following the incubation period, the cells underwent a series of procedures. They were first fixed in 4% formalin for 20 minutes and then rinsed with saline. After fixation, the cells were washed

three times with phosphate-buffered saline (PBS) and stained with Sirius Red for 20 minutes at 37°C using a 20 mL staining solution. This was followed by three additional PBS washes and treatment with 10% acetic acid.

The cells were then subjected to three more PBS washes before being stained with picro-sirius red for one hour. Finally, the samples were dehydrated using ethanol, rinsed with acidified water, and examined under a fluorescent microscope.[9]

RESULTS

Our study obtained the following results:

The relative percentage of alkaline phosphatase (ALP) was higher in erbium-doped hydroxyapatite (Er-HAP) compared to control HA. ALP plays a crucial role in biomineralization by hydrolyzing phosphate-containing substrates, producing orthophosphate, and enhancing calcium uptake in bone. The increased ALP activity in Er-HAP is depicted in Figure 1. The relative calcium content was also found to be higher in Er-HAP than in control HA. Since calcium is a key component of bone, its higher presence in Er-HAP suggests improved bone formation potential, as shown in Figure 2.

Collagen content was observed to be similar in both the control and experimental groups. Collagen forms the organic matrix of bone, providing mechanical support and acting as a scaffold for bone cells. The comparable levels of collagen in both HA and Er-HAP are illustrated in Figure 3.

At all tested concentrations, cell viability in Er-HAP was slightly lower than in control HA. However, as the concentration increased, cell viability remained stable. These findings further support the biocompatibility of Er-HAP for potential bone regeneration applications.

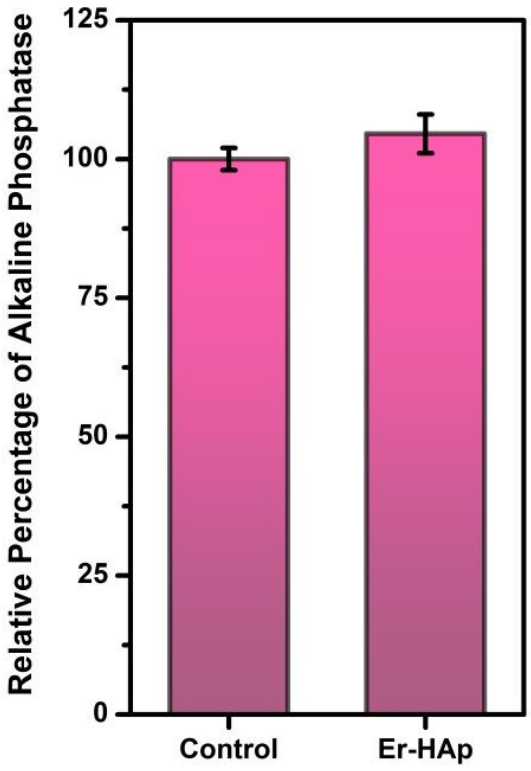

Figure 1: Graph depicting relative percentage of alkaline phosphatase in HA (control) and Erbium doped HA (Er-HAp).

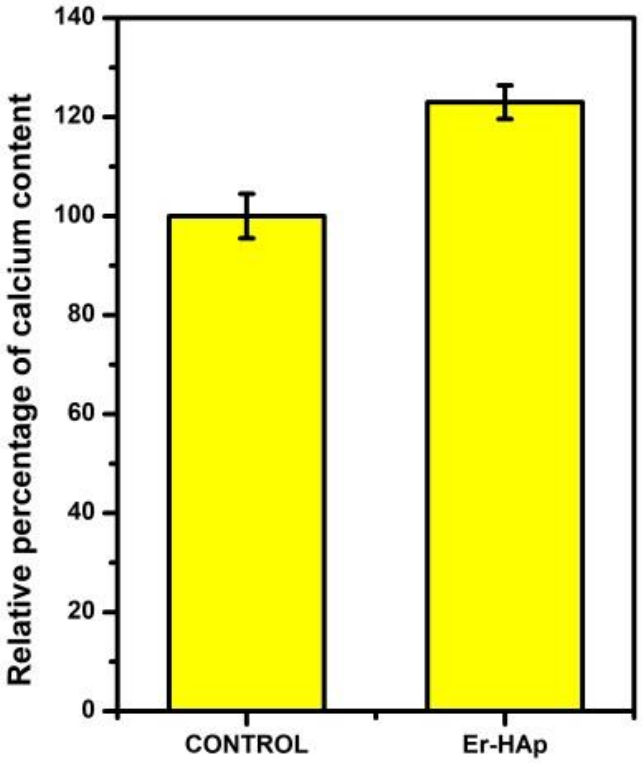

Figure 2: Graph depicting the relative percentage of calcium content in HA and Er-HAp.

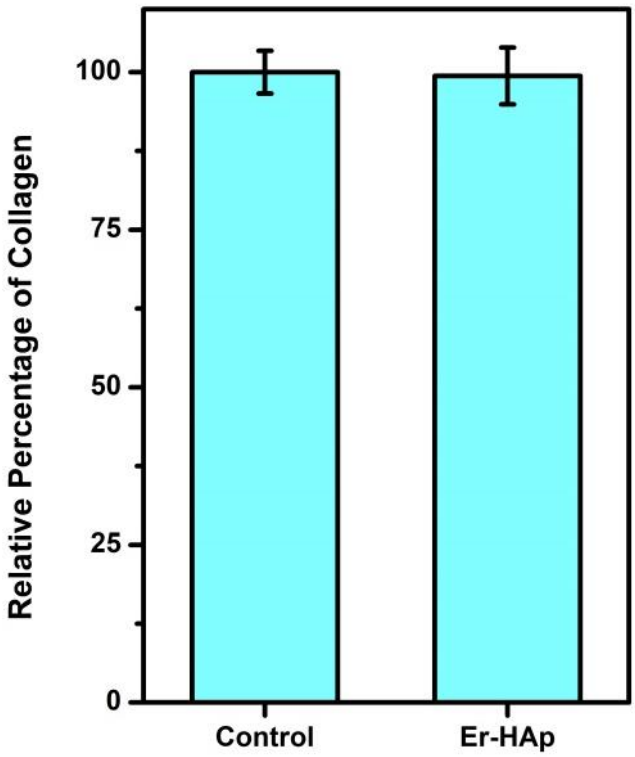

Figure 3: Graph depicting Relative percentage of collagen content in HA and Er-HAp.

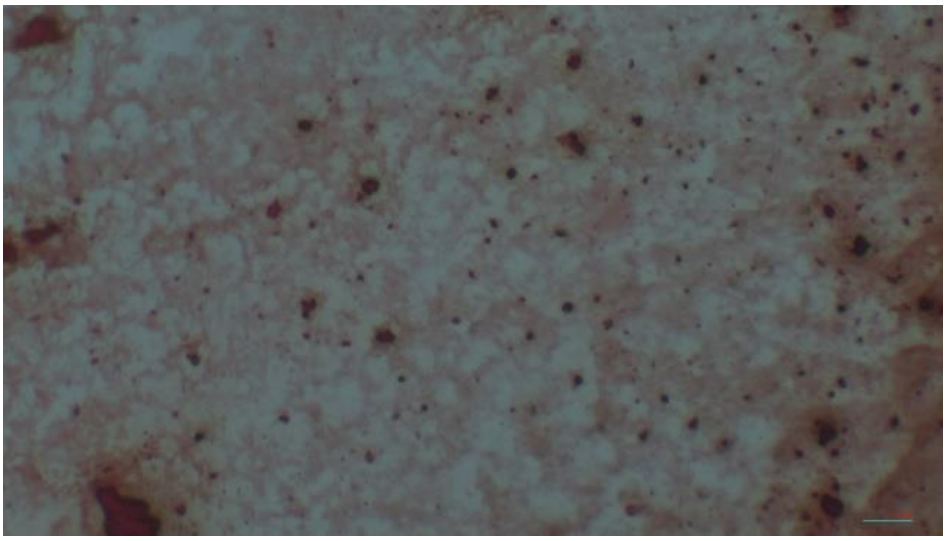

Figure 4: shows ALP activity observed in the microscopic staining panels for erbium doped HA

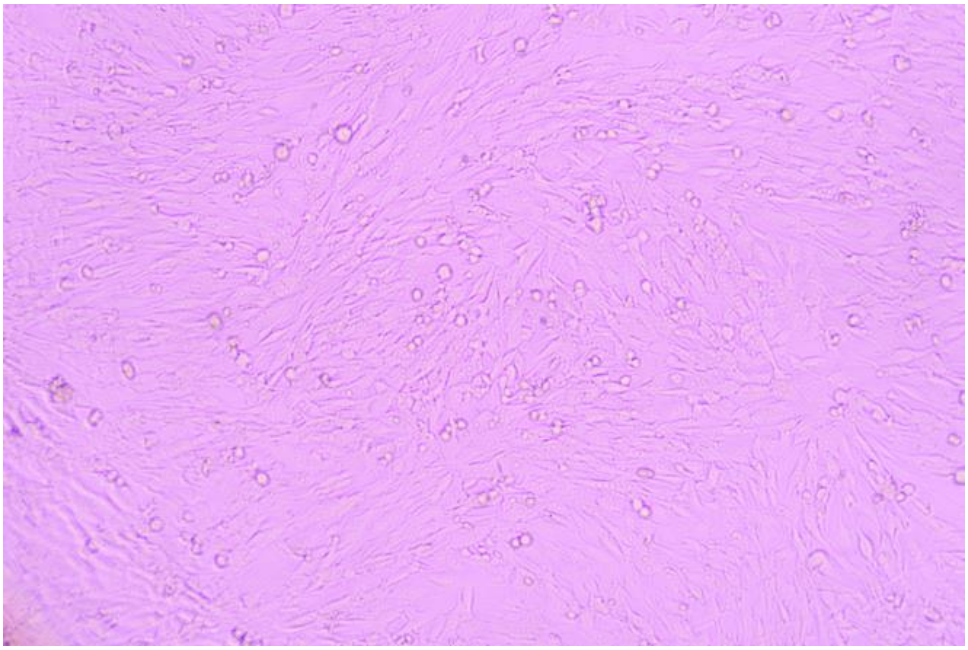

Figure 5: calcium staining observed microscopically in erbium doped HA

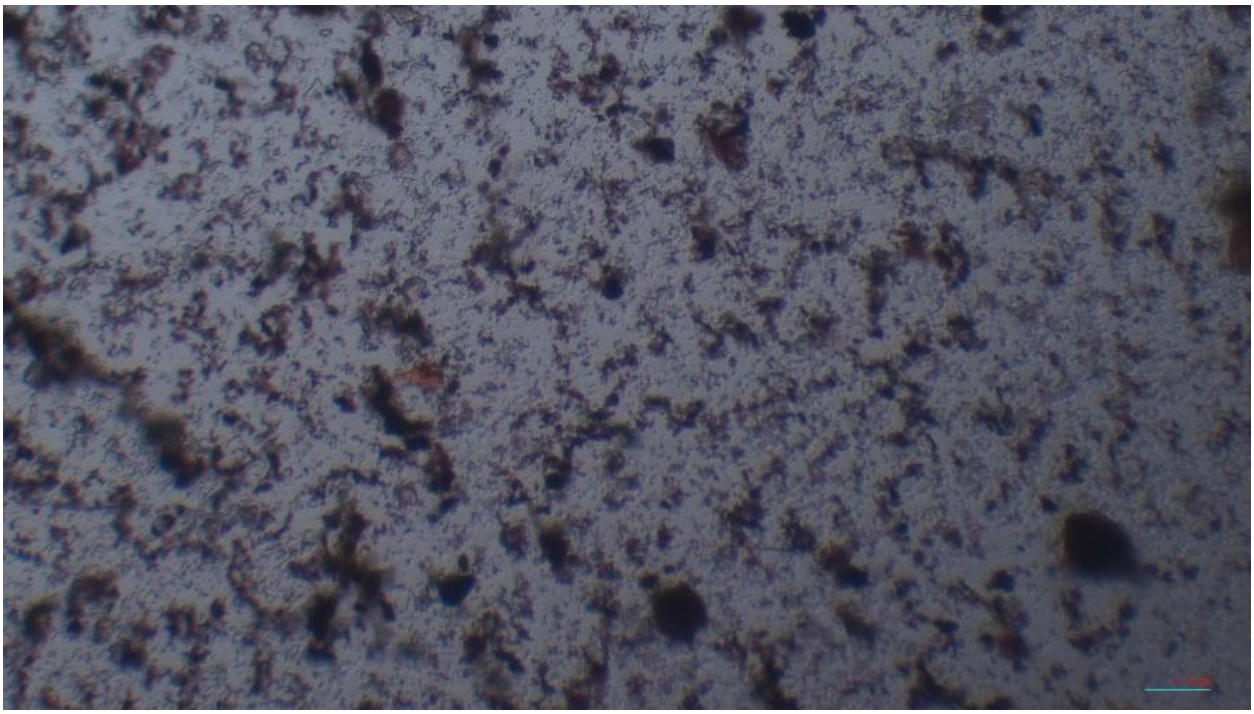

Figure 6:collagen staining observed microscopically in erbium doped HA

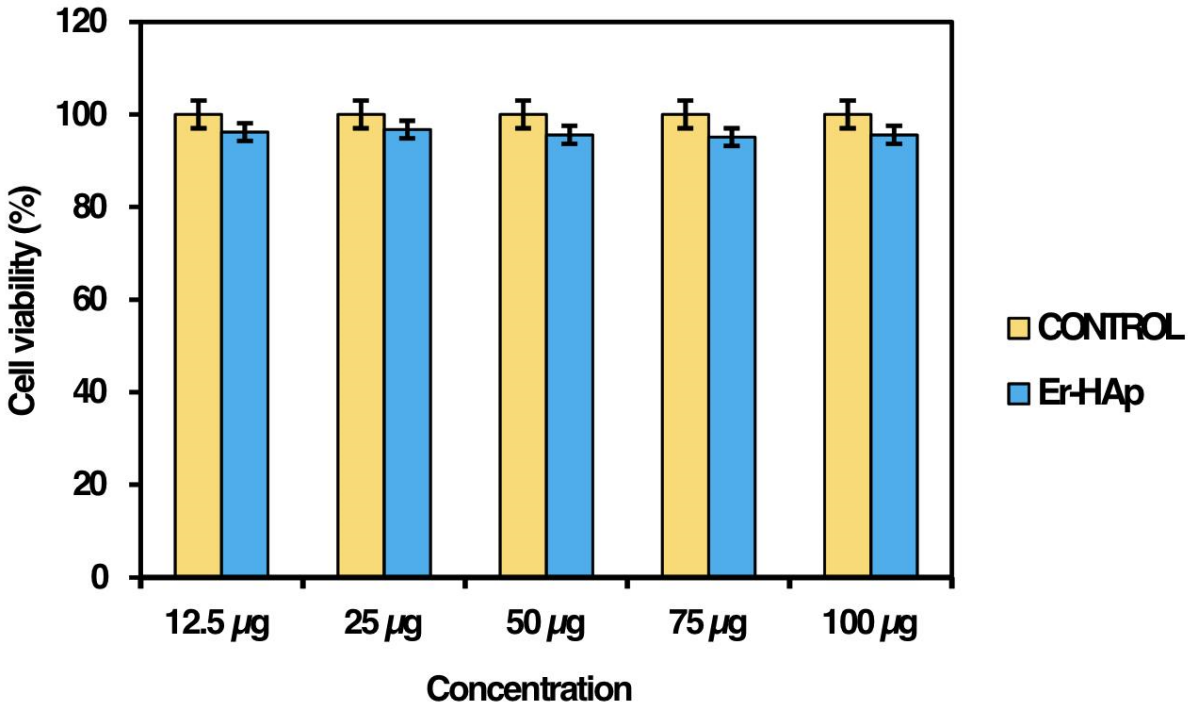

Figure 7: Graph depicting cell viability at increasing concentrations of HA and Er-HAp.

DISCUSSION

In recent years, there has been an increasing emphasis on the exploration of various materials for medical applications, with the goal of improving human health and quality of life. Calcium orthophosphates, essential components of human bones and teeth, contribute significantly to their strength and durability, making them a focal point in biomedical research .[10,11,12]

To optimize both the chemical and optical properties of lanthanide-doped luminescent nanoparticles and expand their biomedical applications, it is crucial to develop simple and efficient synthesis techniques. These methods must allow precise control over nanoparticle composition, morphology, size, and crystal structure. This study successfully demonstrates the synthesis of erbium-doped hydroxyapatite (Er(x)-HAp, where x = 0.1, 0.25, 0.5, 1.0 mol%) with optical activity using the chemical precipitation method.[13,14,15]

In the pursuit of advanced biomaterials for bone healing and regeneration, the incorporation of nickel (Ni) and strontium (Sr) into the hydroxyapatite (HAp) structure has been found to enhance critical thermal, magnetic, and dielectric properties, which are essential for mimicking natural HAp. The inclusion of Ni notably influenced nanoparticle morphology, resulting in a slight increase in agglomeration. To assess cytotoxicity and biocompatibility, human foreskin fibroblasts (HFF) were used, and after a 48-hour exposure, 50–75% of the cells remained viable. These findings indicate that Sr/Ni-HAp nanoparticles hold significant promise for applications in bone regeneration and repair. [16,17,18]

There are substantial differences between biological apatite and synthetic hydroxyapatite in terms of stoichiometry, composition, and crystallinity. Unlike biological apatite, synthetic hydroxyapatite exhibits high crystallinity, which reduces its ability to rapidly bond with bone tissue and stimulate new bone formation. Additionally, synthetic HA has a slow resorption rate, limiting its effectiveness in accelerating patient recovery. Furthermore, due to its slower bone integration and apposition rate, synthetic HA demonstrates lower reactivity within the bone environment.[19]

A study investigating the effects of yttrium doping on erbium-based hydroxyapatites found that as photon energy increased, linear absorption values decreased. The synthesized samples exhibited excellent biocompatibility, with cell viability exceeding 110%, highlighting their potential for biomedical applications.[20]

CONCLUSION

Based on our study, we conclude that while erbium-doped hydroxyapatite (Er-HAP) exhibits slightly lower cell viability compared to pure hydroxyapatite (HAP), it offers significant advantages for bone regeneration. Er-HAP demonstrates higher calcium and alkaline phosphatase content, both of which are essential for bone formation. Additionally, cell viability remains stable even at higher concentrations. Therefore, our findings suggest that Er-HAP is biocompatible and, with further research, holds promise for future applications in bone regenerative procedures.

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