

Full length article

A pure zinc membrane with degradability and osteogenesis promotion for guided bone regeneration: In vitro and in vivo studies



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ABSTRACT

Selection of an appropriate membrane material for guided bone regeneration (GBR) is still ongoing among resorbable and nonresorbable membranes with different characteristics. The major problem with nonresorbable membranes is the inevitable secondary surgery, while resorbable polymer membranes have limitations in providing sufficient mechanical support during the bone repair period due to premature loss of mechanical strength. Pure magnesium foil has been evaluated to explore its feasibility as a resorbable GBR membrane. It exhibited better mechanical properties, whereas poor formability and fast degradation rate were noted. In light of this, pure zinc membrane was developed as a pilot research in this paper. We designed three types of pure zinc membranes: pure Zn without pores, pure Zn with 300 μm diameter and 1000 μm diameter pores, and pure titanium without pores as a control. The mechanical property, in vitro immersion tests, and MC3T3-E1 cell viability assays were tested. Moreover, in vivo behaviors of three type zinc membranes were evaluated by using a rat calvarial critical-sized bone defect model. The experimental results indicated that pure Zn membrane with 300 μm pores showed the most favorable osteogenic capability, comparable to that of titanium membrane without pores. Therefore, considering appropriate degradation rate, adequate mechanical maintenance, and profitable osteogenic capacity, metallic pure zinc is believed to be a promising candidate for barrier membranes in GBR therapy for bone regeneration, and its mechanical property can be enhanced with further alloying.

Statement of Significance

Metallic element zinc plays a pivotal role in the growth and mineralization of bone tissues. As a pilot research, three type of guided bone regeneration (GBR) membranes were developed in the present work: pure Zn without pores, pure Zn with 300 μm -diameter and 1000 μm -diameter pores respectively. The mechanical property, in vitro immersion tests and MC3T3-E1 cell viability assays were tested, with pure titanium without pores as a control, thereafter the in vivo performance were evaluated by using a rat calvarial critical-sized bone defect model. It indicated that pure Zn membrane with 300 μm pores showed the most favorable osteogenic capability, comparable to that of titanium membrane control, and is believed to be a promising material candidate as barrier membrane in GBR therapy for bone regeneration.

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1. Introduction

Over the past century, guided bone regeneration (GBR) technique has advanced constantly, and widely applied to orthopedics and dentistry [1,2], especially in the maxillofacial region [3]. There-

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into, the barrier membrane material plays a key role. By placing the membrane between the soft tissue and bone defect, a physical barrier can be created. The membrane blocks the fibrous connective tissue cells and epithelial cells from the surrounding soft tissue, allowing the osteoblasts with osteogenic potential to preferentially enter the bone defect area and thus have sufficient time to proliferate, ultimately achieving bone regeneration [4].

The currently clinically used GBR membranes can be divided into two categories: nonresorbable and resorbable membranes in terms of their clinical performances [5]. For nonresorbable membranes, mainly titanium mesh and polytetrafluoroethylene (PTFE), they exhibit favorable mechanical stiffness [6–8] and provide high volume stability [9]. Nevertheless, the lack of biodegradability demands a secondary surgery procedure for removal, and the membrane stiffness may cause soft tissue dehiscence, resulting in the likelihood of wound infection [10–12] and extended healing period [4]. Thereafter, resorbable membranes, aiming to obviate the need for additional surgery, have been investigated, which were based on natural or synthetic polymers, such as collagen (e.g. Biogide™, Wollhusen Switzerland), poly glycolic acid (PGA) [13], poly lactic acid (PLA) [14], polycaprolactone (PCL) [15,16] and so on [17–19]. Completed bone regeneration occurs 3–6 months after operation, depending on the size of the bone defect [20]. However, because of the fast resorption and premature loss of mechanical properties, these resorbable membranes have limitations in providing volume-stability during the bone repair period.

Taking into account the demands for resorbability and sufficient mechanical strength maintenance during bone regeneration procedure, biodegradable metals (BMs) might be considered as a new choice. Magnesium, iron and zinc-based BMs have been developed for orthopedic [21–24] and cardiovascular [25–29] applications, due to their favorable biocompatibility, adequate degradability and desirable mechanical property. Among them, zinc and its alloys, which exhibit comparatively appropriate mechanical properties, degradation behaviors and benign biocompatibilities, have become a new option in the last 5 years. Zinc, as an essential trace element in the human body, participates in various basic biological functions including nucleic acid metabolism, signal transduction, apoptosis regulation and gene expression in addition to interacting with a lot of organic ligands [30]. Recent studies have shown that zinc exhibits better in vivo degradation behavior without leaving voluminous corrosion products that are hard to be eliminated by human body [25,31–33]. More importantly, zinc plays a pivotal role in the growth and mineralization of bone tissues. It can directly activate aminoacyl-tRNA synthetase in osteoblastic cells [34] and stimulate cellular protein synthesis [35]. In addition, zinc inhibits osteoclastic bone resorption by inhibiting osteoclast-like cell formation from marrow cells [36]. Zinc also plays an important role in the preservation of the bone mass [37].

In this work, we fabricated three kind of pure zinc membranes and studied the in vitro degradation behavior and cytotoxicity, and in vivo biocompatibility systematically, with pure Ti membrane as a comparison. We aimed to explore the feasibility of pure zinc membrane as a new type of resorbable GBR membrane for bone defect repair.

2. Materials and methods

2.1. Material preparation

Pure zinc foil (99.998% purity, 30 μm thickness, Goodfellow (Shanghai) Trading Co., Ltd., China) was used as zinc membrane materials without pores. By using laser cutting technology (SK-P30 A, 200 W laser power, 1.064 μm wavelength, 20 kHz pulse frequency), the pure zinc membrane was further made into two other types of zinc membrane materials with pores: 300 μm and

1000 μm in diameter, which were uniformly distributed with the distance between two neighboring pores was 1.2 mm. Pure titanium membrane (99.6% purity, 20 μm thickness, Xian Zhongbang titanium biological materials Co., Ltd., China) was used as control group for in vitro and in vivo biocompatibility evaluation, denoted as Ti without pores. For all specimens used in the experiment, they were ultrasonic cleaned in acetone, absolute ethanol and distilled water for 10 min in sequence.

2.2. Mechanical tests

The tensile tests were conducted on a universal test machine (Instron 5969, USA) with a displacement rate of 1 mm/min, at room temperature. Specimens with 10 mm width and 20 mm length, were used for testing. An average of at least 5 parallel samples were taken for each group.

2.3. Immersion tests

In this experiment, zinc membranes were cut into the square samples with surface area of 10 $\times$ 10 mm² and immersed in Hank's solution at 37 °C. The ratio of solution volume to sample surface area was 20 ml/cm² according to ASTM G31-72 standard. Five parallel samples were taken for each group. At different immersion timepoint, a batch of samples was removed from Hank's solution, gently rinsed with distilled water, and dried at room temperature. Then the corrosion products were removed by ultrasonic cleaned in chromic acid solution (200 g/L Cr₂O₃) at 40 °C for 5 min, and the corrosion rates were estimated using the weight loss method. The surface morphologies before and after removing corrosion products were observed using SEM (S-4800, Hitachi, Japan) with energy-disperse spectrometer (EDS) attachment. X-ray diffractometer (XRD, Rigaku DMAX 2400, Japan) was used to identify the constitutional phases of surface corrosion products.

2.4. In vitro cytotoxicity assays

Cell viability and proliferation evaluation was conducted according to ISO 10,993–5: 2009. Mouse osteoblastic cell line (MC3T3-E1) were used and cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin, in a humidified atmosphere, with 5% CO₂ at 37 °C. The extracts of three type of zinc membranes and Ti membrane were obtained by incubating them in the same culture medium mentioned above for 24 h respectively, with an extraction ratio of 1.25 cm²/mL and collecting the supernatants. Subsequently, by adding different amounts of MEM to dilute the 100% extracts, the 50% and 10% concentration extracts were prepared. The cytocompatibility assays were independently evaluated using the 100%, 50%, and 10% concentration extracts of the four kinds of experimental membranes. Cells were incubated in 96-well culture plates at a density of 3 $\times$ 10³ cells/well and incubated for 24 h to allow cell attachment. Then the medium was replaced with the different concentration extracts of the specimens, with the MEM medium as negative control and normal medium plus 10% Dimethyl sulfoxide (DMSO) as positive control. Each group was taken in five copies. After incubating for 1, 3 and 5 days, the cell viability and proliferation were valued with a Cell Counting Kit-8 (CCK-8, Dojindo, Japan). The spectrophotometric absorbance of each well was measured with a microplate reader (Bio-RAD680) at 450 nm wavelength. Zinc ion concentrations in the extracts of three kind of zinc membranes were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, iCAP6300, Thermo, USA).

For cellular live/dead staining assay, cells cultured in different extracts for 24 h were rinsed with phosphate buffer saline (PBS)

solution for three times and incubated with 2 mM Calcein AM and 4 mM PI (Live/Dead Cell Stains, Dojindo, Japan) for 20 min in a humidified incubator. The cells were then rinsed twice by PBS solution and visualized using the confocal laser scanning confocal microscope (A1R-si, Nikon, Japan). Each group was prepared in triplicate for observation.

2.5. *In vivo* animal surgery

2.5.1. Surgical implantation

Animal testing was approved by Peking University Health Science Center (Approval number: LA2016305) and were performed in accordance with the protocol established by the Experimental Animal Ethics Branch. Forty ten-week old male Sprague-Dawley rats were randomly divided into five groups with eight in each group: (1) Zn without pores, (2) Zn with 300 μm pores, (3) Zn with 1000 μm pores, (4) Ti without pores and (5) Sham control (bone defects without membrane). All rats were anesthetized by pentobarbital sodium (50 mg/kg) to reduce potential suffering. The experiment was conducted as described previously [38]. Briefly, one 6-mm-diameter and full-thickness critical-sized defect was made on the right side of each rat's calvaria, using a trephine bur under low-speed drilling. The membranes ($9 \times 9 \text{ mm}^2$ in area) were then implanted into the defects, and the incision was sutured. Postoperatively, all rats were raised in an environmentally controlled animal care house.

2.5.2. Micro-CT analysis

Four animals of each group were killed at 6 and 10 weeks after surgery. The calvaria including the implants and main organs (including heart, liver, spleen and kidney) of animals were harvested and carefully excised, then fixed in 10% neutral buffered formalin for 24 h at room temperature. Senographe essential X-ray apparatus (GE, Fairfield, CT, USA) was used to capture soft X-ray pictures. A high resolution Inveon apparatus (Siemens, Munich, Germany) was used to evaluate bone formation within the bone defect. Images were acquired at an effective pixel size of 8.82 μm . Multimodal three-dimensional (3D) visualization software (Inveon Research Workplace, Siemens, Germany) was used for 3D reconstruction of the images and evaluating new bone volume in the defects by quantifying pixels in these regions.

2.5.3. Histological analysis

After completing the above steps, the specimens were dehydrated with gradient dehydration from 75% to absolute ethanol and then embedded in polymethylmetacrylate (PMMA). Then, the embedded specimens were sectioned into 150- μm -thick sections using a Leica SP1600 saw microtome (Leica, Hamburg, Germany) encompassing the entire defect. The sections were ground and polished to 40–60 μm , followed by staining with Toluidine blue for histological examination.

Specimens were decalcified for 14 days in 10% EDTA solution (pH 7.4) under constant agitation at room temperature. After decalcification, the specimens were embedded in paraffin and sliced into 5- μm -thick serial sections. The main organs of animals were also fixed, dehydrated, embedded, and sliced. Hematoxylin-eosin (H&E) staining was performed for histological examination. The sections were observed and images were obtained using an optical microscope (BX51, Olympus, Japan).

2.5.4. Degradation products (DP) characterization

The hard tissue sections were ground to 7000 grit and then polished with a water soluble 0.5 μm diamond slurry on microfiber. The cross sections were sputter deposited with a thin gold film, and then observed under a scanning electron microscope (SEM,

Hitachi S-4800, Japan) equipped with an energy dispersive spectrometer (EDS, Bruker QUANTAX, Germany). The structure and the element distribution of the degradation products were analyzed.

2.6. Statistical analysis

Data are presented as mean value $\pm$ standard deviation and analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, US). One-way analysis of variance (ANOVA) was performed for data analysis. Statistical significance was defined as *p* value of <0.05 .

3. Results

3.1. Material characterization

Fig. 1 shows the general observation on experimental pure zinc and titanium membranes. As can be seen from the macroscopic view, the surfaces of all experimental membranes are glossy with metallic luster. For pure zinc membranes with 300 μm and 1000 μm pore size, pores were evenly distributed on the entire region. A few laser cutting debris, which mainly composed of zinc and oxygen element from the typical EDX line scan analysis, can be found at the edge of pores, as shown in the higher magnification SEM images (Fig. 1(b) and (c)). This demonstrated that oxidation occurred at the laser-cutting edges during the process of laser drilling, and there were certain heat-affected zones around the pores [39,40], which might slightly influence the degradation behavior of zinc membranes. For the pure zinc membrane without pores (Fig. 1(a)) and titanium membrane without pores (Fig. 1(d)), the surfaces were relatively flat and evenly distributed with fibrous traces caused by rolling procedure.

3.2. Mechanical properties

The mechanical property parameters of experimental pure Zn membranes and Ti membrane are displayed in Fig. 2. For zinc membrane without pores, the yield strength (YS) and ultimate tensile strength (UTS) were $92.16 \pm 8.76 \text{ MPa}$ and $108 \pm 4.87 \text{ MPa}$, respectively, with elongation of $42.82 \pm 2.69\%$. However, for the two type zinc membranes after laser cutting, a significant reduction in tensile strength can be observed, especially for the pure Zn membrane with 1000 μm pores. Ti membrane without pores showed superior strength but inferior plasticity.

3.3. *In vitro* degradation behavior

The degradation behaviors of three kinds of pure Zn membrane after static immersion in Hank's solution for different time were evaluated, with Ti membrane as control (Figs. 3–5). The typical corrosion morphologies of the experimental specimens immersed at different times are shown in Fig. 3(a). From a macroscopic perspective, pure Zn membrane without pores (Fig. 3(a)–(c)) maintained a relatively uniform corrosion mode during the immersion. With the extension of immersion time, the degradation products continuously formed on the surface of the specimens, from a relatively sporadic distribution to a full coverage. For Zn membrane with 300 μm (Fig. 3(d)–(f)) and 1000 μm pores (Fig. 3(g)–(h)), corrosion occurred preferentially at the periphery of pores, with inconspicuous change on pore size. However, obvious fracture of pure Zn membrane with 1000 μm pores (Fig. 3(i)) was detected after 60 days immersion. For Ti membrane without pores (Fig. 3(j)–(l)), very few degradation products was observed.

After removal of corrosion products, the fresh surface of pure Zn membrane without pores (Fig. 4(a) and (b)) was comparatively smooth and integral, with several corrosion pits distributed. Similar surface condition of pure Ti membrane without pores (Fig. 4(g)

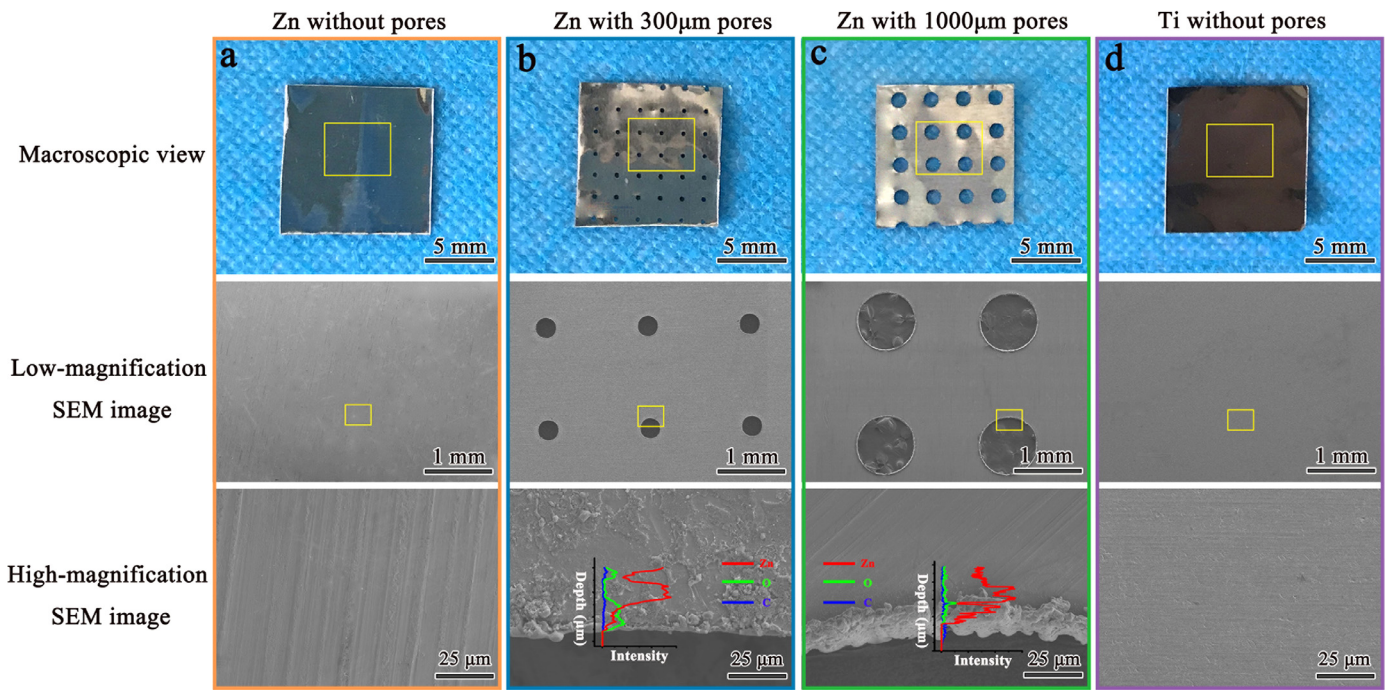


Fig. 1. Original morphologies of three type pure zinc membranes, with pure titanium membrane as control. (a) Macroscopic images of four types of membranes and (b) Scanning electron microscope (SEM) graphs of the corresponding magnified areas of yellow rectangles in (a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

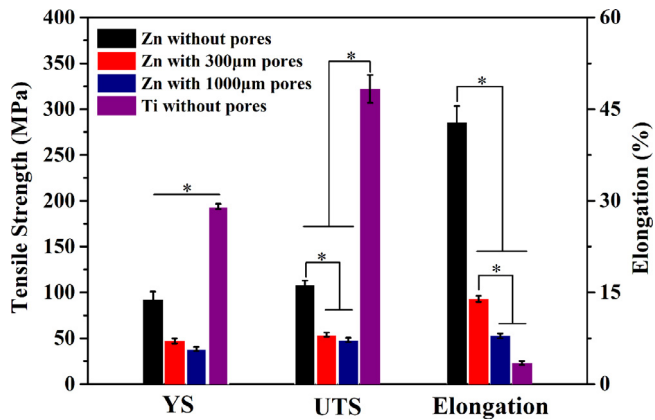


Fig. 2. Mechanical properties of three types of pure Zn membranes, with pure titanium membrane as control.

and (h)) was observed, probably due to the pitting effect of chloride ions. For Zn membrane with 300 μm pores (Fig. 4(c) and (d)), the overall surface remained relatively intact, although obvious corrosion and cracks were found at the pore periphery after 60 days immersion. In pure Zn membrane with 1000 μm pores (Fig. 4(e) and (f)), a noticeable rupture occurred after 28 days of immersion, and severe fragmentation was detected with longer immersion time.

The weight loss ratios and degradation rates during immersion were calculated and displayed in Fig. 5(a) and (b). For pure Zn membrane without pores, the percentage of weight loss increased significantly after immersion for 14 days, and reached to value of $42.11 \pm 0.24\%$ after 60 days' immersion. Furthermore, the degradation rate was slightly low at the initial stage, then accelerated after 14 days immersion, and gradually became stable at 0.042 ± 0.003 mm/year after 60 days. A similar trend was detected for pure Zn membrane with 300 μm pores, with comparable weight loss ratio of $42.71 \pm 3.02\%$ and degradation rate of

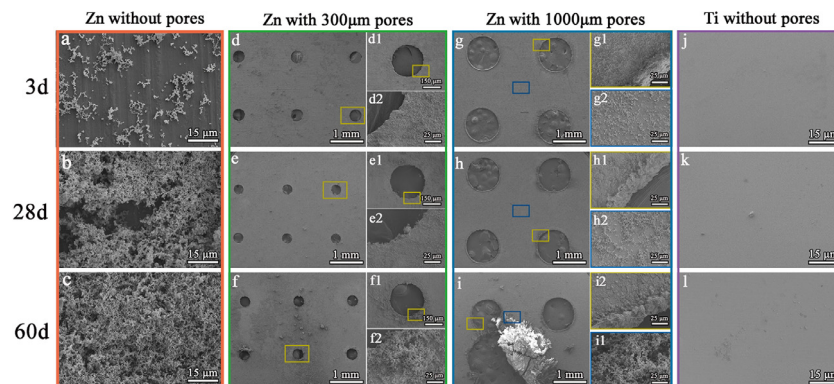


Fig. 3. Typical surface morphologies of three types of pure Zn membranes, (a)–(c) Zn membrane without pores, (d)–(f) Zn membrane with 300 μm pores, (g)–(i) Zn membrane with 1000 μm pores and (j)–(l) Ti membrane without pores after immersion in Hank's solution for different time.

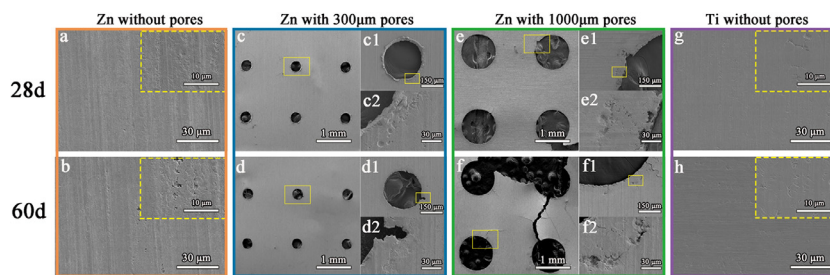


Fig. 4. Representative images of surface morphology of membranes after removal of corrosion products. (a) and (b) Zn membrane without pores, (c) and (d) Zn membrane with 300 µm pores, (e) and (f) Zn membrane with 1000 µm pores and (g) and (h) Ti membrane without pores.

0.046 ± 0.004 mm/year after 60 days immersion. Pure Zn membrane with 1000 µm pores displayed faster degradation rate during the whole immersion time because of premature rupture. For Ti membrane without pores, there was almost no weight change. The evolution of pH values over the whole immersion period is shown in Fig. 5(c). The three types of pure zinc membranes demonstrated parallel trends, in which the pure Zn membrane with 1000 µm pores manifested slightly higher pH value in the initial 10 days. Nevertheless, the difference among three type zinc membranes was gradually reduced and eventually the pH values reached to approximately 8.5 after 60 days immersion. Similar variation trend with time yet lower pH value was exhibited for the Ti membrane without pores. The variation tendency of zinc ion concentration with immersion time was also measured (Fig. 5(d)), in line with that of degradation rate (Fig. 5(b)). The two pure Zn membranes with pores displayed a little bit higher zinc ion concentrations at early stage, in contrast with the Zn membrane without pores. Thereafter, all three kind of pure Zn membranes reached comparable ion concentration value. After immersing for 60 days, the Zn^{2+} concentrations of the pure Zn membrane without pores, Zn membrane with 300 µm pores and 1000 µm pores were 56.2 ± 1.7 µg/ml, 57.4 ± 1.9 µg/ml and 57.6 ± 1.1 µg/ml, respectively.

XRD and FTIR were used to further analyze the chemical compositions of surface degradation products layers after 28 days and 60 days' immersion separately (Fig. 5(e) and (f)). Zn, O, C, P and Ca were the major elements detected in degradation products of pure zinc membranes. Zinc oxide and calcium carbonate as major crystallized products can be revealed by XRD. The presence of PO_4^{3-} and CO_3^{2-} functional groups could be distinguished in degradation products layer on sample's surface, as reflected from FTIR spectra (Fig. 5(f)). The narrow and high intensity peak which was observed at $1000\text{--}1140\text{ cm}^{-1}$, was reported to be ν_3 mode of PO_4^{3-} [6]. The peak at around 580 cm^{-1} was likely related to ZnO [25]. In addition, the defined wavenumber ranges of $1400\text{--}1500\text{ cm}^{-1}$ and $3100\text{--}3600\text{ cm}^{-1}$ were referred to ν_3 mode of CO_3^{2-} [31] and O–H stretching vibration [27], respectively. From the above results, the degradation products for the pure Zn membrane were identified to be mainly zinc oxide, zinc phosphate and calcium carbonate. As immersion time prolonged, the contents of these corrosion products increased obviously, especially for the pure Zn membrane with 1000 µm pores. In Ti membrane without pores, however, scarcely any degradation products resided on the surface, because titanium has high corrosion resistance.

3.4. Cytotoxicity test

To examine the in vitro cytotoxicity of experimental pure zinc membranes, the MC3T3-E1 cell viabilities culturing in the extracts of experimental specimens, without and with 50% and 10% dilution, were evaluated. As shown in Fig. 6(a), the 100% concentration extracts of pure Zn membrane with 1000 µm pores exhib-

ited noticeable toxicity with a significant low cell viability, despite the slightly improved cytocompatibility after culturing for 5 days. However, cells cultured in extracts with 50% and 10% dilution displayed obviously improved cell viabilities. For the two groups of pure Zn membrane without pores and with 300 µm pores, all the cell viabilities measured with different concentration extracts surpassed the minimum threshold of acceptable cytotoxicity. Moreover, cell viabilities in 10% dilution extracts of these two type pure Zn membranes were higher than that of Ti membrane group, which is considered to have good biocompatibility [9].

Fig. 6(b) revealed the live/dead staining images of cells culturing in 100% concentration extracts of three types of pure Zn membranes and pure Ti membrane, as well as in normal MEM medium served as negative control. For the pure Zn membrane without pores, rare death of cells were observed, comparable to the groups of pure Ti membrane without pores and negative control, implying favorable biocompatibility. In the group of pure Zn membrane with 300 µm pores, negligible cell death was detected, while a certain number of cells were dead for the pure Zn membrane with 1000 µm pores group.

The zinc ion concentrations of the three type of pure zinc membranes (Fig. 6(c)) were 14.4 ± 1.5 µg/mL, 19.1 ± 0.9 µg/mL, 21.3 ± 0.8 µg/mL respectively. The pure Zn membrane with 1000 µm pores displayed comparatively higher Zn^{2+} concentration, which was in accordance with previous results in Hank's solution.

3.5. Animal testing

In this work, rat calvarial bone defect model was used for in vivo animal experiment, identical to references [41,42], without taking mechanical stresses into consideration [43], because the main focus areas are in vivo degradation behavior and osteogenic ability of experimental materials. A similar situation can be found in references [38,44–48].

3.5.1. Micro-CT analysis

Representative micro-CT results of rat calvarias covered with three kinds of pure zinc membranes at week 6 and week 10 are displayed in Fig. 7(a), with titanium membrane as control. For the sham control group, very few bone regeneration evidence could be found at the marginal area of the calvarial defect after 6 and 10 weeks, whereas newly formed bones were clearly observed at the margins of the defect sites in the three types of pure zinc membranes after 6 weeks implantation, in which the Zn membrane with 300 µm pores indicated the highest osteogenic capability. Judging from the cross-sectional micro-CT images, all of the pure Zn membranes and Ti membrane were well attached to the cranial defect area. However, because of the corrosion characteristic of zinc, some degradation products were formed at zinc membrane surface. With the prolongation of implantation time, the new bone formations were prominently increased at week 10, and

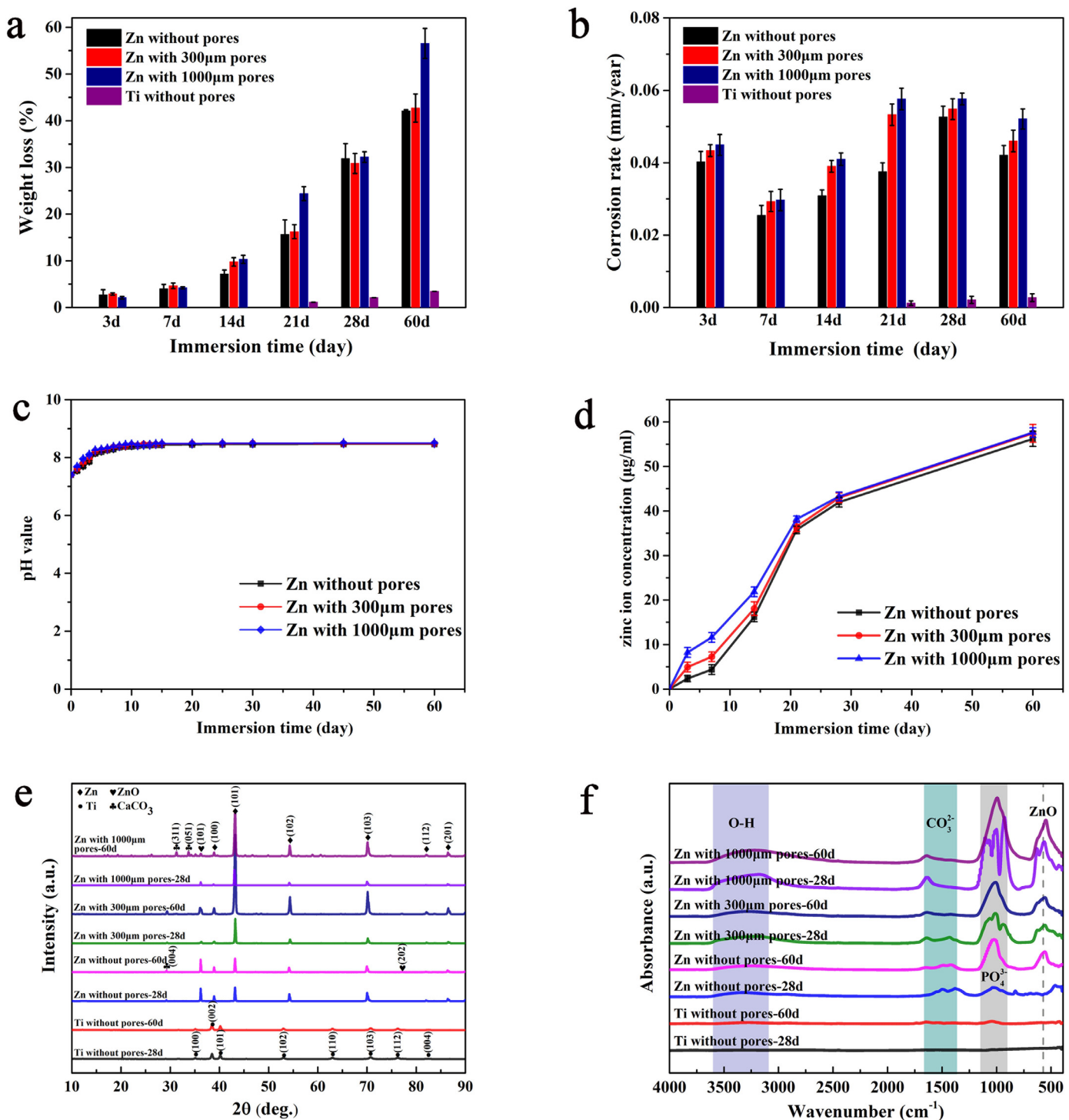


Fig. 5. In vitro degradation behavior of pure zinc membranes immersed in Hank's solution. (a) Weight loss ratio and (b) degradation rate of membranes. (c) Evolution of pH values and (d) zinc ion concentrations of three type zinc membranes during immersion time. (e) X-ray diffraction pattern and (f) FTIR spectrum of degradation products after immersion for 28 days and 60 days respectively.

pure Zn membrane with 300 µm pores manifested maximum new bone formation, while pure Zn membrane with 1000 µm pores showed minimal newly formed bone evidence. As demonstrated in cross-section micro-CT images at week 10, the Zn membrane without pores, Zn membrane with 300 µm pores, and Ti membrane without pores still maintained comparatively adequate mechanical strength and volume stability, which were beneficial and essential to the recovery and repair of bone [49]. In contrast, the pure Zn membrane with 1000 µm pores partially collapsed at week 6 due

to fast degradation rate and lack of sufficient mechanical integrity, and almost broken down at week 10.

According to the analysis of micro-CT images, the quantitative bone volume ratios (BV/TV) of new bone formation were calculated and depicted in Fig. 7(b). The pure Zn membrane with 300 µm pores showed relative higher BV/TV value of $29.6 \pm 3.7\%$ at week 6, compared with other two kind Zn membranes, while the Ti membrane without pores exhibited the maximum BV/TV value of $31.3 \pm 2.6\%$. Nonetheless, there was no significant difference in

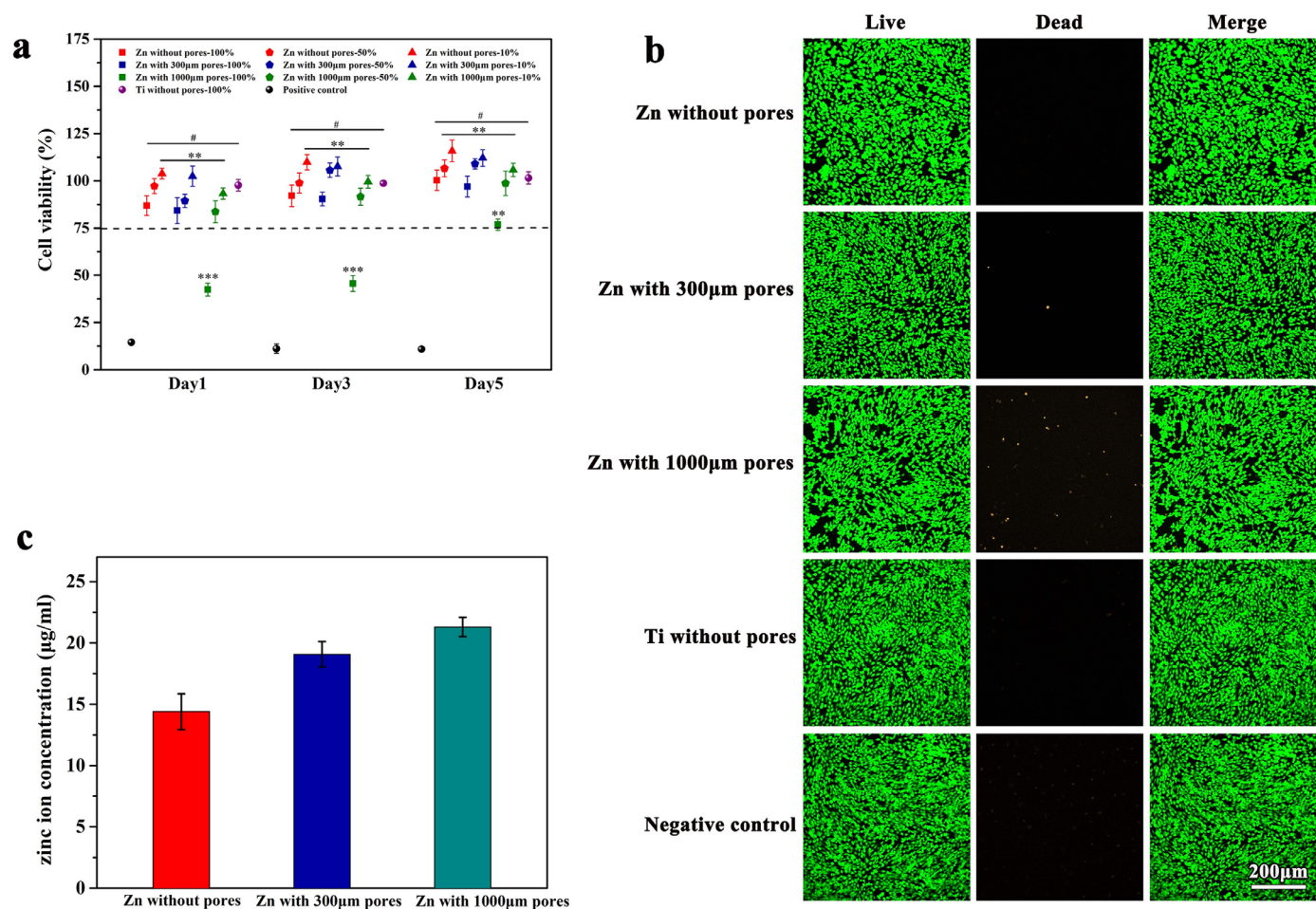


Fig. 6. (a) In vitro cytotoxicity test of MC3T3-E1 culturing in three kinds of pure zinc membranes' extracts with and without dilution, with cells culturing in the normal MEM medium plus 10% DMSO as positive control. # $p < 0.001$ compared to positive control, ** $p < 0.01$, *** $p < 0.001$ compared between groups. (b) Live/Dead fluorescence images of cells cultured in 100% concentration extracts of different kinds of membranes. (c) Zinc ion concentrations of three types of pure zinc membranes after immersion in culture medium for 24 h at 37 °C, 5% CO₂.

new bone formation between the pure Zn membrane with 300 μm pores and Ti membrane without pores. After implantation for 10 weeks, the pure Zn membrane with 300 μm pores presented pronounced new bone formation ability, and even a bit higher than that of Ti membrane group. On the flipside, the pure Zn membrane with 1000 μm pores displayed the minimal BV/TV value.

3.5.2. Histological analysis of bone regeneration

The sections of calvarias after membranes implantation for 6 weeks and 10 weeks were displayed in Fig. 8. It demonstrated that new bone formed mainly from the periphery of bone defect area to center, and Zn membrane with 300 μm pores seemingly manifested evident osteogenic capacity, which was comparable to titanium membrane without pores. At 6 weeks post-implantation of Zn membrane with 300 μm pores (Fig. 8(a)), the fibrous connective tissue was attached to the outside surface of zinc membrane, while new bone formed under the inside surface of membrane and more cellular components were arranged and gathered around the new bone. The Ti membrane without pores also showed favorable bone-promoting ability, and there was no significant difference between these two membranes. The pure Zn membrane without pores and with 1000 μm pores showed less new bone regeneration. Predominantly fibrous tissue filled the bone defects was observed in the sham control group with only a small amount of new bone detected.

After 10 weeks of implantation, the Zn membrane with 300 μm pores appeared to maintain the leading position in osteogenic capacity (Fig. 8(b)). The joint connection was depicted between new bone and old bone, and the thickness of new bone tissues was increased. At the interface of the zinc membrane and bone defect area, it mainly contained new bone and degradation products of zinc. The titanium membrane without pores manifested commendable new bone growth as well. However, the Zn membrane with 1000 μm pores exhibited the weakest bone regeneration ability due to lack of sufficient mechanical strength and collapse into bone defect area, thus probably delaying the rate of bone repair. Arranging bone regeneration capacity from strong to weak, the order was Zn membrane with 300 μm pores, Ti membrane without pores, Zn membrane without pores and Zn membrane with 1000 μm pores in sequence. From the H&E-stained images of organs of different membrane groups (Fig. 9), no obstruction, histopathological changes and accumulation of degradation products were found, which indicated that the biocompatibility of zinc membranes and no adverse effect on the metabolism of animals.

3.5.3. Degradation products analysis

The morphologies of the bone-implant cross-sections of Zn membrane with 300 μm pores and Ti membrane without pores after implantation for 10 weeks are shown in Fig. 10, together with the corresponding elemental mappings. In general, zinc membrane underwent a relative macroscopic uniform degradation mode,

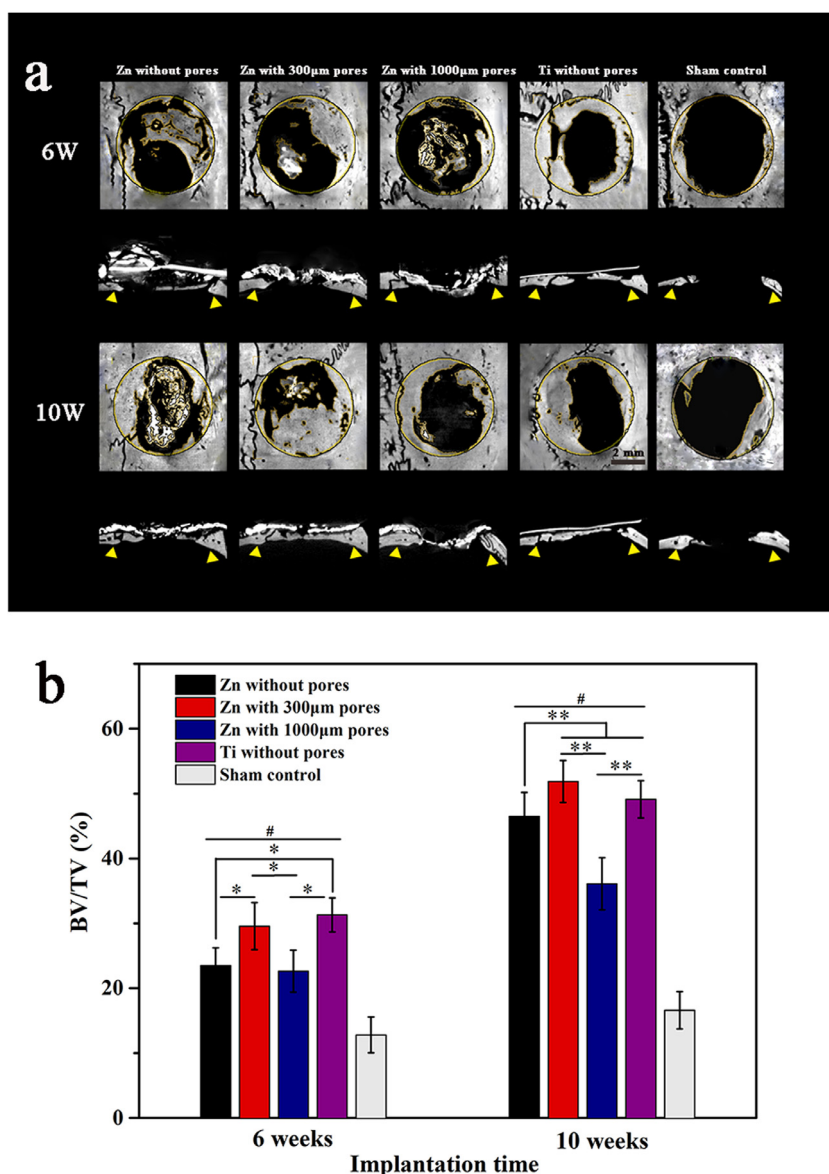


Fig. 7. In vivo rat calvaria bone defect repair using three kinds of pure zinc membranes, with pure titanium membrane as control, and bone defect without membrane as sham control group. (a) Representative micro-CT images and (b) Quantitative bone volume ratios (BV/TV) calculating new bone formation of four kinds of membranes after implantation for 6 and 10 weeks, respectively (# $p < 0.001$ compared to sham control group, ** $p < 0.01$, *** $p < 0.001$ compared between groups). New bone formation areas were circled by yellow lines, and the edges of bone defect area were marked with yellow arrowheads. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

without obvious localized corrosion (Fig. 10(a)). Corrosion products were visible and distributed around the implant. More corrosion products seemed to deposit on the side contacting with the soft tissues. The interface between implant and calvaria consisted of residual zinc matrix, degradation products and newly formed bone (Fig. 10(a)).

The EDS mapping (Fig. 11(a)) illustrated that the degradation products of zinc membrane exhibited two-layer structure. The thin inner layer adjacent to the surface of zinc matrix was rich in elements Zn and O, while the outer layer composed mainly by elements P and Ca. Bone was rich in Ca and P. The atomic percentages of major elements in degradation products of three type zinc membranes are shown in Fig. 11(b). As can be seen, with the prolongation of implantation time, the contents of Ca kept increasing in degradation products of all three zinc membrane groups. Among these, degradation products formed on the surface of Zn membrane with 300 µm pores contained more P and Ca elements, compared to the other two zinc membranes.

After 10 weeks of implantation, the thickness of the remaining zinc membrane was approximately 21.5 ± 0.2 µm (Fig. 11(a)), that is, the degradation rate of in vivo was 0.044 ± 0.003 mm/year. Thus, the zinc membrane might attain complete degradation after 7–8 months of implantation.

4. Discussion

4.1. Feasibility of pure Zn membrane for GBR application in view of material design

The basic principle of GBR is to isolate the bone defect area from the surrounding connective tissue by placing a mechanical membrane between them, thus providing a secluded space for facilitating the growth of the slower-growing osteoblasts to achieve bone regeneration [50]. In light of this, an ideal GBR membrane ought to fulfill four fundamental criteria [51] from the perspec-

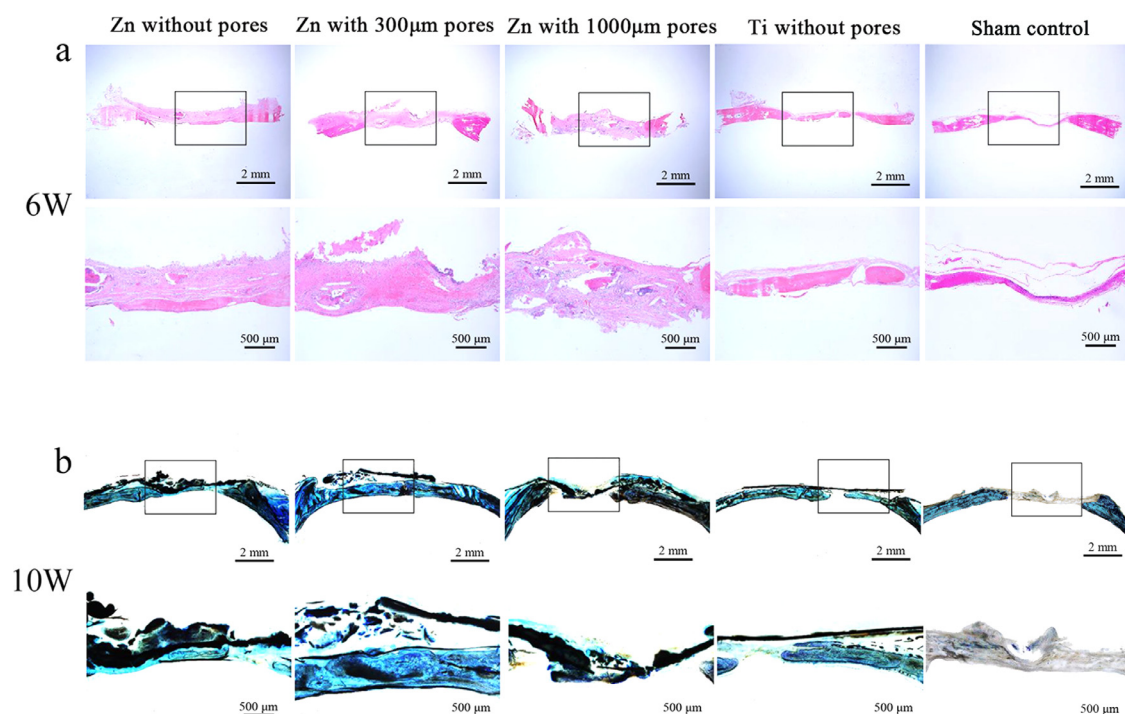


Fig. 8. Histological characterization of newly formed bone and materials. (a) H&E staining of experimental membranes after 6 weeks implantation and (b) Toluidine blue staining of specimens after 10 weeks implantation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

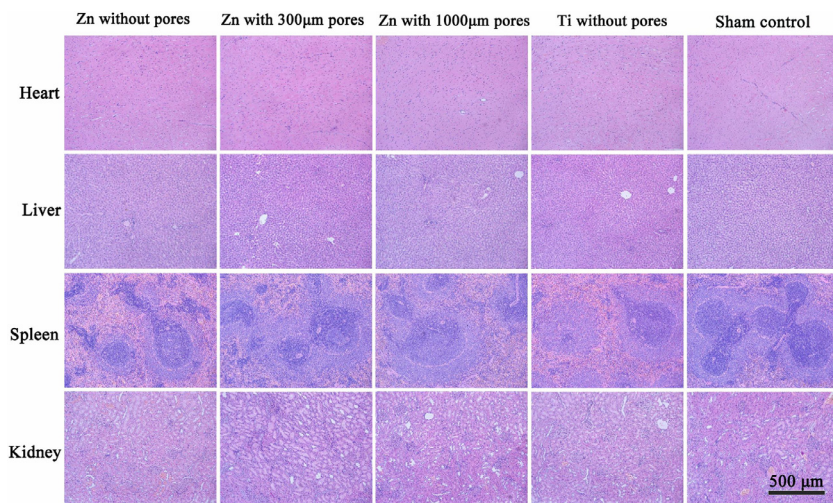


Fig. 9. Histological characterization of important organs of SD rats by H&E staining after 10 weeks postoperatively, including heart, liver, spleen and kidney.

tive of material design: (i) benign biocompatibility, (ii) sufficient mechanical property and stability, (iii) reasonable biodegradability, along with (iv) clinical manageability [52], as illustrated in Fig. 12(a). To our knowledge, this is the first study to examine the in vitro and in vivo performances of pure zinc membrane for potential GBR membrane application.

4.1.1. Biocompatibility

When considering a material as a medical device, biocompatibility is one of the most pivotal factors to be evaluated. The interaction between the membrane and host tissues must avoid affecting the surrounding tissue disadvantageously. With respect to degradable membranes, the degradation products formed during the degradation process should also pledge an acceptable level of biocompatibility. For the pure Zn membranes evaluated

in the present study, favorable biocompatibilities of the membranes and its degradation products were testified by in vitro and in vivo experiments, which displayed acceptable MC3T3-E1 cell compatibility (Fig. 6(a)), pronounced osteogenic capacity (Figs. 7 and 8) and no adverse effect on animal metabolism (Fig. 9).

Zinc, as one of the essential trace elements, has been reported to play a crucial role in inducing the proliferation and differentiation of osteoblasts and new bone formation. Significantly enhanced mineralization of extracellular matrix (ECM) and human bone marrow mesenchymal cells (hBMCs) osteogenic differentiation, as well as increased expression of bone-related genes when cultured with zinc or zinc-modified materials have been recently reported [34, 53–57]. Eluted zinc ions were beneficial for osteoblast differentia-

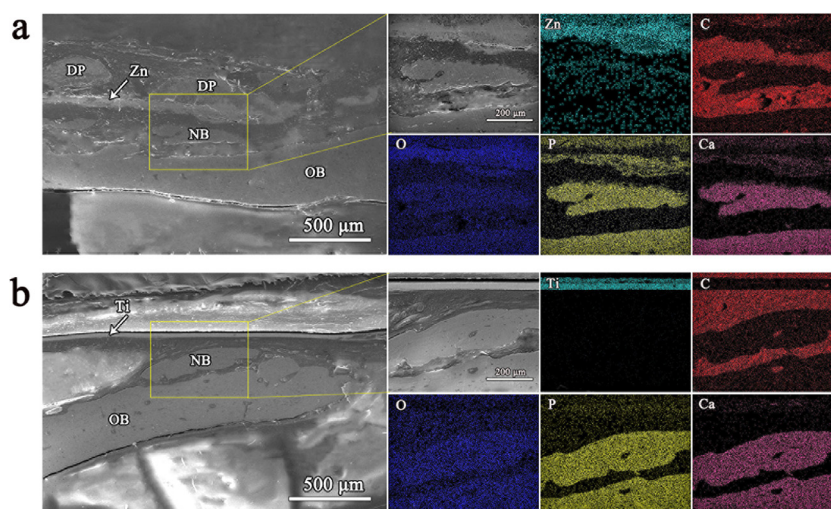


Fig. 10. SEM images of the cross sections. (a) Zn membrane and (b) Ti membrane after implantation for 10 weeks, with magnified images (yellow rectangles) and corresponding elemental distribution in view. (Zinc (green), Carbon (red), Oxygen (blue), Phosphate (yellow), Calcium (yellow), NB (new bone), OB (old bone), DP (degradation products)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tion of human dental pulp stem cells (DPSCs) as well, which exhibited the potential application to bone disease treatment [58]. In vivo animal experiments also demonstrated favorable bone formation, enhanced osseointegration capacities in zinc-incorporated materials and zinc-modified material surfaces [31,56,59,60]. However, zinc ions show biphasic effects on cell viability, proliferation, and migration [61,62]. Relatively high concentration of released zinc ions ($14.4 \pm 1.5 \mu\text{g/mL}$) could destroy the viability of MC3T3-E1 cells, as shown in Fig. 3(a). When extracts diluted to lower zinc ion concentrations, favorable cell viabilities displayed, indicating benign cytotoxicity of zinc membrane. Previous studies have confirmed the cytotoxicity of zinc ions at high concentrations as well. Proliferation of human osteoblasts was increased on the surface of 5% zinc containing apatite cement (Zn-TCP) with 0.085 ppm released zinc ion concentration, while significantly decreased on the surface of 5% Zn-TCP group, with value of 0.0085 ppm [63]. Nevertheless, zinc-containing tricalcium phosphate (Zn-TCP) promoted osteogenic differentiation of hBMSCs and regulated osteoclastic differentiation of monocyte/macrophages in vitro, as well as enhanced new bone formation in the paraspinal muscle of canines at a relatively high zinc content [64]. The promotion of new bone formation of pure Zn membrane with 300 μm pores discovered in this work may be correlated with the release of zinc ions as well. Although the observed bone regeneration cannot be attributed solely to zinc ion release, and the clear mechanism for osteogenic capacity of zinc membrane remains vague, we believe that proper zinc ion concentration is pivotal and ought to be considered in the bone regeneration process.

4.1.2. Mechanical property

Adequate mechanical property and sufficient strength maintenance during healing period is another vital requirement for membranes, according to the basic principle of GBR [5,52]. The membrane ought to have a sufficient mechanical strength to create and maintain suitable spaces for intended osteoblasts growth, as well as withstand some external forces [13], such as compression of the overlying soft tissue. If the membrane were too soft to collapse into the defect area, the space for bone regeneration would reduce; thus, an optimal clinical outcome would fail to be attained [5]. Fig. 12(b) illustrated the mechanical properties of typical non-resorbable membranes (Ti, PTFE membrane), resorbable polymer membranes, developing Mg membrane [65] and Zn membrane. As can be seen, resorbable natural polymers [66,67] showed low

mechanical strength, at most 50 MPa. Resorbable synthetic polymers [68–71] exhibited improved elongation and similar strength. Comparing the metallic membranes, pure Zn displayed relative lower strength but favorable plasticity. However, the in vivo animal testing results (Fig. 7) demonstrated that the pure Zn membrane seemed to provide sufficient mechanical support during healing period. In this regard, the initial mechanical property of GBR membrane may not need to be particularly high, as long as the basic requirements are met.

4.1.3. Biodegradability

To refrain from secondary surgery, resorbable membranes have been developed extensively in recent years. An ideal biodegradable material or device should fit perfectly to the healing process of damaged tissues, provide sufficient mechanical support during the repair stage, and completely dissolve in a longer period of time, with the acceptable degradation rate for human body to withstand [72]. Therefore, for a potential biodegradable barrier membrane, the change of mechanical property during implantation time is a critical factor to be monitored. Fig. 12(c) summarizes the mechanical loss time and total degradation time of some typical type GBR membranes. The natural biodegradable polymers, including the collagen-based [67], chitosan-based [18,73] and alginate-based membranes [74,75], exhibit relative low mechanical properties, with initial strength in the range of 10–30 MPa. However, a significant loss of mechanical strength of the natural polymer membranes occurred for up to 8 weeks and usually degraded completely in about 12 weeks [76–78]. For the synthetic biodegradable polymers, such as polyesters-based polymer membranes, their mechanical strength and strength maintenance are improved by various means [79–81]. Nevertheless, the mechanical integrity loss still proceed prematurely, despite their adequate long full degradation cycle [82]. For the zinc-based membrane, the initial tensile strength is significantly superior to that of polymers. During the whole implantation time, it displayed a relative uniform degradation mode (Fig.11) and better mechanical integrity, manifesting potential for a novel barrier membrane.

4.1.4. Clinical manageability

The membrane should also be easy to handle and adequately malleable to allow bending, contouring and cutting to fit various bone defect area [5], related to material ductility. Meanwhile, it

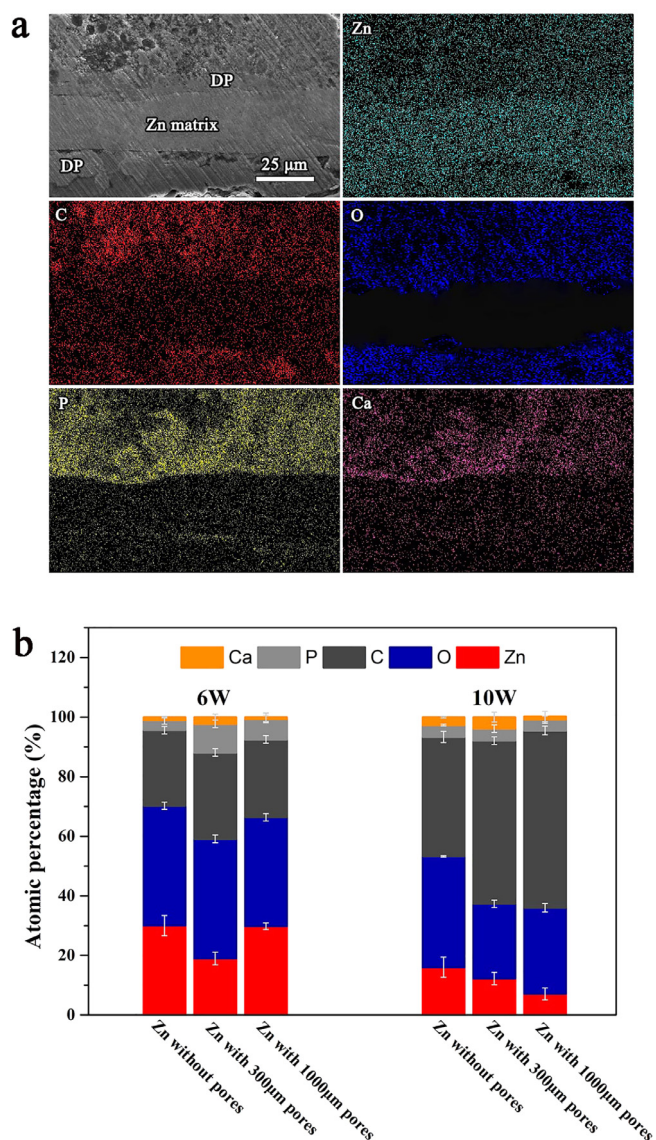


Fig. 11. EDS mapping and analysis of zinc membrane degradation from a cross-section view. (a) Representative EDS mapping of zinc membrane after 10 weeks of implantation. (b) Atomic percentages of degradation products deposited on surfaces of three kind experimental zinc membranes. (Zinc (green), Carbon (red), Oxygen (blue), Phosphate (yellow), Calcium (magenta), DP (degradation products)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ought to be rigid enough to maintain the space for bone regeneration. Therefore, an equilibrium between these mechanical properties is required to attain pleasing operability. For pure Zn membranes in this work, they were cut into pieces easily, sterilized, and attached to the margin of bone defect areas readily without displacement during operation, demonstrating favorable clinical manageability.

In addition, an appropriate pore size is reported to play a role in desirable occlusivity of GBR membrane [83,84]. The property of occlusivity refers to obstruction of the ingrowth of fibrous tissues into bone defect site while allow the transfer of oxygen, fluids and other nutrients in the favor of bone regeneration, which is correlated with membrane plasticity and porosity [52]. Current clinical used titanium mesh membranes are generally designed with macroporosity, which is thought to enhance wound stability by improving tissue integration and ensure nutrients diffusion [84]. Furthermore, other researchers indicated that more bone formed in

healing area when increasing the internodal distance from 8 µm to 100–300 µm in the PTFE domes, which assumed to permit soft-tissue invasion [43]. Besides, placement of e-PTFE containing 300 µm pores associated with Ti implants was shown to provide adequate space and significant vertical bone augmentation [44]. In this work, the pure Zn membrane with 300 µm pores manifested prominent osteogenic capability, comparable to titanium (Fig. 7). Indeed, the optimal pore size and porosity of membranes are still obscure, which could vary from macro-pores to micro-pores with different materials; therefore, further investigation is required to determine whether the membrane has to be porous, and the role of membrane porosity during bone regeneration.

4.2. Comparisons between the performance of zinc membrane and previously reported GBR membranes

Hitherto, various type membranes have been developed, including nonresorbable and resorbable membranes. The present study focused on experimental studies on pure zinc membranes with different porosities and solid titanium. Although we have not compared zinc with other clinically used materials, an early but short discussion on the possible pros and cons of zinc in relation to other clinically available membrane materials, is considered appropriate (Fig. 12). Natural polymers such as collagen-based membranes are commonly used for regenerative purposes in the clinic. Nevertheless, the degradation rate of collagen membranes has been considered more rapid than that of bone regeneration [85]. As a result, it is unable to ensure sufficient space maintenance ascribing to unpredictable degradation rate and thus premature loss of mechanical integrity, which would affect the bone repair process prominently. Therefore, collagen membrane is mainly applicable to small bone defect repair. In addition, the high cost is unfriendly to patients [86]. The same is true of biodegradable synthetic polymers. Despite their improved mechanical properties, slower degradation rates and acceptable biocompatibility [87], the release of acid byproducts and oligomers might induce remarkable inflammation reaction in vivo during implantation time [88], which is still an obstacle for synthetic polymer membranes.

Magnesium and its alloys, as one type of the promising biodegradable metals for orthopedics [89–92] and cardiovascular [93–95] applications, have drawn much public attention over the years, due to favorable biocompatibility and biodegradability. Lately, some researchers focus on their potential for dentistry application, such as acting as a GBR membrane for bone repair [49,65,96]. Peng et al. [96] investigated the calcium-phosphate-coated Mg membrane for barrier membrane application, demonstrating the neoosteogenesis capability of the membrane but too rapid degradation. The degradation of Mg causes localized corrosion, abrupt rise of local pH value and Mg^{2+} concentration, as well as hydrogen gas generation [91,97]. Small gas cavities might affect little on the body system, because of quick transfer with surrounding environment [98], whereas large gas cavities with excessive pressure could induce callus formation and disturb bone regeneration, and even decrease survival rate in some more serious cases [99]. Moreover, in order to satisfy demand for sufficient mechanical strength, Mg membranes are generally thick due to limitation of intrinsic structure, which is adverse to tissue integration. For zinc membrane fabricated in this paper, the degradation rate is more moderate than Mg, with milder increase of pH value, negligible gas production, and comparable bone regeneration.

In Fig. 12d, a summary of the known properties of used materials in a clinical context is provided. It should be noted that the properties attributed to zinc are based on the limited experimental results in the present study and therefore have not yet been shown in the clinic. Although promising, our results indicate the need for further studies and verifications of zinc as a new in-

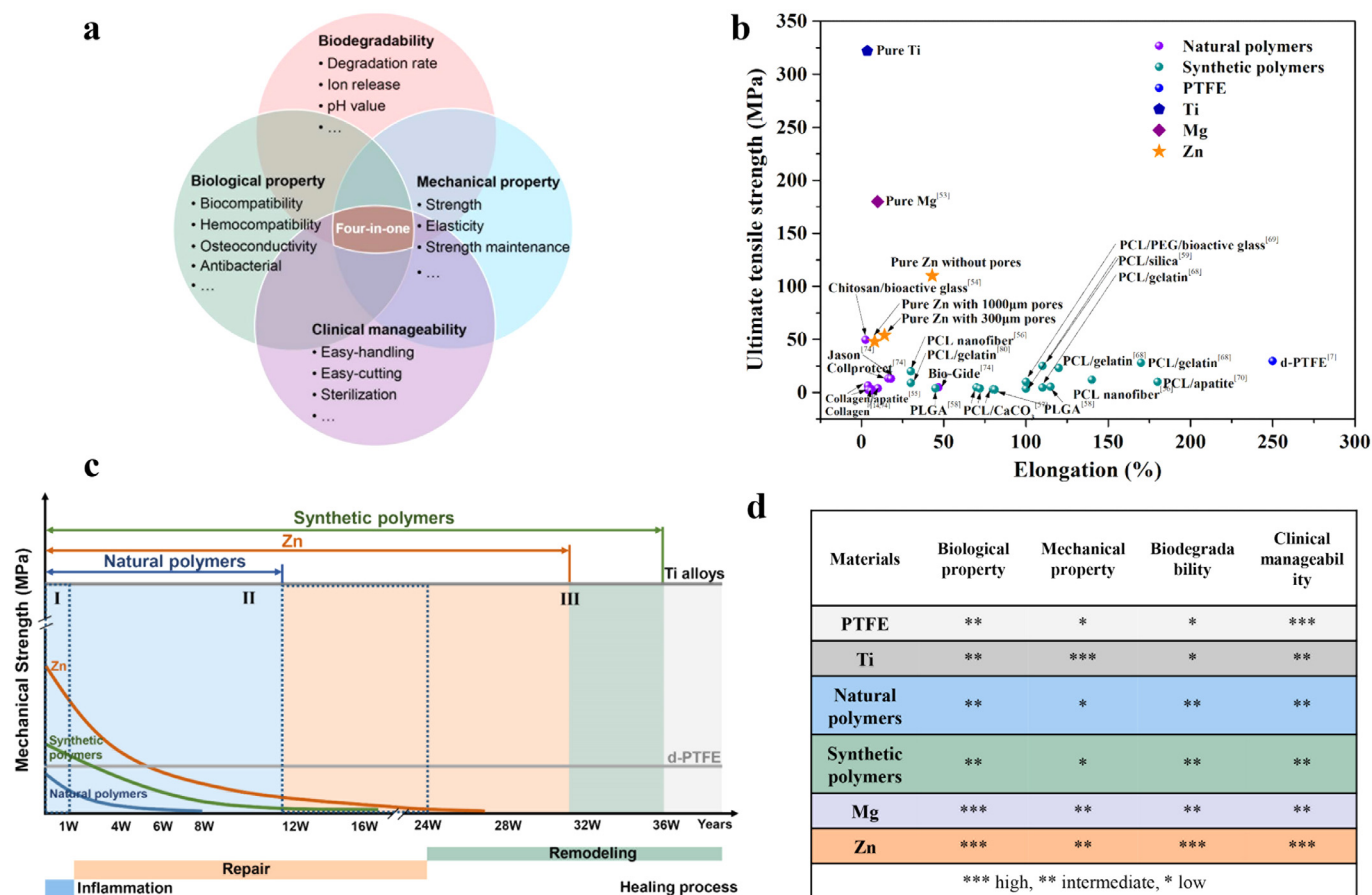


Fig. 12. (a) Schematic diagram of four main criteria that an ideal GBR membrane should fulfill. (b) Illustration of mechanical properties of different types of GBR membranes, including permanent membranes (Ti, D-PTFE), natural and synthetic resorbable polymers, new-developing Mg-based and Zn-based membranes. (c) Schematic diagram of mechanical loss time and total degradation time of different types of GBR membranes. (d) Comprehensive performance evaluation for pure Zn membrane and other currently-reported GBR membranes according to the criteria. Each criterion was ranked as *** high, ** intermediate, * low.

teresting material for GBR applications. There are potential drawbacks and limitations with the current zinc material as used by us. One example is that the complete degradation of the pure Zn membrane may take about 8 months, in terms of the degradation rate (0.044 ± 0.003 mm/year) obtained from this research. In future work, we will reduce the thickness of zinc membrane on the premise of ensuring adequate mechanical properties. The mechanical property can be further improved by alloying with elements such as Li and Mg [21,32,100], and the degradation rate can be accelerated by micro-oxidation treatment [101]. Furthermore, surface modifications [102] can also be applied to enhance functional properties of zinc-based membranes, such as osteogenic and antibacterial ability. In addition, the mechanisms underlying interactions between the degradation of zinc membrane and related cells, and the metabolic pathway of degradation products are insufficiently explained and need further exploration in detail.

5. Conclusions

In the present study, we first fabricated three kinds of pure zinc membranes, without pores, with 300 μm and 1000 μm diameter pores, to explore their feasibility as guided bone regeneration (GBR) membranes. The main conclusions can be drawn as follows:

- Pure zinc membrane without pores exhibited favorable mechanical property, with ultimate tensile strength (UTS) of 108 ± 4.87 MPa and $42.82 \pm 2.69\%$ elongation. Laser cutting reduced the mechanical properties of the membranes.

- Pure zinc membrane without pores and with 300 μm pores showed a relatively suitable degradation rates in vitro degradation tests, while the pure zinc membrane with 1000 μm pores degraded quickly and collapsed after 60 days immersion.
- Pure zinc membrane with 300 μm pores displayed acceptable MC3T3-E1 cytocompatibility in vitro and favorable osteogenic ability in vivo, demonstrating promising potential application in GBR membrane for bone regeneration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] J.K. Park, J. Yeom, E.J. Oh, M. Reddy, J.Y. Kim, D.-W. Cho, H.P. Lim, N.S. Kim, S.W. Park, H.-I. Shin, D.J. Yang, K.B. Park, S.K. Hahn, Guided bone regeneration by poly(lactic-co-glycolic acid) grafted hyaluronic acid bi-layer films for periodontal barrier applications, *Acta Biomater.* 5 (9) (2009) 3394–3403.
- [2] J.-T. Kaliterna, N.A.N. Nik Malek, M.R. Abdul Kadir, In vitro degradation of triple layered poly (lactic-co-glycolic acid) composite membrane composed of nanoapatite and lactic acid for guided bone regeneration applications, *Mater. Chem. Phys.* 221 (2019) 501–514.
- [3] A. Turri, I. Elgali, F. Vazirani, A. Johansson, L. Emanuelsson, C. Dahlin, P. Thomsen, O. Omar, Guided bone regeneration is promoted by the molecular events in the membrane compartment, *Biomaterials* 84 (2016) 167–183.
- [4] P. Gentile, V. Chiono, C. Tonda-Turo, A.M. Ferreira, G. Ciardelli, Polymeric membranes for guided bone regeneration, *Biotechnol. J.* 6 (10) (2011) 1187–1197.
- [5] Y.D. Rakhmatia, Y. Ayukawa, A. Furuhashi, K. Koyano, Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications, *J. Prosthodont. Res.* 57 (1) (2013) 3–14.
- [6] T. Korzinskas, O. Jung, R. Smeets, S. Stojanovic, S. Najman, K. Glenske, M. Hahn, S. Wenisch, R. Schnettler, M. Barbeck, In vivo analysis of the biocompatibility and macrophage response of a non-resorbable PTFE membrane for guided bone regeneration, *Int. J. Mol. Sci.* 19 (10) (2018).
- [7] M. Trobos, A. Juhlin, F.A. Shah, M. Hoffman, H. Sahlin, C. Dahlin, In vitro evaluation of barrier function against oral bacteria of dense and expanded polytetrafluoroethylene (PTFE) membranes for guided bone regeneration, *Clin. Implant. Dent. Relat. Res.* 20 (2018) 738–748.
- [8] A. Cucchi, E. Vignudelli, A. Napolitano, C. Marchetti, G. Corinaldesi, Evaluation of complication rates and vertical bone gain after guided bone regeneration with non-resorbable membranes versus titanium meshes and resorbable membranes. A randomized clinical trial, *Clin. Implant. Dent. Relat. Res.* 19 (5) (2017) 821–832.
- [9] S. Her, T. Kang, M.J. Fien, Titanium mesh as an alternative to a membrane for ridge augmentation, *J. Oral Maxillofac. Surg.* 70 (4) (2012) 803–810.
- [10] P.J. Temprow, J. Nalbandian, Colonization of retrieved polytetrafluoroethylene membranes: morphological and microbiological observations, *J. Periodontol.* 64 (3) (1993) 162–168.
- [11] D. Buser, U. Bragger, N.P. Lang, S. Nyman, Regeneration and enlargement of jaw bone using guided tissue regeneration, *Clin. Oral Implant. Res.* (1) (1990) 22–32.
- [12] V.W.J. Wecke, S. Fath, J.-P. Bernimoulin, The occurrence of trepanomes and their spherical bodies on polytetrafluoroethylene membranes, *Oral Microbiol. Immunol.* 10 (1995) 278–283.
- [13] M.C. Bottino, V. Thomas, G. Schmidt, Y.K. Vohra, T.M. Chu, M.J. Kowolik, G.M. Janowski, Recent advances in the development of GTR/GBR membranes for periodontal regeneration—a materials perspective, *Dent. Mater.* 28 (7) (2012) 703–721.
- [14] E. Zhang, C. Zhu, J. Yang, H. Sun, X. Zhang, S. Li, Y. Wang, L. Sun, F. Yao, Electrospun PLLA/PLGA composite membranes for potential application in guided tissue regeneration, *Mater. Sci. Eng. C. Mater. Biol. Appl.* 58 (2016) 278–285.
- [15] R. Shi, J. Xue, H. Wang, R. Wang, M. Gong, D. Chen, L. Zhang, W. Tian, Fabrication and evaluation of a homogeneous electrospun PCL–gelatin hybrid membrane as an anti-adhesion barrier for craniectomy, *J. Mater. Chem. B* 3 (19) (2015) 4063–4073.
- [16] M.A. Basile, G.G. d'Ayala, M. Malinconico, P. Laurienzo, J. Coudane, B. Nottelet, F.D. Ragione, A. Oliva, Functionalized PCL/HA nanocomposites as microporous membranes for bone regeneration, *Mater. Sci. Eng. C. Mater. Biol. Appl.* 48 (2015) 457–468.
- [17] Z. Sheikh, J. Qureshi, A.M. Alshahrani, H. Nassar, Y. Ikeda, M. Glogauer, B. Ganss, Collagen based barrier membranes for periodontal guided bone regeneration applications, *Odontology* 105 (1) (2017) 1–12.
- [18] M.J.A. Echazú, M.V. Tuttolomondo, M.L. Foglia, A.M. Mebert, G.S. Alvarez, M.F. Desimone, Advances in collagen, chitosan and silica biomaterials for oral tissue regeneration: from basics to clinical trials, *J. Mater. Chem. B* 4 (43) (2016) 6913–6929.
- [19] S. Turkan, A.E. Pazarcevir, D. Keskin, N.E. Machin, O. Duygulu, A. Tezcaner, Nanosized CaP-silk fibroin-PCL-PEG-PCL based bilayer membranes for guided bone regeneration, *Mater. Sci. Eng. C. Mater. Biol. Appl.* 80 (2017) 484–493.
- [20] D.G.K. Jie Liu, Mechanisms of guided bone regeneration: a review, *Open Dent. J.* 8 (2014) 56–65.
- [21] H. Li, H. Yang, Y. Zheng, F. Zhou, K. Qiu, X. Wang, Design and characterizations of novel biodegradable ternary Zn-based alloys with IIA nutrient alloying elements Mg, Ca and Sr, *Mater. Des.* 83 (2015) 95–102.
- [22] X. Yu, D. Zhao, S. Huang, B. Wang, X. Zhang, W. Wang, X. Wei, Biodegradable magnesium screws and vascularized iliac grafting for displaced femoral neck fracture in young adults, *BMC Musculoskelet. Disord.* 16 (2015) 329.
- [23] P. Han, P. Cheng, S. Zhang, C. Zhao, J. Ni, Y. Zhang, W. Zhong, P. Hou, X. Zhang, Y. Zheng, Y. Chai, In vitro and in vivo studies on the degradation of high-purity Mg (99.99 wt.%) screw with femoral intracondylar fractured rabbit model, *Biomaterials* 64 (2015) 57–69.
- [24] N.S. Murni, M.S. Dambatta, S.K. Yeap, G.R. Froemming, H. Hermawan, Cytotoxicity evaluation of biodegradable Zn–3Mg alloy toward normal human osteoblast cells, *Mater. Sci. Eng. C. Mater. Biol. Appl.* 49 (2015) 560–566.
- [25] H. Yang, C. Wang, C. Liu, H. Chen, Y. Wu, J. Han, Z. Jia, W. Lin, D. Zhang, W. Li, W. Yuan, H. Guo, H. Li, G. Yang, D. Kong, D. Zhu, K. Takashima, L. Ruan, J. Nie, X. Li, Y. Zheng, Evolution of the degradation mechanism of pure zinc stent in the one-year study of rabbit abdominal aorta model, *Biomaterials* 145 (2017) 92–105.
- [26] W. Lin, L. Qin, H. Qi, D. Zhang, G. Zhang, R. Gao, H. Qiu, Y. Xia, P. Cao, X. Wang, W. Zheng, Long-term in vivo corrosion behavior, biocompatibility and bioreabsorption mechanism of a bioresorbable nitrided iron scaffold, *Acta Biomater.* 54 (2017) 454–468.
- [27] T. Kraus, F. Moszner, S. Fischerauer, M. Fiedler, E. Martinelli, J. Eichler, F. Witte, E. Willbold, M. Schinhammer, M. Meischel, P.J. Uggowitzer, J.F. Löffler, A. Weinberg, Biodegradable Fe-based alloys for use in osteosynthesis: outcome of an in vivo study after 52 weeks, *Acta Biomater.* 10 (7) (2014) 3346–3353.
- [28] J. Liu, B. Zheng, P. Wang, X. Wang, B. Zhang, Q. Shi, T. Xi, M. Chen, S. Guan, Enhanced in vitro and in vivo performance of Mg–Zn–Y–Nd alloy achieved with apes pretreatment for drug-eluting vascular stent application, *ACS Appl. Mater. Interfaces* 8 (28) (2016) 17842–17858.
- [29] Y. Shi, L. Zhang, J. Chen, J. Zhang, F. Yuan, L. Shen, C. Chen, J. Pei, Z. Li, J. Tan, G. Yuan, In vitro and in vivo degradation of rapamycin-eluting Mg–Nd–Zn–Zr alloy stents in porcine coronary arteries, *Mater. Sci. Eng. C. Mater. Biol. Appl.* 80 (2017) 1–6.
- [30] M. Dermience, G. Lognay, F. Mathieu, P. Goyens, Effects of thirty elements on bone metabolism, *J. Trace Elements Medicine Biol.* 32 (2015) 86–106.
- [31] H. Yang, X. Qu, W. Lin, C. Wang, D. Zhu, K. Dai, Y. Zheng, In vitro and in vivo studies on zinc-hydroxyapatite composites as novel biodegradable metal matrix composite for orthopedic applications, *Acta Biomater.* 71 (2018) 200–214.
- [32] E. Mostaed, M. Sikora-Jasinska, J.W. Drelich, M. Vedani, Zinc-based alloys for degradable vascular stent applications, *Acta Biomater.* 71 (2018) 1–23.
- [33] P.K. Bowen, E.R. Shearier, S. Zhao, R.J. Guillory, F.Z. 2nd, J. Goldman, J.W. Drelich, Biodegradable metals for cardiovascular stents: from clinical concerns to recent Zn-alloys, *Adv. Healthc. Mater.* 5 (10) (2016) 1121–1140.
- [34] H.J. Seo, Y.E. Cho, T. Kim, H.I. Shin, I.S. Kwun, Zinc may increase bone formation through stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblastic MC3T3-E1 cells, *Nutr. Res. Pract.* 4 (5) (2010) 356–361.
- [35] S.I.S.H. Storriea, Cellular response to zinc-containing organoapatite: an in vitro study of proliferation, alkaline phosphatase activity and biomineralization, *Biomaterials* 26 (2005) 5492–5499.
- [36] C.J. Frederickson, J.Y. Koh, A.I. Bush, The neurobiology of zinc in health and disease, *Nat. Rev. Neurosci.* 6 (6) (2005) 449–462.
- [37] M. Yamaguchi, Role of zinc in bone formation and bone resorption, *J. Trace Elements Exp. Med.* 11 (2–3) (1998) 119–135.
- [38] L. Dupoirieux, D. Pourquier, M.C. Picot, M. Neves, Comparative study of three different membranes for guided bone regeneration of rat cranial defects, *Int. J. Oral. Maxillofac. Surg.* 30 (1) (2001) 58–62.
- [39] R. Patwa, H. Herfurth, C. Bratt, M. Christophersen, B.F. Philips, Laser drilling of micro-hole arrays in tantalum, *J. Laser Appl.* 27 (S2) (2015) S28006.
- [40] A. Weck, T.H.R. Crawford, D.S. Wilkinson, H.K. Haugen, J.S. Preston, Laser drilling of high aspect ratio holes in copper with femtosecond, picosecond and nanosecond pulses, *Appl. Phys. A* 90 (3) (2007) 537–543.
- [41] C. Bosch, B. Melsen, K. Vargervik, Importance of the critical-size bone defect in testing bone-regenerating materials, *J. Craniofac. Surg.* 9 (1998) 310–316.
- [42] K.A. Schlegel, F.J. Lang, K. Donath, J.T. Kulow, J. Wiltfang, The monocortical critical size bone defect as an alternative experimental model in testing bone substitute materials, *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.* 102 (1) (2006) 7–13.
- [43] J.C.K. Jeffrey, O. Hollinger, The critical size defect as an experimental model to test bone repair materials, *J. Craniofac. Surg.* 2 (1990) 237–243.
- [44] M.N. Hassan, M.A. Yassin, S. Suliman, S.A. Lie, H. Gjengedal, K. Mustafa, The bone regeneration capacity of 3D-printed templates in calvarial defect models: a systematic review and meta-analysis, *Acta Biomater.* 91 (2019) 1–23.
- [45] J. Li, Y. Man, Y. Zuo, L. Zhang, C. Huang, M. Liu, Y. Li, In vitro and in vivo evaluation of a nHA/PA66 composite membrane for guided bone regeneration, *J. Biomater. Sci. Polym. Ed.* 22 (1–3) (2011) 263–275.
- [46] E.J. Lee, S.H. Teng, T.S. Jang, P. Wang, S.W. Yook, H.E. Kim, Y.H. Koh, Nanostructured poly(epsilon-caprolactone)-silica xerogel fibrous membrane for guided bone regeneration, *Acta Biomater.* 6 (9) (2010) 3557–3565.
- [47] J.K. Park, J. Yeom, E.J. Oh, M. Reddy, J.Y. Kim, D.W. Cho, H.P. Lim, N.S. Kim, S.W. Park, H.I. Shin, D.J. Yang, K.B. Park, S.K. Hahn, Guided bone regeneration by poly(lactic-co-glycolic acid) grafted hyaluronic acid bi-layer films for periodontal barrier applications, *Acta Biomater.* 5 (9) (2009) 3394–3403.
- [48] Y. Xue, M. Wu, Z. Liu, J. Song, S. Luo, H. Li, Y. Li, L. Jin, B. Guan, M. Lin, F. Chen, C. Jin, D. Liu, Y. Li, X. Zhang, In vitro and in vivo evaluation of chitosan scaffolds combined with simvastatin-loaded nanoparticles for guided bone regeneration, *J. Mater. Sci. Mater. Med.* 30 (4) (2019) 47.
- [49] R. Amberg, A. Elad, D. Rothamel, T. Fienitz, G. Szakacs, S. Heilmann, F. Witte, Design of a migration assay for human gingival fibroblasts on biodegradable magnesium surfaces, *Acta Biomater.* 79 (2018) 158–167.
- [50] I. Elgali, A. Turri, W. Xia, B. Norlindh, A. Johansson, C. Dahlin, P. Thomsen, O. Omar, Guided bone regeneration using resorbable membrane and different bone substitutes: early histological and molecular events, *Acta Biomater.* 29 (2016) 409–423.
- [51] M. Sanz, C. Dahlin, D. Apatzidou, Z. Artzi, D. Bozic, E. Calcilari, H. De Bruyn, H. Dommiss, N. Donos, P. Eickholz, J.E. Ellingsen, H.J. Haugen, D. Herrera, F. Lambert, P. Layrolle, E. Montero, K. Mustafa, O. Omar, H. Schliephake, Biomaterials and regenerative technologies used in bone regeneration in the

- craniomaxillofacial region: consensus report of group 2 of the 15th European workshop on periodontology on bone regeneration, *J. Clin. Periodontol.* 46 (Suppl 21) (2019) 82–91.
- [52] J. Caballe-Serrano, A. Munar-Frau, O. Ortiz-Puigpelat, D. Soto-Penaloza, M. Penarocha, F. Hernandez-Alfaro, On the search of the ideal barrier membrane for guided bone regeneration, *J. Clin. Exp. Dent.* 10 (5) (2018) e477–e483.
- [53] D. Zhu, Y. Su, M.L. Young, J. Ma, Y. Zheng, L. Tang, Biological responses and mechanisms of human bone marrow mesenchymal stem cells to Zn and Mg biomaterials, *ACS Appl. Mater. Interfaces* 9 (33) (2017) 27453–27461.
- [54] K. Huo, X. Zhang, H. Wang, L. Zhao, X. Liu, P.K. Chu, Osteogenic activity and antibacterial effects on titanium surfaces modified with Zn-incorporated nanotube arrays, *Biomaterials* 34 (13) (2013) 3467–3478.
- [55] K. Yusa, O. Yamamoto, M. Fukuda, S. Koyota, Y. Koizumi, T. Sugiyama, In vitro prominent bone regeneration by release zinc ion from Zn-modified implant, *Biochem. Biophys. Res. Commun.* 412 (2) (2011) 273–278.
- [56] Y. Yu, G. Jin, Y. Xue, D. Wang, X. Liu, J. Sun, Multifunctions of dual Zn/Mg ion co-implanted titanium on osteogenesis, angiogenesis and bacteria inhibition for dental implants, *Acta Biomater.* 49 (2017) 590–603.
- [57] G. Jin, H. Cao, Y. Qiao, F. Meng, H. Zhu, X. Liu, Osteogenic activity and antibacterial effect of zinc ion implanted titanium, *Colloids Surf. B* 117 (2014) 158–165.
- [58] K. Yusa, O. Yamamoto, M. Iino, H. Takano, M. Fukuda, Z. Qiao, T. Sugiyama, Eluted zinc ions stimulate osteoblast differentiation and mineralization in human dental pulp stem cells for bone tissue engineering, *Arch. Oral Biol.* 71 (2016) 162–169.
- [59] Z.S. Tao, W.S. Zhou, X.W. He, W. Liu, B.L. Bai, Q. Zhou, Z.L. Huang, K.K. Tu, H. Li, T. Sun, Y.X. Lv, W. Cui, L. Yang, A comparative study of zinc, magnesium, strontium-incorporated hydroxyapatite-coated titanium implants for osseointegration of osteopenic rats, *Mater. Sci. Eng. C Mater. Biol. Appl.* 62 (2016) 226–232.
- [60] G. Jin, H. Qin, H. Cao, S. Qian, Y. Zhao, X. Peng, X. Zhang, X. Liu, P.K. Chu, Synergistic effects of dual Zn/Ag ion implantation in osteogenic activity and antibacterial ability of titanium, *Biomaterials* 35 (27) (2014) 7699–7713.
- [61] J. Ma, N. Zhao, D. Zhu, Endothelial cellular responses to biodegradable metal zinc, *ACS Biomater. Sci. Eng.* 1 (11) (2015) 1174–1182.
- [62] E.R. Shearier, P.K. Bowen, W. He, A. Drelich, J. Drelich, J. Goldman, F. Zhao, In vitro cytotoxicity, adhesion, and proliferation of human vascular cells exposed to zinc, *ACS Biomater. Sci. Eng.* 2 (4) (2016) 634–642.
- [63] K. Ishikawa, Y. Miyamoto, T. Yasab, A. Itoc, M. Nagayama, K. Suzuki, Fabrication of Zn containing apatite cement and its initial evaluation using human osteoblastic cells, *Biomaterials* 23 (2002) 423–428.
- [64] X. Luo, D. Barbieri, N. Davison, Y. Yan, J.D. de Bruijn, H. Yuan, Zinc in calcium phosphate mediates bone induction: in vitro and in vivo model, *Acta Biomater.* 10 (1) (2014) 477–485.
- [65] S.H. Byun, H.K. Lim, S.M. Kim, S.M. Lee, H.E. Kim, J.H. Lee, The bioresorption and guided bone regeneration of absorbable hydroxyapatite-coated magnesium mesh, *J. Craniofac. Surg.* (2017).
- [66] J. Mota, N. Yu, S.G. Caridade, G.M. Luz, M.E. Gomes, R.L. Reis, J.A. Jansen, X.F. Walboomers, J.F. Mano, Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration, *Acta Biomater.* 8 (11) (2012) 4173–4180.
- [67] J.H. Song, H.E. Kim, H.W. Kim, Collagen-apatite nanocomposite membranes for guided bone regeneration, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 83 (1) (2007) 248–257.
- [68] J. Xue, M. He, Y. Niu, H. Liu, A. Crawford, P. Coates, D. Chen, R. Shi, L. Zhang, Preparation and in vivo efficient anti-infection property of GTR/GBR implant made by metronidazole loaded electrospun polycaprolactone nanofiber membrane, *Int. J. Pharm.* 475 (1–2) (2014) 566–577.
- [69] K. Fujihara, M. Kotaki, S. Ramakrishna, Guided bone regeneration membrane made of polycaprolactone/calcium carbonate composite nano-fibers, *Biomaterials* 26 (19) (2005) 4139–4147.
- [70] I. Yoshimoto, J.I. Sasaki, R. Tsuboi, S. Yamaguchi, H. Kitagawa, S. Imazato, Development of layered PLGA membranes for periodontal tissue regeneration, *Dent. Mater.* 34 (3) (2018) 538–550.
- [71] A.G.B. Castro, M. Diba, M. Kersten, J.A. Jansen, J. van den Beucken, F. Yang, Development of a PCL-silica nanoparticles composite membrane for guided bone regeneration, *Mater. Sci. Eng. C Mater. Biol. Appl.* 85 (2018) 154–161.
- [72] Y.F. Zheng, X.N. Gu, F. Witte, Biodegradable metals, *Mater Sci Eng.* 77 (2014) 1–34.
- [73] C. Xu, C. Lei, L. Meng, C. Wang, Y. Song, Chitosan as a barrier membrane material in periodontal tissue regeneration, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 100 (5) (2012) 1435–1443.
- [74] H. Jiang, H. Hong, S. Lieping, G. Genghua, Comparison of calcium alginate film with collagen membrane for guided bone regeneration in mandibular defects in rabbits, *J. Oral Maxillofac. Surg.* 60 (12) (2002) 1449–1454.
- [75] H. He, J. Huang, F. Ping, G. Sun, G. Chen, Calcium alginate film used for guided bone regeneration in mandible defects in a rabbit model, *Cranio* 26 (1) (2008) 65–70.
- [76] D. Rothamel, F. Schwarz, M. Sager, M. Hertten, A. Sculean, J. Becker, Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat, *Clin. Oral Implants. Res.* 16 (3) (2005) 369–378.
- [77] S.B. Qasim, S. Najeeb, R.M. Delaine-Smith, A. Rawlinson, I. Ur Rehman, Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration, *Dent. Mater.* 33 (1) (2017) 71–83.
- [78] E.J. Lee, D.S. Shin, H.E. Kim, H.W. Kim, Y.H. Koh, J.H. Jang, Membrane of hybrid chitosan-silica xerogel for guided bone regeneration, *Biomaterials* 30 (5) (2009) 743–750.
- [79] M. Masoudi Rad, S. Nouri Khorasani, L. Ghasemi-Mobarakeh, M.P. Prabhakaran, M.R. Foroughi, M. Kharaziha, N. Saadatkish, S. Ramakrishna, Fabrication and characterization of two-layered nanofibrous membrane for guided bone and tissue regeneration application, *Mater. Sci. Eng. C Mater. Biol. Appl.* 80 (2017) 75–87.
- [80] K. Ren, Y. Wang, T. Sun, W. Yue, H. Zhang, Electrospun PCL/gelatin composite nanofiber structures for effective guided bone regeneration membranes, *Mater. Sci. Eng. C Mater. Biol. Appl.* 78 (2017) 324–332.
- [81] S. Soltani Dehnavi, M. Mehdikhani, M. Rafienia, S. Bonakdar, Preparation and in vitro evaluation of polycaprolactone/PEG/bioactive glass nanopowders nanocomposite membranes for GTR/GBR applications, *Mater. Sci. Eng. C Mater. Biol. Appl.* 90 (2018) 236–247.
- [82] F. Yang, S.K. Both, X. Yang, X.F. Walboomers, J.A. Jansen, Development of an electrospun nano-apatite/PCL composite membrane for gtr/gbr application, *Acta Biomater.* 5 (9) (2009) 3295–3304.
- [83] G. Zellin, A. Linde, Effects of different osteopromotive membrane porosities on experimental bone neogenesis in rats, *Biomaterials* 17 (7) (1996) 695–702.
- [84] R. Gutta, R.A. Baker, A.A. Bartolucci, P.J. Louis, Barrier membranes used for ridge augmentation: is there an optimal pore size? *J. Oral Maxillofac. Surg.* 67 (6) (2009) 1218–1225.
- [85] K.J. Shields, M.J. Beckman, G.L. Bowlin, J.S. Wayne, Mechanical properties and cellular proliferation of electrospun collagen type II, *Tissue Eng.* 10 (9–10) (2004) 1510–1517.
- [86] D.N. Tatakis, A. Promsudthi, U.M.E. Wikesjö, Devices for periodontal regeneration, *Periodontol.* 2000 19 (1999) 59–73.
- [87] J. Xue, M. He, H. Liu, Y. Niu, A. Crawford, P.D. Coates, D. Chen, R. Shi, L. Zhang, Drug loaded homogeneous electrospun PCL/gelatin hybrid nanofiber structures for anti-infective tissue regeneration membranes, *Biomaterials* 35 (34) (2014) 9395–9405.
- [88] J.E. Bergsma, W.C. de Bruijn, F.R. Rozema, R.R.M. Bos, G. Boering, Late degradation tissue response to poly(L-lactide) bone plates and screws, *Biomaterials* 16 (1995) 25–31.
- [89] S. Agarwal, J. Curtin, B. Duffy, S. Jaiswal, Biodegradable magnesium alloys for orthopaedic applications: a review on corrosion, biocompatibility and surface modifications, *Mater. Sci. Eng.* 68 (2016) 948–963.
- [90] F. Amerstorfer, S.F. Fischerauer, L. Fischer, J. Eichler, J. Draxler, A. Zitek, M. Meischel, E. Martinelli, T. Kraus, S. Hann, S.E. Stanzl-Tschegg, P.J. Unggowitz, J.F. Löffler, A.M. Weinberg, T. Prohaska, Long-term in vivo degradation behavior and near-implant distribution of resorbed elements for magnesium alloys WZ21 and ZX50, *Acta Biomater.* 42 (2016) 440–450.
- [91] D. Dziuba, A. Meyer-Lindenberg, J.M. Seitz, H. Waizy, N. Angrisani, J. Reifensath, Long-term in vivo degradation behaviour and biocompatibility of the magnesium alloy ZEK100 for use as a biodegradable bone implant, *Acta Biomater.* 9 (10) (2013) 8548–8560.
- [92] D. Tie, R. Guan, H. Liu, A. Cipriano, Y. Liu, Q. Wang, Y. Huang, N. Hort, An in vivo study on the metabolism and osteogenic activity of bioabsorbable Mg-1Sr alloy, *Acta Biomater.* 29 (2016) 455–467.
- [93] Y. Yue, L. Wang, N. Yang, J. Huang, L. Lei, H. Ye, L. Ren, S. Yang, Effectiveness of biodegradable magnesium alloy stents in coronary artery and femoral artery, *J. Interv. Cardiol.* 28 (4) (2015) 358–364.
- [94] E. Wittchow, N. Adden, J. Riedmüller, C. Savard, R. Waksman, M. Braune, Bioresorbable drug-eluting magnesium-alloy scaffold: design and feasibility in a porcine coronary model, *EuroIntervention* 8 (12) (2013) 1441–1450.
- [95] R. Waksman, R. Pakala, P.K. Kuchulakanti, R. Baffour, D. Hellinga, R. Seabron, F.O. Tio, E. Wittchow, S. Hartwig, C. Harder, R. Rohde, B. Heublein, A. Andrae, K.H. Waldmann, A. Haverich, Safety and efficacy of bioabsorbable magnesium alloy stents in porcine coronary arteries, *Catheter Cardiovasc. Interv.* 68 (4) (2006) 607–617 discussion 618–9.
- [96] W. Peng, J.-X. Chen, X.-F. Shan, Y.-C. Wang, F. He, X.-J. Wang, L.-L. Tan, K. Yang, Mg-based absorbable membrane for guided bone regeneration (GBR): a pilot study, *Rare Metals* 38 (6) (2019) 577–587.
- [97] Y. Yu, H. Lu, J. Sun, Long-term in vivo evolution of high-purity Mg screw degradation - Local and systemic effects of Mg degradation products, *Acta Biomater.* 71 (2018) 215–224.
- [98] J. Kuhlmann, I. Bartsch, E. Willbold, S. Schuchardt, O. Holz, N. Hort, D. Hoche, W.R. Heineman, F. Witte, Fast escape of hydrogen from gas cavities around corroding magnesium implants, *Acta Biomater.* 9 (10) (2013) 8714–8721.
- [99] D. Noviana, D. Paramitha, M.F. Ulum, H. Hermawan, The effect of hydrogen gas evolution of magnesium implant on the postimplantation mortality of rats, *J. Orthop. Translat.* 5 (2016) 9–15.
- [100] Y. Zhang, Y. Yan, X. Xu, Y. Lu, L. Chen, D. Li, Y. Dai, Y. Kang, K. Yu, Investigation on the microstructure, mechanical properties, in vitro degradation behavior and biocompatibility of newly developed Zn-0.8%Li-(Mg, Ag) alloys for guided bone regeneration, *Mater. Sci. Eng. C Mater. Biol. Appl.* 99 (2019) 1021–1034.
- [101] W. Yuan, B. Li, D. Chen, D. Zhu, Y. Han, Y. Zheng, Formation mechanism, corrosion behavior, and cytocompatibility of microarc oxidation coating on absorbable high-purity zinc, *ACS Biomater. Sci. Eng.* 5 (2) (2018) 487–497.
- [102] O. Omar, I. Elgali, C. Dahlin, P. Thomsen, Barrier membranes: more than the barrier effect? *J. Clin. Periodontol.* 46 (Suppl 21) (2019) 103–123.