In *vitro* and in *vivo* assessments of pure Zn and Zn-1Mg alloy as biodegradable metals

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Introduction: In recent years, magnesium and its alloys have attracted considerable attention as potential biodegradable implant materials [1,2]. However, the practical usage of biodegradable magnesium alloys faces some challenges. Firstly, their exceedingly fast corrosion rate would deteriorate and cause rapid reduction of mechanical integrity of the implants to bone before healing. In addition, the rapid corrosion causes an abrupt pH increase, which may damage the neighboring tissues, and bring about abrupt generation of H_2 gas [3,4]. Zinc is a more noble metal than Mg and is widely used as the alloying element in magnesium alloys to positively enhance the corrosion resistance and mechanical property. Zn is very important in the human body for biological functions as it is involved in various aspects of cellular metabolism. In this study, the in *vitro* and in *vivo* mechanical and corrosion performance, degradation behavior and biocompatibility of pure Zn and Zn-1Mg alloy have been studied.

Methods: A mixture of pure elements (>99.9 wt%) of Zn and Mg were melted in a high purity graphite crucible and cast into a steel mold. An X-ray diffractometer was employed to identify the crystal structure of the phases. The tensile tests were performed before and after immersion test. The immersion tests and electrochemical tests were carried out carried out in Hank's solution. Human osteosarcoma cells (MG63) were used in the *vitro* cell culture experiment. For the in *vivo* evaluation, the alloys were intramedullary implanted into 3-month-old male healthy C57BL/6 mice. Radiographic and micro-CT evaluation, histological evaluations and element distribution analysis were employed.

Results: Pure Zn has quite low tensile strength (10 MPa) and hardness (37 HV). Zn-1Mg alloy has enhanced tensile strength (175 MPa) and hardness (80 HV) as adding the Mg alloying element has refined the grain size and formed eutectic network (Zn and Mg_2Zn_{11}). After immersion test for 2 month, the tensile strength of Zn-1Mg alloy still remains 90%. The influence of Mg on the corrosion rate is smaller compared with the influence on the mechanical properties. The corrosion products mainly contain Zn and Mg hydroxides, carbonates and phosphates. The Zn-1Mg alloy shows better cytocompatibility than pure Zn, owing to the influence of the Mg element. For the indirect cell culture, cells cultured in Zn-1Mg alloy extracts exhibited higher cell viability than pure Zn; for the direct cell culture, cells cultured on the surface of pure Zn didn't spread out and remained in a sphere morphology after 1 day, however, cells cultured on the Zn-1Mg surface exhibited a well elongated, spindle-shaped morphology, stretching out filopodia. The in *vivo* test demonstrated that Zn-1Mg alloy maintained its central integrity after two months' implantation. No degraded debris was found around the metal alloy rods. Gas evolution wasn't observed throughout the whole implantation period. In the diaphyseal region new bone formation was observed in the fluorescence images of both the Zn-1Mg group and control group. A high density of Ca and P was found in the white fraction region within the bone tunnel, indicating the new bone formation, which is in accordance with the histological results. There was no obvious inflammation could be found around the Zn-1Mg rods and no mice died after the operation and throughout the whole implantation period.

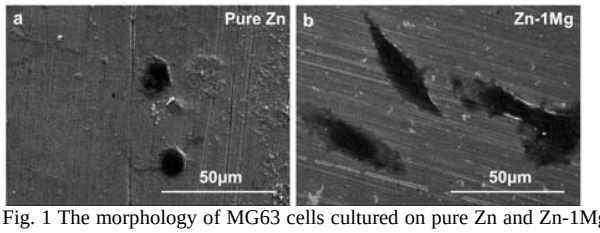

Fig. 1 The morphology of MG63 cells cultured on pure Zn and Zn-1Mg alloy samples.

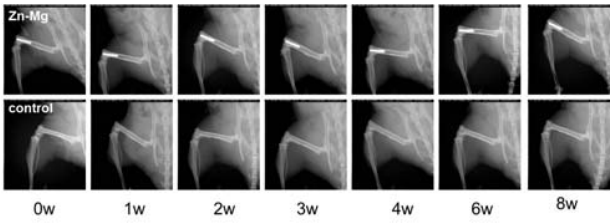

Fig. 2 Radiographs of mice distal femora with and without Zn-1Mg rod implants for different periods after surgery.

Conclusions: Zn-1Mg alloy shows much higher tensile strength and hardness compared with pure Zn, mainly due to grain refinement and second phase formation. The in *vitro* cell experiment indicated that Zn-Mg alloy exhibited better cytocompatibility and increased ALP activity than pure Zn. The in *vivo* results showed that the degrading rate of Zn-1Mg alloy is slow and still remain structure integrity after 2 months' implantation. The Zn-1Mg alloy can promote bone mineralization and peri-implant new bone formation without inducing any significant adverse effects. Therefore, the Zn-1Mg alloy is one of the promising degradable biomaterials for clinical application.

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