Rubidium doped nano-hydroxyapatite with cytocompatibility and antibacterial

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ABSTRACT
Hydroxyapatite (HAp) is a widely used biomaterial because it is the main inorganic component of human bone and teeth. Rubidium (Rb) is a vital trace metal element in human body, and has been reported to possess antibacterial ability and can promote osteoblasts proliferation and differentiation. In this work, nanoscale hydroxyapatite particles doped with different contents of Rb (Rb-nHAp) were successfully prepared by hydrothermal method. X-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FT-IR) were used to characterize the structure and morphology of the synthesized Rb-nHAp. XRD and FT-IR results show that the powders were pure HAp with good crystal structure and no other phase or impurity. SEM and TEM observations indicate that Rb-nHAp powders exhibit a homogeneous rod-like morphology, which were 50 ~ 300 nm in size. The in vitro results show that 3% and 5% Rb-nHAp exhibited enhanced proliferation and differentiation to MG-63 cells than pure HAp at day 7 or longer. In vitro bacterial proliferation assay shows that Rb-nHAp powders exhibit higher inhibition against the both bacteria. In conclusion, doping Rb could simultaneously endow nano-hydroxyapatite (nHAp) with favorable cytocompatibility and antibacterial capacity. Rb-nHAp can be a promising material for biomedical applications.

1. Introduction
Inorganic biomaterials based on calcium orthophosphate have been widely used in tissue engineering [1–4]. It is worth to mention that hydroxyapatite (Ca_{10}(PO_{4})_{6}(OH)_{2}, HAp) is a natural construction material for bones and teeth and therefore is considered as bio-compatible, bioactive and osteoconductive [5]. HAp has been used to fill up a wide range of bone defects in orthopedic and maxillofacial surgeries and dentistry [6–8]. It also has been widely used as a coating material for metallic prostheses to improve their biological properties [9–11].

Bacterial infection after the replacement of implant is a primary reason for implant failure. The adherent bacteria on biomaterial surface can multiply, differentiate and secrete some polysaccharide matrices and cause implantation failure. The traditional treatment of inflammation around the implant includes mechanical debridement of the surface of the implant using curettes or ultrasound equipment [12], and a combination of local or systemic antibiotics and antibacterial agents. However, according to the existing evaluation data, this treatment either has limited improvement in the main clinical parameters with a clear trend of disease recurrence [13] or causes resistance to antibiotics. Therefore, many multidisciplinary research groups directed their scientific interests toward inorganic antibacterial elements for endowing biomaterials with antibacterial capacity [14,15]. Various kinds of elements such as magnesium [16–18], strontium [18,19], silicon [20], zinc [21], lithium [22] and iron [23] have been used as doping elements, which can promote the proliferation and differentiation of osteoblasts. But only limited elements have antibacterial effect, such as silver, copper and zinc. HAp structure is flexible to both cationic and anionic substitutions, which yields the possibility of functionalizing the structure and modulating the number of acid-base sites in the HAp crystal. Besides, doping element has been proved to be a successful strategy to further improve the functions of HAp.

Rubidium (Rb) is an essential trace element. In animals and plants, it exists at a certain concentration range to stimulate or accelerate growth [24]. In terms of metabolism, there may be a balance and regulation of Rb in the body [25]. Recent studies showed that the potassium channels are involved in the proliferation and differentiation of pre-osteoblasts. The differentiation and mineralization of infantile osteoblasts were reported to be enhanced when the potassium channels were inhibited by inhibitor at a relatively low concentration [26]. CsCl is a common inhibitor of potassium channels since the radius of Cs⁺ is significantly bigger than that of K⁺. Since Rb and Cesium (Cs) belong to the same main group, they are considered to have some similar physicochemical and biological
properties [27]. However, the regulatory effects of Rb on cell response and bacteria multiplication have been rarely reported. Our previous work has proved that Rb can endow the biomaterial multifunction, such as osteogenesis, angiogenesis, and most importantly, antibacterial [15,24].

This work intends to prepare and characterize the multifunctional nano-hydroxyapatite powders doped with Rb; investigate the regulatory effects of Rb on MG-63 cell response and antibacterial capacity against escherichia coli (E. coli) and staphylococcus aureus (S. aureus).

2. Materials and methods

2.1. Preparation of Rb-nHAp powders

All the reagents for synthesis including ammonium dihydrogen phosphate [(NH₄)₂ HPO₄], calcium nitrate [Ca (NO₃)₂· 4H₂O], and rubidium nitrate (RbNO₃) (Alpha Aesar) were purchased and used without further purification.

The pure hydroxyapatite ceramic powder was prepared for comparing using Ca (NO₃)₂· 4H₂O and (NH₄)₂ HPO₄ by hydrothermal method (Ca/P molar ratio = 5:3). A designed amount of ammonium dihydrogen phosphate [(NH₄)₂ HPO₄] was dissolved in deionized water to form a 0.3 mol/L solution. A designed amount of calcium nitrate tetrahydrate [Ca (NO₃)₂· 4H₂O] was also dissolved in deionized water to form a 0.5 mol/L solution. The P-containing solution was added drop-by-drop into the Ca-containing solution and stirred for 2 h by a mechanical stirrer at 60°C. The pH was constantly adjusted by diluted ammonia and kept at 9 during the reaction. After the reaction, the deposited mixtures were washed several times with absolute ethanol and deionized water. The resulting material was dried at 120°C for 12 h in an electrical air oven.

Rb-nHAp nanoparticles were prepared by setting the atomic ratio of Rb/(Rb+Ca) at 1%, 3%, 5%, 7%, 10% and (Rb+Ca)/P as 5:3. According to the proportion, the Ca (NO₃)₂· 4H₂O and RbNO₃ were dissolved in deionized water to obtain a 300 mL (Rb+Ca)-containing solution. On the other hand, the (NH₄)₂ HPO₄ was dissolved in deionized water to make a 300 mL P-containing solution. The (Rb+Ca)-containing solution stirred at 60°C for 30 min. Meanwhile, the pH of P-containing solution was adjusted to 9 by diluted ammonia and stirred continuously for 30 min. The P-containing solution was added drop-by-drop into the (Rb+Ca)-containing solution and stirred for 2 h and the pH was constantly adjusted by diluted ammonia and kept at 9 during the reaction. After the reaction, the deposited mixtures were washed several times with absolute ethanol and deionized water. The resulting material was dried at 120°C for 12 h.

2.2. Material characterizations

X-ray diffraction (XRD) was performed on a Bruker D8 Advance diffractometer, with nickel-filtered Cu Kα (λ = 1.5418 Å) radiation, and a high-efficiency one-dimensional detector (Lynx Eye type) operated in integration mode. The diffraction patterns were collected in the 2θ range 15°- 140°, with a step size of 0.02° and 34 s measuring time per step. The structure and morphology of the powder samples were observed using an FEI Quanta 250 FEG-type scanning electron microscope (SEM), operating at 25 kV in vacuum and transmission electron microscopy (TEM, Tecnai G2 20S-Twin). The specimen for TEM imaging was prepared from the particle suspension in deionized water. A drop of well-dispersed supernatant was placed on a carbon-coated 200 mesh copper grid, followed by drying the sample at ambient conditions before it is attached to the sample holder on the microscope. The functional groups present in the prepared powders were identified by Fourier transform infrared spectroscopy (FT-IR, Nicolet NEXUS-670). For this, 1% of the powder was mixed and ground with 99% KBr. Tablets of 10 mm diameter for FT-IR measurements were prepared by pressing the powder mixture at a load of 5 tons for 2 min and the spectrum was taken in the range of 400–4000 cm⁻¹ with resolution 4 and 128 times scanning. VG ESCA 3 MK II XPS installation (Eᵦₒ = 1486.7 eV) was used to study the elemental ratios in the surface region of Rb-nHAp powders.

2.3. Ion release

The samples were soaked in simulated body fluid (SBF) at 37°C, and the ratio of the samples to solution was 1 g to 10 mL. The concentration of Rb⁺ in different solutions was analyzed by inductively coupled plasma atomic emission spectrometer (ICP-AES, IRZS Advantage 1000) after 24 h. The pH of obtained immersion solution was tested three times.

2.4. Cell culture

MG-63 cells (Human osteosarcoma cells, purchased from China Infrastructure of Cell Line Sources, China) were used as cell models in the current work. The cells were maintained in normal culture medium consisting of minimum Eagle’s medium Alpha (MEM-Alpha) modified culture medium (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin under standard culture condition (37°C, 5% CO₂ and 100% humidity). The media was refreshed every 48 h. The powder samples were disinfected in an autoclave at 120°C for 30 min and irradiated by ultraviolet light for 20 min.
2.5. Cell proliferation

Cell proliferation was performed by Cell Counting Kit-8 (CCK-8; Dojindo, Japan) tests. The leaching solution was prepared with Rb-nHAp powders in the culture medium for 48 h under standard culture condition and kept the concentration at 100 μg/mL. Each culture plate hole was seeded with $5 \times 10^5$ MG-63 cells and cultured in a different Rb leaching solution. After 1, 3, 5 and 7 days, a 10 vol.% CCK-8 mixed with normal medium was added into each well and incubated for 1 h at 37°C. The optical density (O.D.) of the solution was measured by a microplate reader (Biorad, USA) at a wavelength of 450 nm. The experiments were carried out in triplicate.

2.6. Cell differentiation

The ALP activity, collagen synthesis, and calcium deposition are osteogenic markers which correspond to the early, middle and late stages of osteoblast differentiation, respectively. Under alkaline conditions, the protein reduces Cu$^{2+}$ to Cu$^{+}$. Cu$^{+}$ forms a purple complex with the BCA reagent, and two molecules of BCA chelate a Cu$^{+}$. By comparing the absorbance value of the water-soluble complex at 570 nm with the standard curve, the concentration of the protein to be tested can be calculated. Each culture plate hole was seeded with $5 \times 10^5$ MG-63 cells and cultured in a different Rb leaching solution. After 7 and 14 days, the ALP activity of the cells was tested using an ALP-testing kit (Beyotime Biotechnology of Shanghai, China) and then normalized protein content of samples compared to the total protein solution were measured by a BCA Protein Assay Kit (Beyotime Biotechnology of Shanghai, China). The process of osteogenesis induction was to deposit calcium ions on the cell surface in the form of calcium salts known as “calcium nodules”. The complex of alizarin red can be used to identify the successful transformation to osteoblasts. Under the same cultured condition, the Alizarin Red staining test was done using an ARS-testing kit (Beyotime Biotechnology of Shanghai, China).

2.7. In vitro antibacterial capacity

E. coli and S. aureus (ATCC 8739, ATCC 6538) were used to test the antibacterial capacity of the specimens. Luria-Bertani (LB) broth was prepared by dissolving 10 g tryptone, 5 g yeast extract, 10 g sodium chloride (NaCl) and 20 g solid medium plus agar powder in 1000 ml distilled water. The pH was adjusted to 7.2 using 5 mol/L NaOH. Finally, the LB broth was sterilized at 121°C for 30 min. The bacteria were cultured at 37°C, washed with 10 mmol/L phosphate buffer saline twice and adjusted to a concentration of $2 \times 10^8$ CFU/ml (colony-forming units). The samples were transferred into a 48-well plate, and then 0.2 ml of the bacterial suspension was added. Thereafter, the bacteria-added specimens were further incubated in an incubator with 37°C, 5% CO$_2$ and 90% humidity for 24 h, respectively. After culture, the specimens were carefully washed by 2 ml sterilized physiological saline solution. The bacteria-containing physiological saline solution was then diluted 100 times. After that, 0.2 ml of the diluted solution was inoculated onto the LB plates and cultured at 37°C for 24 h under a humidity of 90%. Thereafter, the number of the bacterial colony on the LB plates was counted. The antibacterial rate K was calculated by the following formula: $K = (C_{\text{Control}} - C_{\text{Sample}})/C_{\text{Control}} \times 100\%$. Here, $C_{\text{Control}}$ and $C_{\text{Sample}}$ are the numbers of the bacterial colony for pure HAp group and Rb-nHAp groups, respectively.

2.8. Statistical analysis

Analysis was performed using SPSS 25.0. All quantitative data were expressed as the mean ± standard deviation. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by post-hoc comparison using the least significant difference (LSD) method. All assays of in vitro study were repeated three times and p < 0.05 was considered statistically significant. (*p < 0.05, **p < 0.01, ***p < 0.001.)

3. Results and discussions

3.1. Material characterizations

The XRD patterns, presented in Figure 1, indicate the characteristic peaks of hydroxyapatite for each sample and no other crystalline phases are detected beside this phase according to ICDD-PDF NO. 09–0432. It also demonstrates that nHAp and Rb-nHAp powders made by hydrothermal method exhibit the apatite characteristics with good crystal structure. The overall diffraction pattern of the Rb-nHAp groups shifts slightly to the left. This is because the doping of Rb causes the original HAp unit cell to expand.

In order to get the microstructural characteristics (crystallite size and volume), the whole powder patterns atomic structural was refined by the Rietveld method for Rb-nHAp groups. The results are shown in Table 1. For the pure nHAp, the length of the average crystallite (the average column size parallel to the c-axis) is around 0.9389 nm, the width (the average column size perpendicular to the c-axis) is around 0.6865 nm and the mean volume is 0.5241 nm$^3$. For Rb-nHAp, with the increase of Rb content, the length gradually increases from 0.6872 nm to 0.6879 nm, and the width increases from 0.9401 nm to 0.9425 nm. Therefore, the volume gradually increases from 0.5261 nm$^3$ to 0.5290 nm$^3$. The reason is that the
radius of Rb is larger than that of Ca, so the incorporation of Rb causes the expansion of the original unit cell slightly, which is similar to the results of doping Ag into HAp [28].

FT-IR spectroscopy was performed to investigate the functional groups present in pure nHAp and Rb-nHAp samples obtained by hydrothermal method, as shown in Figure 2. These data clearly reveal that the presence of the various vibrational modes corresponding to phosphates and hydroxyl groups. For all the samples, the presence of strong OH⁻ vibration peak at 3573 cm⁻¹ could be noticed. The broad bands in the regions 1600–1700 and 3200–3600 cm⁻¹ correspond to H-O-H bands of lattice water [29–31]. The large bands at 3200–3600 cm⁻¹ which were attributed to adsorbed water diminished for Rb-nHAp samples. The changes are attributed to the substitution of Rb⁺ from Ca²⁺ into the lattice of apatite. Band characteristics of the phosphate and hydrogen phosphate groups in apatite environment were observed: 565, 603, 634, 962, and 1000–1100 cm⁻¹ for the PO₄³⁻ groups [32] and at 875 cm⁻¹ for the HPO₄²⁻ ions [33]. Moreover, it

Table 1. The microstructural characteristics of Rb-nHAp.

| Sample       | a (b, nm) | c (nm) | α (β, °) | γ (°) | Volume (nm³) | Density (g/cm³) |
|--------------|-----------|--------|----------|-------|--------------|-----------------|
| HAp          | 0.9389    | 0.6865 | 90       | 120.0 | 0.5241       | 3.1830          |
| 1% Rb-nHAp   | 0.9401    | 0.6872 | 90       | 120.0 | 0.5261       | 3.1707          |
| 3% Rb-nHAp   | 0.9402    | 0.6874 | 90       | 119.9 | 0.5265       | 3.1680          |
| 5% Rb-nHAp   | 0.9405    | 0.6877 | 90       | 120.0 | 0.5268       | 3.1665          |
| 7% Rb-nHAp   | 0.9414    | 0.6877 | 90       | 120.0 | 0.5275       | 3.1624          |
| 10% Rb-nHAp  | 0.9425    | 0.6879 | 90       | 120.0 | 0.5290       | 3.1532          |

Figure 1. XRD patterns of Rb-nHAp with different Rb content.

Figure 2. Transmittance infrared spectra of pure nHAp and Rb-nHAp samples.
should be noted that the HPO$_4^{2-}$ band is present in all the spectra but for high values of Rb-nHAp atomic ratio, the band diminished. The small -COO- band was presented in the spectra of all samples at 1460 cm$^{-1}$ [33]. It shows that during the sample preparation process, the trace CO$_2$ dissolved in the reaction solution may have participated in the reaction. The bands at 1636 cm$^{-1}$ of Rb-nHAp show the acromial and bimodal peaks shifted to the right due to interaction between -OH and Rb [34]. In general, because elemental Rb has no infrared absorption, the infrared spectra of Rb-nHAp synthesized with different Rb content are not significantly different from those of nHAp.

Bands observed in the FT-IR spectroscopies are characteristic of crystallized apatite phase. These results agree with the XRD patterns, evidencing the crystallized apatite phase and the apatite phase is the only one detected.

XPS technique has been tested as a useful tool for qualitatively determining the surface components and composition of the samples. Figure 3(a) shows the survey XPS general scan spectra of the selected 10% Rb-HAp powder and XPS narrow scan spectra of Rb element. In the XPS spectrum of 10%Rb-nHAp (Figure 3(a)), the binding energy of Ca (2p$_{3/2}$, 347.3 eV) and Ca (2p$_{1/2}$, 350.7 eV), O (1s, 532.1 eV), and P (2p, 133.09 eV) can obviously be found. The peaks of Rb (3d, 110.5 eV and 3d$_{5/2}$, 111.3 eV) agree well with the literature [35]. XPS narrow scan spectra of Ca, P and Rb element in Rb-nHAp with different contents of Rb are presented in Figure 3(b), (c) and (d). The chemical states of relevant elements in Rb-nHAp groups were further analyzed to illustrate the chemical composition. For Rb-nHAp groups, peaks positioned at 347.3 eV for Ca 2p$_{3/2}$ and 350.7 eV for Ca 2p$_{1/2}$ were assigned to Ca in Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ and CaO. The P$_{2p}$ peaks consistently showed the presence of P in CaHPO$_4$(133.1 eV) and Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ (133.2/133.3 eV). Three peaks were noticed for Rb, indicating the existence of Rb. The peak positioned at 110.5 eV was assigned the characteristic peak of Rb$_2$PO$_4$. The others located at 111.3 and 111.8 eV were corresponding to Rb in Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ [36,37].

In addition, as the content of Rb increases, the characteristic peak of Rb shifts slightly to the right. This is because the deviation of the characteristic peak is related to the electron density of the outer layer of the element. When the Rb combines with O, the electronegativity of the O is greater, which causes the lone electron pair on the Rb to shift toward the direction of O. The decrease in the electron density of Rb will increase the binding ability of Rb atoms to the extranuclear electrons. The greater the amount of Rb being doped, the more the binding energy will

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**Figure 3.** XPS general spectrum of 10%Rb-nHAp powder (a). XPS narrow scan spectra for Ca (b), P(c) and Rb(d) of Rb-nHAp powders with different contents of Rb.
increase and the greater the deviation will become [38]. XPS results provide the additional evidence for the successful doping of Rb⁺ in Rb-nHAp.

SEM images of pure HAp and Rb-nHAp are shown in Figure 4. The results suggest that the doping Rb⁺ has little influence on the morphology of the HAp. The series samples show a typical elliptic-cylinder shape. Rb-nHAp powders are longer than pure HAp, which is consistent with the c-axis variable length of the unit cell after HAp doped with Rb. The morphology identifications indicated that the nanoparticles with good crystal structure could be made by hydrothermal method.

The XRD and XPS results confirmed that the Rb-doped nano-hydroxyapatite powder was successfully prepared. During the process of hydrothermal treatment, nHAp and Rb-nHAp can be formed by the following actions ($2x = 0, 0.01, 0.03, 0.05, 0.07, 0.10$):

$$
10\text{Ca(NO}_3)_2 + 6(\text{NH}_4)_2\text{HPO}_4 + 8\text{NH}_3\cdot\text{H}_2\text{O} \rightarrow \text{Ca}_{10}\text{(PO}_4)_6\text{(OH)}_2 + 20\text{NH}_4\text{NO}_3 + 6\text{H}_2\text{O} (1)
$$

**Figure 4.** SEM images of Rb-nHAp with different Rb content: (a) pure nHAp, (b)1%Rb-nHAp, (c)3%Rb-nHAp, (d)5%Rb-nHAp, (e)7% Rb-nHAp, (f)10%Rb-nHAp.

**Figure 5.** TEM micrographs of Rb-nHAp with different Rb content: (a) pure nHAp, (b)1%Rb-nHAp, (c)3%Rb-nHAp, (d)5%Rb-nHAp, (e)7% Rb-nHAp, (f)10%Rb-nHAp.
3.2. Cell proliferation and differentiation

Figure 7(a) shows the CCK-8 assay results about the viability and proliferation of MG-63 cells cultured in various sample leaching solution for 1, 3, 5 and 7 days. For day 1 and 3, the viability of cells followed the trend generally: control>nHAp>1% Rb-nHAp>3% Rb-nHAp>5% Rb-nHAp>7% Rb-nHAp>10% Rb-nHAp, showing that increasing the Rb content inhibited the cytocompatibility of materials for short term. However, with longer durations, the viability of MG-63 cells got significant growth on various leaching solution, especially for 3%Rb-nHAp at 5 and 7 days. But MG-63 cells cultured in 10%Rb-nHAp were lower than that of control and pure nHAp groups during the whole period. The result indicated that compared to control and pure nHAp groups, Rb⁺ at an appropriate concentration range can promote the proliferation of MG-63 cells. There is a limit of Rb content under which it will have a positive effect on the cell proliferation. However, if Rb addition is overdose, like 10% Rb in this work, it will inhibit the cell proliferation.

Figure 7(b) shows the ALP activity of MG-63 cells cultured on various sample leaching solution for 7 and 14 days. The ALP activity can imply the differentiation of osteoblasts at early stage. It is commonly defined as the amount of phenol catalyzed by 1 mg of proteins per minute at 37°C (U/mg protein). Little significant differences are found between the different groups for 7 days. For 14 days, compared to control and pure nHAp groups, Rb⁺ at 1%–5% range exhibit stimulatory effect on the differentiation of MG-63 cells. However, 7% and 10% Rb-nHAp has a negative impact on the ALP activity compared with pure nHAp group after 14 days. The results indicate that the Rb doping in appropriate concentration range could influence the early differentiation of MG-63 cells to some extent.

Figure 7(c) shows the BCA protein simulation function of MG-63 cells cultured on various samples leaching solution for 7 days, which was compared to the actual measured BCA standard solution concentration. According to the simulation function Y = 0.9735X + 0.0046, the protein concentrations of the control group, 0, 1%, 3%, 5%, 7%, and 10% Rb-nHAp are 0.1275, 0.1238, 0.1356, 0.1277, 0.1228, 0.1269, and 0.1300 g/L, respectively, after removing the background values. Therefore, after cell disruption, 3% and 5% of Rb-nHAp have close to or even exceed the protein content of the control group without inhibiting cell proliferation. This is also consistent with the ALP assay results shown in Figure 7(b).

Calcium ions play a vital role in the process of bone remodeling. Low extracellular calcium ions (2–4 mmol/L) can activate the intracellular mechanism and increase the expression of insulin growth factor by affecting the calcium-sensitive receptors of osteoblasts. It is suitable for the survival and proliferation of osteoblasts. A moderate concentration of calcium ions (6–8 mmol/L) can promote osteogenic differentiation. When the concentration of calcium ions is higher than 10 mmol/L, it will be toxic to cells [39–41]. In addition, extracellular calcium ions can promote the release of glutamate from osteoblasts, and the glutamine signaling pathway plays an important role in bone mechanical sensitivity [42]. In order to determine whether the ion exchange of nHAp can replace calcium ions in the solution, the calcium ions in the buffer were quantitatively analyzed. It was found that both nHAp and each group of Rb-nHAp had calcium ions sustained release, and the release amount was not statistically different shows that nHAp is not a source of free calcium ions in neutral solution. However, in the liquid cell culture medium, the powder itself absorbs water and swells and occurs structure degradation, and the liquid impact force of replacing the culture medium accelerates the degradation rate, which may
eventually cause the nHAp particles to directly contact the cells with the extraction liquid. These particles may be absorbed and degraded by cells through endocytosis or non-endocytosis, and release calcium ions, thereby regulating the intracellular calcium ion concentration or further regulating the osteogenic activity of cells [43]. It remains to be verified whether these nHAp particles will anchor to the contacting cells and then produce osteoinductive effects. The in vitro mineralization of MG-63 cells cultured in various Rb-nHAp leaching solution for 14 days are evaluated (figure 7(d)). The calcium deposition is commonly regarded as a marker for the late stage of osteoblast differentiation. The results showed that pure nHAp and Rb-nHAp promoted the in vitro mineralization compared to control group. Figure 7(e) further shows that the staining of MG-63 cells after 14 days, each group had different degrees of calcium salt deposition. Among them, the staining degree of Rb-nHAp groups was significantly higher than that of the control group,
indicating that the extracellular calcium salt deposition was much higher than that of the control group. It means that the mineralization degree of extracellular matrix was significantly higher than that of the control group. Therefore, nHAp and Rb-nHAp can promote the MG-63 cell mineralization by releasing Ca^{2+}.

### 3.3. *In vitro* antibacterial activity

E. coli and S. aureus are common disease-causing bacteria that are used as experimental models to test the antibacterial performance of biomaterials. Figure 8(a) shows the growth of E. coli incubated on the different Rb-nHAp groups for 24 h and Figure 8(c1) shows the calculated antibacterial rates of different samples for 24 h. A large number of bacteria were found for control and pure nHAp groups. However, less bacteria were found for the Rb-doped groups. Figure 8(b) shows the growth of S. aureus incubated on the different Rb-nHAp groups for 24 h and Figure 8(c2) shows the calculated antibacterial rates of different specimens for 24 h. For E. coli, the antibacterial rates of nHAp, 1%Rb-nHAp, 3%Rb-nHAp, 5%Rb-nHAp, 7%Rb-nHAp, 10% Rb-nHAp were 7.8%, 42.3%, 57.2%, 65.9%, 72.7% and 78.6%, respectively. For S. aureus the antibacterial rates were 3.8%, 47.1%, 63.5%, 70.6%, 78.5% and 83.9% for nHAp, 1%Rb-nHAp, 3%Rb-nHAp, 5%Rb-nHAp, 7% Rb-nHAp, 10%Rb-nHAp, respectively. No significant antibacterial capacity was found for pure HAp group, while the Rb-doped HAp groups exhibited excellent antibacterial capacity.

The ion channels widely exist not only in mammalian cells but also in bacteria. The communication of bacterial communities is also dependent on ion channels [44]. The growth and metabolism of bacteria can induce the release of potassium from bacteria, causing other bacteria to depolarize, thereby affecting the growth and metabolism of other bacteria [45]. Potassium channels are one group of the ion channels that control the formation of biofilm. Bacteria use potassium ion-channel-mediated electrical signals to coordinate metabolism within the biofilm [46]. Here, we speculated that Rb\(^+\) may possess antibacterial capacity by inhibiting the potassium channels of bacteria. In addition, Rb\(^+\) has a positive charge, while the surface of the bacteria is negatively charged. It is inferred that Rb\(^+\) could be adsorbed on bacteria surface and subsequently lead to damaged cell wall of the bacteria, thus achieving the antibacterial effect.

### 4. Conclusions

In this article, Rb-doped nHAp nano-powders were successfully prepared by a simple and low-cost hydrothermal method. Rb\(^+\) partially substitutes for calcium and enters the structure of hydroxyapatite. *In vitro* assay showed that Rb-nHAp powders exhibited excellent cytocompatibility in appropriate
concentration range and could influence the early differentiation of MG-63 cells to some extent. When the content of Rb was 3%, Rb-nHA-p was optimal to promote proliferation and ALP activity of MG-63 cells. In vitro bacterial adhesion, study showed a significantly reduced number of E. coli and S. aureus on different Rb-nHAp powders, which indicate that the Rb-nHAp powders show strong antibacterial activity. In conclusion, we used a highly facile and simple hydrothermal method for preparing rubidium-doped hydroxyapatite nano-powder, which could simultaneously endow materials with favorable cytocompatibility and anti-bacterial capacity.

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