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In vitro degradation and biocompatibility of Mg-Nd-Zn-Zr alloy

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In this study, *in vitro* degradation and biocompatibility of Mg-Nd-Zn-Zr (NZK) alloy were investigated to determine its suitability as a degradable medical biomaterial. Its corrosion properties were evaluated by static immersion test, electrochemical corrosion test, scanning electron microscopy (SEM), and energy dispersive spectroscopic (EDS) analysis, and *in vitro* biocompatibilities were assessed by hemolysis and cytotoxicity tests. Pure magnesium was used as control. The results of static immersion test and electrochemical corrosion test in simulated body fluid (SBF) demonstrated that the addition of alloying elements could improve the corrosion resistance. The hemolysis test found that the hemolysis rate of calcium phosphate coated NZK alloy was 4.8%, which was lower than the safe value of 5%. The cytotoxicity test indicated that NZK alloy extracts did not significantly reduce MC_3T_3-E_1 cell viability. Hemolysis test and cytotoxicity test display excellent hemocompatibility and cytocompatibility of NZK alloy *in vitro*. Our data indicate that NZK alloy has excellent biocompatibility and thus can be considered as a potential degradable medical biomaterial for orthopedic applications.

magnesium alloy, degradation, biocompatibility, *in vitro*

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Metallic materials have been widely used as bone implant materials for many years, and they are more suitable for load bearing applications because of their high mechanical strength, fracture toughness and plasticity. However, the commonly used metallic materials, including titanium alloys, stainless steels and cobalt based alloys will cause some problems such as stress shielding effects which lead to decreased bone strength and delay in bone healing, and release of toxic ions or particles which can result in chronic inflammation and bone dissolution [1–4]. Moreover, the metallic bone implants are permanent and most of them need to be taken out via a second operation, which not only raises the costs, but also brings unwanted suffering to patients. Degradable biomaterials can solve these problems, so they are much desired for orthopedic applications.

Magnesium alloys are easily corroded in physiological environments, and have become a promising degradable medical biomaterial, attracting much attention in recent years [5–8] not only because of their degradability, but also their good biocompatibility, low density, and suitable mechanical properties such as high specific strength and elastic modulus approximating that of human bone [9–11]. Previous studies had shown that screws and plates made of magnesium alloy corroded too quickly because of impurities in the alloy, which may have resulted in abandoning magnesium alloys for medical biomaterials [12,13]. In recent years, along with development in metallurgy, magnesium alloys have gained renewed interest as appropriate degradable biomaterials. Up until now, many different kinds of magnesium alloy materials, such as bulk materials [8–11], scaffolds [14,15], and composites [16–18], have been investi-

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gated as potential implant materials. Unfortunately, fast degradation of magnesium alloys via corrosion in the human environment may limit their clinical applications [1,2]. On the one hand, for magnesium alloys to be used as viable implant materials, their degradation rates should not exceed the healing rate of the affected tissue. They should remain present in the body and maintain their mechanical integrity until the affected tissue has healed. On the other hand, the toxicity and biocompatibility of magnesium alloys must be taken into consideration during the magnesium alloy design and selection of main alloying elements. To gain better corrosion resistance and biocompatibility than that of commercial magnesium alloys used industrially, a new, patented magnesium alloy, Mg-Nd-Zn-Zr (NZK) alloy, has been designed in our laboratory [19]. To our knowledge, NZK alloy has not been systematically studied as degradable biomaterials *in vitro* or *in vivo*. The aim of the present study was to characterize the *in vitro* degradation behavior and biocompatibility of NZK alloy. It is expected that this work may help to confirm the medical safety of NZK alloy and obtain a new promising degradable magnesium alloy.

1 Materials and methods

1.1 Materials

NZK alloy and pure magnesium were obtained from the National Engineering Research Center of Light Alloy Net Forming, Shanghai Jiao Tong University. The composition of the alloy is listed in Table 1 [19]; note the low impurity content of the alloy.

The alloy was formed into discs, 12 mm in diameter and 3 mm in height; the discs were ground with Al_2O_3 abrasive paper up to 1000 grits, polished with 1 μm diamond abrasive paste, followed by ultrasound washing in ethanol for 10 min and sterilizing with ethylene oxide for 12 h.

Simulated body fluid (SBF) was used as the degradation medium. Its composition was: NaCl 6.800 g/L, $CaCl_2$ 0.200 g/L, KCl 0.400 g/L, $MgSO_4$ 0.100 g/L, $NaHCO_3$ 2.200 g/L, Na_2HPO_4 0.126 g/L and NaH_2PO_4 0.026 g/L [20]. The pH value of SBF was adjusted to 7.4±0.2 with NaOH and HCl solutions before experiments.

The New Zealand white rabbits, clean grade and weighing 2.5±0.3 kg were supplied by the Shanghai Jiesijie Lab. Animal Co., Ltd., China [license No. SCXK (hu) 2010-0026].

MC_3T_3-E_1 cells were provided by the Chinese Academy of Sciences Type Culture Collection (China).

1.2 Microstructures of NZK alloy

Microstructures of NZK alloy and pure magnesium were characterized using scanning electron microscopy (SEM, Hitachi S-4800, Japan) equipped with an energy disperse spectrometry (EDS) system.

1.3 Static immersion test

The immersion tests were carried out at 37°C in a thermostatic bath. After 3, 7 and 30 d static immersion, the samples were taken out from the SBF, gently rinsed with deionized water, cleaned with ethanol, and dried at room temperature. Then the surface morphologies before and after immersion were characterized by SEM. At the end, the samples were cleaned with 200 g/L chromic acid to remove the corrosion products, and the degradation rates were obtained according to the weight loss method [21]:

$$\text{Corrosion rate} = \left(K \times W\right) \Big/ \left(A \times T \times D\right), \tag{1}$$

where the coefficient K=8.76×10^4, W is the weight loss (g), A is the sample area exposed to solution (cm^2), T is the exposure time (h), and D is the density of the material (g/cm^3).

1.4 Electrochemical test

The electrochemical tests were carried out at room temperature in SBF on an electrochemical workstation (PARSTAT-®2273, Ametek). A standard three-electrode system was used: a saturated calomel electrode (SCE) as reference, a platinum electrode as the counter and the samples (NZK alloy and pure magnesium) as the working electrodes. The electrochemical polarization was performed at a scanning rate of 1 mV/s. An average of three measurements was taken for each group.

1.5 Hemolysis test

To evaluate the hemocompatibility of calcium phosphate coated NZK alloy, according to the ISO standard [22], 5 g samples were soaked in 10 mL normal physiological saline in a tube to give the test group. The negative and positive control groups were 10 mL normal physiological saline and

Table 1 Chemical composition of the NZK alloy

Materials	Chemical composition (wt.%)								
	Mg	Nd	Zn	Zr	Si	Ni	Cu	Fe	Al
NZK alloy	balance	2.5	0.2	0.5	0.0016	0.0025	0.0195	0.1038	–
Pure magnesium	⩾99.99	–	–	–	⩽0.0016	⩽0.0001	⩽0.0004	⩽0.0014	⩽0.0005

10 mL distilled water respectively. All tubes were put into a 37°C thermostatted water bath case for 30 min. The 8 mL arterial blood from a healthy New Zealand white rabbit that contained 0.5 mL potassium oxalate (20 g/L) anticoagulant was diluted by 10 mL normal physiological saline. After that 0.2 mL diluted blood was added into the tube and kept at 37°C for 60 min. Then the tube was centrifuged at 1500 r/min for 5 min. Finally, the optical density (OD) was obtained using aspectrophotometer (722S, Shanghai Precise Science Instrument Company, China) at 545 nm wavelength. Six paralleled samples were laid in each group. The mean value of the six samples was taken as the group OD value.

The hemolysis ratio (HR) was expressed as a percentage and was calculated according to the equation:

$$\mathrm{HR}=\left[\left(\mathrm{OD_t}-\mathrm{OD_n}\right)/\left(\mathrm{OD_p}-\mathrm{OD_n}\right)\right]\times 100\%. \quad (2)$$

The OD_t means the OD value of the tested group. OD_n and OD_p are the OD values of the negative and positive control groups respectively. According to the ISO standard [22], the OD value of the negative control group should be less than 0.03 and the OD value of the positive control group should be 0.8±0.3. The test material was considered to be hemolytic if the hemolysis rate was greater than 5%.

1.6 Cytotoxicity test

MC_3T_3-E_1 cells were chosen to assess the cytotoxicity of NZK alloy. The cytotoxicity test was carried out by indirect contact, where the cells were cultured in extracts from the NZK alloy.

(1) Preparation of extracts. Extracts were prepared using α-MEM cell culture medium as the extraction medium, and with a ratio of surface area of samples to volume of extraction medium of 1.25 cm^2/mL in a humidified incubator at 95% relative humidity and 5% CO_2 at 37°C for 24 h [23,24]. After that, diluted extracts of 10% and 50% concentration were prepared by dilution with fresh α-MEM medium. Simple α-MEM medium was chosen as the negative control and α-MEM medium containing 0.64% phenol was the positive control.

(2) Cell culture. MC_3T_3-E_1 cells were cultured in α-MEM medium supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator at 95% relative humidity and 5% CO_2 at 37°C. The growth medium was changed every 3 d. When adherent cells reached 80% confluence, they were passaged.

(3) Cell morphology. MC_3T_3-E_1 cells were incubated in 96-well cell culture plates (Costar, USA) at 5×10^4 cells/mL in each well. There were six wells per group and cells were incubated for 24 h to allow attachment. The medium was then replaced with 100 μL of either 10%, 50% or 100% extract. After incubating the cells in a humidified atmosphere with 5% CO_2 at 37°C for 1, 4 and 7 d, the cell morphology was evaluated under an inverted phase contrast microscope (Olympus IX 71, Japan).

(4) MTT assay. The cytotoxicity was assessed by the MTT test according to the ISO standard [23]. MTT (Sigma, USA) was prepared as 5 g/L in phosphate buffered saline (PBS) at 37°C just before use. After 1, 4 and 7 d culture, 20 μL MTT was added to each well and incubated at 37°C for 4 h in dark. After incubation, the MTT was aspirated, and 150 μL dimethyl sulfoxide (DMSO) (Sigma, USA) was added to each well, and the plates were shaken for 15 min. The optical density (OD) at 490 nm was measured with a spectrophotometer (Wellscan MK3, Labsystem, Finland). The cell relative growth rate (RGR) was calculated according to the following equation:

$$\mathrm{RGR}(\%)=\left(\mathrm{OD_t}/\mathrm{OD_n}\right)\times 100\%, \quad (3)$$

where OD_t is the OD value of the tested group, and OD_n is the OD value of the negative group.

1.7 Statistic analysis

Statistical analysis was conducted with the software SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) to evaluate the differences in each group; all data were expressed as mean ± SD. The experimental values were analyzed using the Student's *t*-test. The statistical significance was defined as P below 0.05.

2 Results

2.1 Microstructure of NZK

Figure 1(a) demonstrates a typical optical microstructure for the NZK alloy. As shown in Figure 1(a), the grain boundaries are barely recognizable due to the more homogeneous microstructure of NZK alloy. Therefore, alloying can remarkably change the microstructure of NZK alloy, which could cause different degradation behavior.

The SEM micrograph in Figure 1(b), showing the microstructure of NZK alloy, illustrates the uniform distribution of all particulates within the NZK alloy.

2.2 Static immersion test

The degradation rates of NZK alloy and pure magnesium after 3, 7 and 30 d static immersion in SBF are shown in Figure 2. It is found that NZK alloy degraded slower than pure magnesium at each time point. As shown in Figure 1(c), it is obvious that there is a corrosion layer on the surface of NZK alloy after 30 d immersion in SBF. To determine the elemental compositions of the particles formed on the surface of NZK alloy during the static immersion test, EDS analysis was performed, which revealed that these particles were mainly composed of oxygen, carbon, sodium,

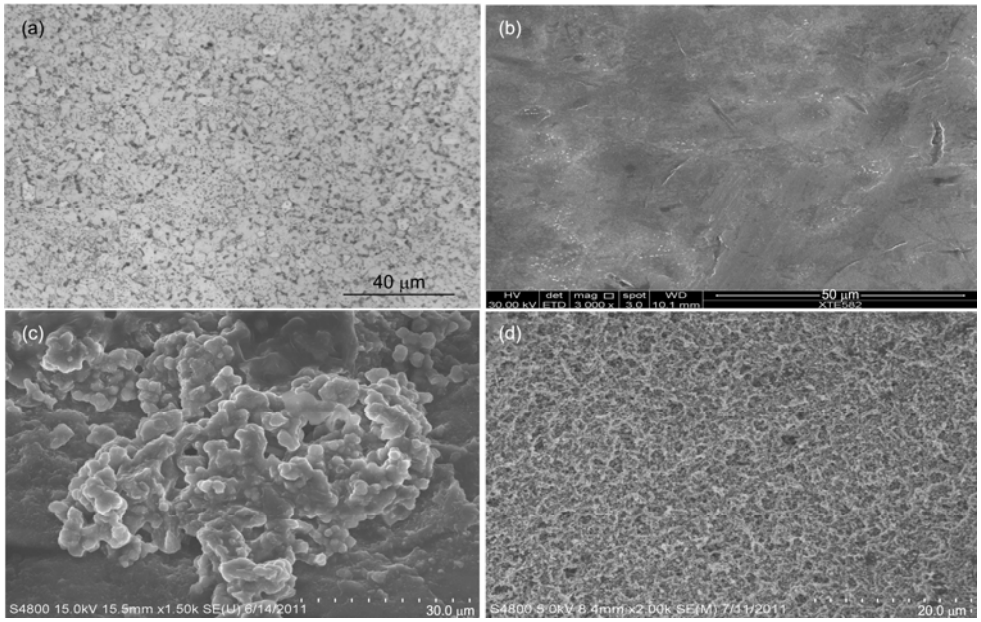

Figure 1 Microstructure and degradation behaviour of NZK alloy. (a) Optical micrograph of NZK alloy; (b) SEM micrographs of NZK alloy; (c) the surface of the NZK alloy was rough with a layer of off-white degradation products after 30 d immersion in SBF; (d) corrosion morphologies of NZK alloy after removing degradation products using chromic acid.

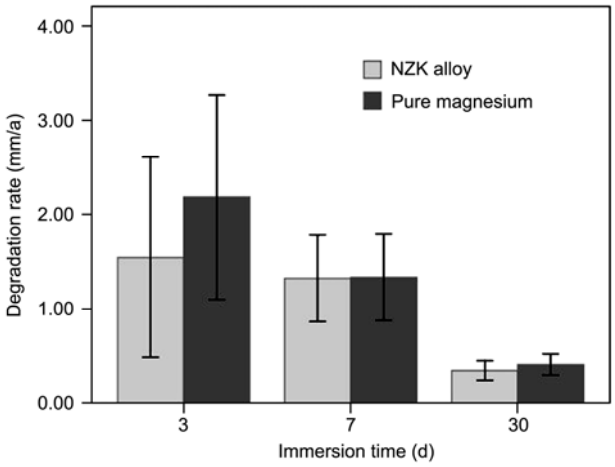

Figure 2 *In vitro* degradation rate of NZK alloy and pure magnesium after 3, 7 and 30 d immersion in SBF.

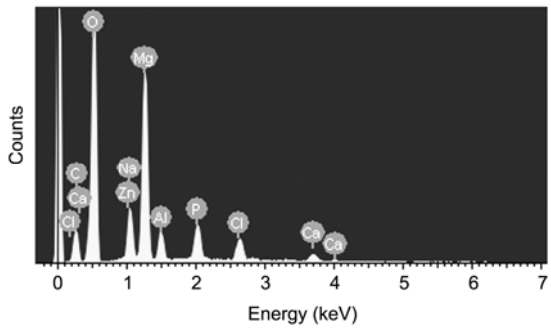

Figure 3 EDS of the degradation products on the surface of NZK alloy, as seen in Figure 1(c).

Table 2 *In vitro* degradation rates of NZK alloy (mm/a)

	3 d	7 d	30 d
NZK alloy	1.54±0.53	1.32±0.23	0.34±0.05
Pure magnesium	2.50±0.54	1.33±0.23	0.40±0.06

magnesium, calcium, phosphate and chlorine (Figure 3). After the corrosion products were removed by 200 g/L chromic acid, corrosion pits can be clearly seen on the surface of NZK alloy (Figure 1(d)). However, the corrosion pits on the surface of NZK alloy were smaller and more even than those on the surface of pure magnesium. This shows that the degradation modes of the NZK alloy investigated in the present study and pure magnesium are distinctly different.

It should be noted that the degradation rate of NZK alloy after 30 d immersion is lower than that after 3 d (Table 2), owing to the corrosion layer formed on the surface of the NZK alloy.

2.3 Electrochemical test

The typical electrochemical polarization curves for the NZK alloy and pure magnesium are shown in Figure 4; the corrosion potential of NZK alloy is nobler than that of pure magnesium, indicating that the NZK alloy is less susceptible to corrosion than pure magnesium. The breakdown potential value of the NZK alloy is greater than that of pure magnesium, and the corrosion current of the NZK alloy is lower than that of pure magnesium, suggesting that the

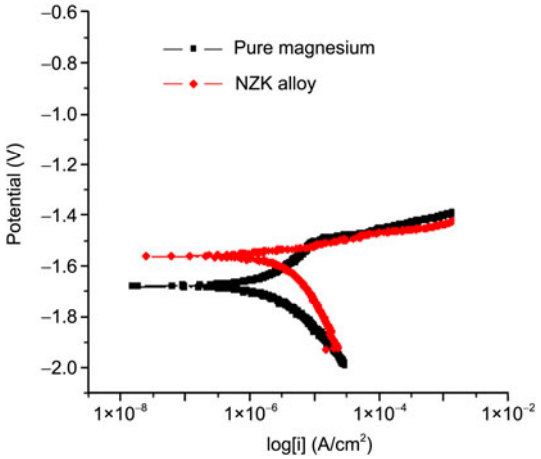

Figure 4 Potentiodynamic polarization curves of NZK alloy and pure magnesium immersed in SBF.

addition of alloying elements can also increase the breakdown potential value and reduce the corrosion current. The electrochemical test results confirm that the NZK alloy has better corrosion resistance than pure magnesium, which is in agreement with the static immersion test results.

2.4 Hemolysis test of NZK

As shown in Table 3, the hemolysis rate of calcium phosphate coated NZK alloy extract is 4.8%, which is below 5%. This means that the calcium phosphate coated NZK alloy will not lead to severe hemolysis according to the ISO standard [22]. Therefore, it is suggested that *in vitro* degradation of calcium phosphate coated NZK alloy has no significant destructive effect on erythrocytes, and thus the alloy displays excellent hemocompatibility *in vitro*.

2.5 Cytotoxicity of NZK

(1) Cell culture and cell morphology. MC_3T_3-E_1 cells were cultivated in different extracts, and cell morphologies were observed prior to MTT assay at 1, 4 and 7 d culture under an inverted phase contrast microscope. Figure 5 shows the cell morphologies cultured in 10%, 50% and 100% extraction medium for 1 and 7 d. MC_3T_3-E_1 cells had low density and were mostly well attached to the bottom of the culture plate at 1 d. The outline of cells was clear with healthy flattened spindle shape and had a normal ratio of nucleus to cytoplasm. The cells proliferated rapidly and had normal morphologies at 4 d. The number of cells increased significantly and grew into conglobation at 7 d. A few aging cells were observed with vacuoles or particulate matters in their cytoplasm. The morphologies of cells in different test groups had no obvious difference compared with the negative control group.

Table 3 Results of hemolysis test for the calcium phosphate coated NZK alloy (n=6)

Group	OD value (mean±SD)	Hemolysis (%)
Test groups	0.054±0.0020	4.8
Negative groups	0.018±0.0024	0
Positive groups	0.767±0.0317	100

(2) Cytotoxicity of NZK. MTT assay used the optical density (OD), relative growth rate (RGR) and grade of cytotoxicity were measured and are listed in Table 4. The cell viabilities cultured in 10%, 50% and 100% extraction medium show non-statistically significant differences ($P>0.05$) after 1, 4 and 7 d compared with the negative control groups. The RGR of different extracts are all above 75%, and the cell proliferation viabilities cultured in identical extracts show notable differences ($P<0.05$) after 4 and 7 d compared with that of after 1 d (Figure 6). The grade of cytotoxicity is 0 to 1 at 1, 4 and 7 d. Therefore, this suggests that the *in vitro* degradation of NZK alloy has no significant destructive effect on MC_3T_3-E_1 cells, and thus NZK alloy displays excellent cytocompatibility *in vitro*.

3 Discussion

3.1 Degradation behavior of NZK alloy

Magnesium and its alloys are easily corroded in solutions, especially in the presence of chloride ions, and their degradation behavior is affected by various chemical, physical and electrochemical factors. When magnesium and its alloys are exposed to SBF, the following reaction will take place [25]:

$$Mg + 2H_2O = Mg(OH)_2 + H_2 \quad (4)$$

The precise reaction processes are

$$Mg = Mg^{2+} + 2e^- \quad \text{anodic reaction} \quad (5)$$

$$2H_2O + 2e^- = H_2 + 2OH^- \quad \text{cathodic reaction} \quad (6)$$

Usually, magnesium and its alloys produce oxide films in corrosive media. MgO and/or $Mg(OH)_2$ are porous and loose in nature and cannot effectively protect magnesium and its alloys. The speed of degradation changes with alloying element, surrounding temperature, pH value and negative ion concentration. Magnesium and its alloys react easily with many solutions, especially chloride ion solution, thus they are easily corroded in physiological environments. Cl^- can transform $Mg(OH)_2$ into $MgCl_2$, and then $MgCl_2$ will dissolve to Mg^{2+} and 2 Cl^-, which will be located on the surface of the sample [7].

$$Mg(OH)_2 + 2Cl^- = MgCl_2 \quad (7)$$

$$MgCl_2 = Mg^{2+} + 2Cl^- \quad (8)$$

It is well known that alloying is a convenient and effective method to alter the microstructure and improve the

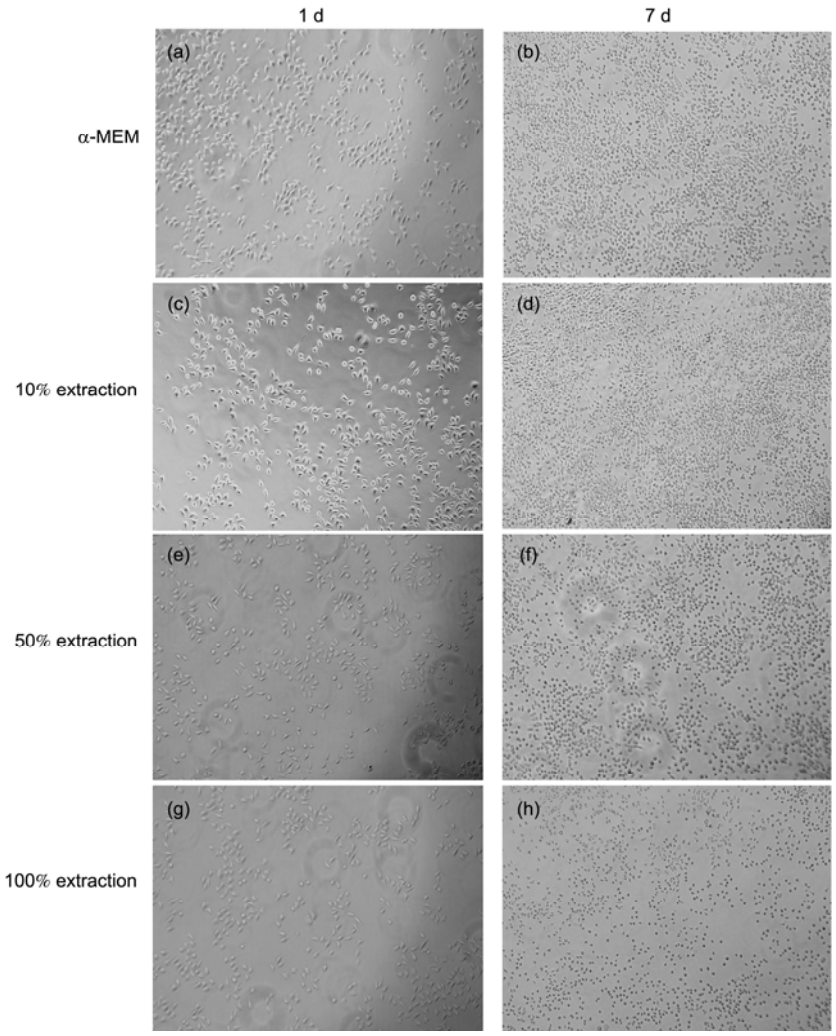

Figure 5 Cell morphology cultured in different concentration extractions of NZK alloy extract. Viewed under an inverted phase contrast microscope (×100).

Table 4 The optical density (OD) and relative cell growth rate (RGR) in different groups (n=6)

Group	1 d		4 d		7 d	
	OD (mean±SD)	RGR (%)	OD (mean±SD)	RGR (%)	OD (mean±SD)	RGR (%)
Negative	0.180±0.027	100	0.323±0.072	100	0.374±0.062	100
10% NZK	0.156±0.035	86.7	0.355±0.072[a)]	109.9	0.388±0.072[b)]	103.7
50% NZK	0.154±0.014	85.6	0.333±0.011[a)]	103.1	0.347±0.048[b)]	92.8
100% NZK	0.128±0.036	76.1	0.319±0.013[a)]	98.8	0.327±0.029[b)]	87.4

a) $P<0.05$ vs. 1 d; b) $P<0.05$ vs. 4 d.

corrosion behavior of magnesium. So in the present research, neodymium, zinc and zirconium were chosen as the alloying elements in magnesium alloy for medical applications. The results from the static immersion test showed that the NZK alloy possessed a higher corrosion resistance than that of the pure magnesium (Figure 2). Thus, it can be suggested that the corrosion resistance of our NZK alloy has been elevated by the addition of alloying elements neodymium, zinc and zirconium to magnesium. Witte et al. [26] added rare earth elements to magnesium and performed *in vivo* degradation tests, which indicated that the addition of rare earth elements may be suitable for the control of the corrosion rate of magnesium alloy. Zinc is a common alloying element in magnesium, and helps overcome the harmful corrosive effect of impurities such as iron and nickel, thus improving the corrosion resistance of magnesium

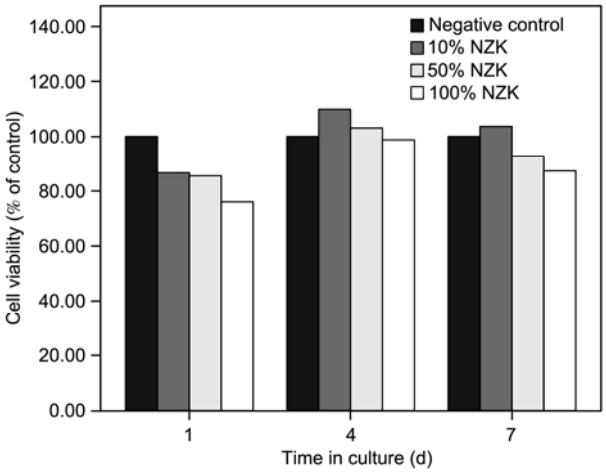

Figure 6 Cell viability expressed as a percentage of the viability of cells in the control group after 1, 4 and 7 d culture in different concentrations of extract of NZK alloy.

alloys [27]. Xu et al. [28] also proposed that the addition of zinc into magnesium could improve the corrosion resistance. Zirconium is usually used as a grain refiner in magnesium alloys without aluminum, thereby contributing to the strength of these alloys and reducing the adverse effects of iron contaminants on the corrosion resistance of magnesium alloys [29,30]. The superior corrosion resistance of NZK alloy may be attributed to the beneficial effects of certain amounts of neodymium, zinc and zirconium on the corrosion resistance by overcoming the harmful corrosive effect of impurities and leading to finer grain sizes. Meanwhile, the corrosion products that form a protective layer on the surface of NZK alloy (Figure 1(c)) may be an important factor in decreasing the corrosion rate.

3.2 Biocompatibility of NZK alloy

Implant materials must have good biocompatibility because they will directly contact tissues and cells after implanting into the human body. As a new degradable medical biomaterial for orthopedic application, it is important to evaluate the biocompatibility and biosafety of NZK from experimental studies prior to clinical application.

In this study, NZK alloy as degradable medical biomaterial for potential orthopedic implants was presented, and its *in vitro* biocompatibilities were assessed using hemolysis and cytotoxicity tests. Hemolysis is a phenomenon of hemoglobin release from erythrolysis. The hemolysis test is based on the degree of the erythrolysis and hemoglobin dissociation while the material contacts with erythrocytes *in vitro*. Besides the endogenic hemolytic factors such as abnormity of erythrocyte membranes and hemoglobin, there are some kinds of extrinsic factors such as physical agents on the material surface which can lead to cytotoxicity or may result in mechanical damage to erythrocytes. In this study, fresh anticoagulant-treated New Zealand white rabbit blood was added into the test, negative and positive control groups. The results showed that the hemolysis rate of calcium phosphate coated NZK alloy was 4.8%, lower than 5%, implying that the calcium phosphate coated NZK alloy will not cause severe damage to erythrocytes. A previous study [16] has demonstrated that untreated pure magnesium and other magnesium alloys (e.g. Mg-Zn-Mn alloy) had obvious hemolytic effects on human erythrocytes. It is believed that the magnesium and its alloys degraded too fast and the high concentration of magnesium in the solution was responsible for the high hemolysis rate [16]. The calcium phosphate coated NZK alloy had no obvious hemolysis, probably because neodymium, zinc and zirconium can improve corrosion resistance and thus slow the release rate of magnesium ions into blood fluid.

The MTT test is considered to be a good method to evaluate the cytotoxicity of medical material *in vitro*; it is sensitive to toxicity of material leaching into the liquor and is consistent with toxicity results in animal experiment. In the present paper, the cytotoxicity of NZK alloy can be assessed from the MTT test results on the l0%, 50% and l00% extract solutions. The RGR of MC_3T_3-E_1 cells cultured in different extracts are all above 75% and cytotoxicity is grade 0–1 at 1, 4 and 7 d. This suggests that the degradation of NZK alloy has no significant destructive effect on MC_3T_3-E_1 cells, and thus NZK displays excellent cytocompatibility *in vitro*. The morphologies of MC_3T_3-E_1 cells cultured in NZK alloy extracts had no significant differences compared with the control group: the cells attached well to the bottom of the culture plates proliferated quickly, and the quantity increased with time. There were no significant differences in the quantity and morphology of MC_3T_3-E_1 cells between the NZK alloy group and negative group at 1, 4 and 7 d, which demonstrated that the NZK alloy had no apparent cytotoxicity and could promote cell proliferation without affecting their normal function.

In summary, the results from this study suggest that the NZK alloy can be resorbed gradually, and has excellent biocompatibility *in vitro*. However, there were some limitations to the current study. First, static immersion tests may not exactly simulate the actual physiological conditions in the human body, because the human body fluid circulates dynamically and, moreover, ion concentrations differ in different parts of the human body [31]. In addition, although the novel NZK alloy has excellent hemocompatibilities and cytocompatibilities *in vitro*, *in vivo* studies will be needed to validate and supplement the *in vitro* results.

4 Conclusions

In the present study, the *in vitro* degradation behavior, hemocompatibilities, and cytocompatibilities of NZK alloy were investigated in order to explore its potential as a degradable medical biomaterial. From the results of the cur-

rent experiments, the following conclusions can be drawn: (1) The elements neodymium, zinc and zirconium in magnesium can increase the corrosion resistance of magnesium; the *in vitro* degradation rate of NZK alloy was lower than that of pure magnesium in SBF. (2) Certain corrosion products can precipitate on the surface of NZK alloy and form a protective layer after immersion in SBF. (3) The hemolysis rate of calcium phosphate coated NZK alloy was 4.8%, suggesting that the calcium phosphate coated NZK alloy did not produce significant hemolysis. (4) The cytotoxicity test showed that NZK alloy extracts did not reduce cell viability of MC_3T_3-E_1 cells.

In conclusion, the addition of alloying elements of neodymium, zinc and zirconium in magnesium could improve the corrosion resistance of magnesium alloy. Hemolysis and cytotoxicity tests reveal excellent biocompatibility *in vitro*. The results obtained from these investigations suggest that NZK alloy could be a promising material for orthopedic applications.

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