In vitro biodegradation and mechanical characteristics of a novel biliary stent made of magnesium alloy

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Abstract
BACKGROUND: Numerous evidence has demonstrated that the magnesium alloy with excellent mechanical properties can degrade in vivo, and can be used as vascular stent materials, bone fixation materials, porous materials for bone repair. But it is not reported in the biliary stent.

OBJECTIVE: To observe the degradation procedures and evaluate the changes of mechanical characteristics of biliary stents made of magnesium alloy (AZ 31B) in human bile in vitro.

METHODS: The balloon-expandable biliary stents were made of magnesium alloy with laser sculpture. Then, 15 stents treated with micro-arc oxidation on the surface served as experimental group and another 15 stents without micro-arc oxidation as control group. A dynamic numerical simulation system was established in vitro to simulate the internal environment of human biliary tract. All of the biliary stents were placed into this system. Their shapes were observed, and their qualities and radical forces were measured every 20 days. At the same time, their surfaces were scanned by scanning electron microscope.

RESULTS AND CONCLUSION: (1) The degradation speeds of the stents in the experimental group were obviously slower than those in the control group. The fracture of the connecting rods in control group and experimental group appeared 20 days and 40 days later, respectively. The peak time of degradation in the control group and experimental group were 30 days and 50 days, respectively. The stents were fully biodegraded within 70 days in the control group while within 90 days in the experimental group. With time, the stent surface became more rough, and the density, area and depth of etch pits were all increased in the two groups. However, the degradation speed of stents in the experimental group was much slower than that in the control group. In summary, the degradation speed of the biliary stents made of magnesium alloy treated with micro-arc oxidation is appropriate and can meet the clinical requirement for the degradation time of biliary stents. This novel material could potentially be used for the preparation of biliary stents.

Subject headings: biocompatible materials; magnesium; biliary tract; histocompatibility
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INTRODUCTION
Biliary stricture is a disease commonly and frequently occurring in hepatobiliary surgery, and it is also the thorniest issue for hepatobiliary surgeons. With the popularity of endoscopic techniques and interventional radiology techniques, biliary stent drainage is becoming the most important and simplest way for treatment of biliary stricture. It is widely accepted by the majority of doctors and patients because of less trauma, simple operation, draining effect, and low cost. Metal and plastic biliary stents commonly used are both permanent stents, which have many flaws that severely limit the widespread application of biliary stents. In recent years, a great progress has been made in the studies of biodegradable polymer scaffolds, but the scaffolds cannot be widely used clinically because of the poor mechanical performance. Therefore, choosing a biodegradable scaffold material with excellent mechanical properties is the main problem currently.

Growing studies have shown that the magnesium alloy with excellent mechanical properties can degrade in vivo, and its biological performance has been deeply analyzed as vascular stent materials, bone fixation materials, porous materials for bone repair in cerebrovascular and orthopedic fields [1-7], especially as vascular stent materials [8-9]. These stents made of magnesium alloy have been widely used in clinic [10-14] and obtained good clinical effects. But it is rarely reported as the biliary stent.
Here, we designed an appropriate biliary stent made of magnesium alloy that is suitable for human body according to the physical and anatomical characteristics of the biliary tract, and evaluated the stent performance by in vitro experiments to provide an experimental basis for further clinical application.

MATERIALS AND METHODS

Design
Randomized controlled, observational study.

Time and setting
The experiment was completed at the Surgery dreamworks, the First Affiliated Hospital, Xi’an Jiaotong University, from August 2013 to October 2013.

Materials
Magnesium alloy stent was provided by the Northwest Institute for Non-ferrous Metal Research.

Methods
Preparation process of balloon-expandable biliary stents made of magnesium alloy
Alloy tube billet forming→magnesium alloy tube heat treatment (vacuum heat treatment at 260–520 °C for 10–90 minutes, and then furnace cooling)→magnesium alloy billet surface treatment (washed with acid liquor, immersed in ethanol, and then dried)→laser settings (laser energy, spot size, speed and other parameters)→stent prepared using laser cutting under argon atmosphere→magnesium alloy stent grinding→magnesium alloy stent finishing→magnesium alloy stent surface treatment (magnesium alloy workpiece→chemical degreasing→washing→micro-arc oxidation→washing→postprocessing→inspection of finished product)→finished stent. The stent, 30 mm in length, 5 mm in outer diameter, 0.3 mm in thickness, was dried in vacuo, weighed, and sterilized by ethylene oxide via fumigation.

Stent grouping and handling
Thirty balloon-expandable biliary stents which were hollowed-out and engraved by laser were taken, including 15 bare stents and 15 stents undergoing surface micro-arc oxidation treatment. All these stents were divided into control group and experimental group. The biliary stents were dried in vacuum, weighed by a balance, and fumigated by ethylene oxide fumigation followed by implantation into the numerical simulation system. The stents were taken out at days 20, 40, 60 after the beginning of the experiment. The surface of stents was repeatedly washed with ethanol to remove surface deposits and corrosion products, and the stents were then placed in the oven at 37 °C for 24 hours. Thereafter, the stents were weighed and recorded. Three stents were randomly selected as electron microscopy specimens, and the remaining stents were placed again into the numerical simulation systems (Figure 1) and observed continuously.

Main outcome measures
(1) General morphology, integrity and color of the stents.
(2) Scanning electron microscope observation of surface cracks, crack size, ofetch pit density, area and depth, and other degradation conditions. (3) Measurement of stent dry mass. (4) Mechanical test (radial strength): A computer-controlled universal testing machine made in Shenzhen Sansi Technology Co., Ltd. was used to determine the radial support force of the stents using planar compression method (the radial force is recorded as the force when 1 mm displacement occurs) and to assess the supporting strength of the stents.

Statistical analysis
SPSS 13.0 statistical software was used for data processing, and the quality and radial force of the stents were expressed as mean ± SD.

RESULTS
Morphological changes of the stents in the two groups
(1) The variation in the general morphology of the stent in the two groups followed the same pattern: intact shape→connecting rod fracture→softened→tube wall collapse→cleaved into big fragments→cleaved into smaller fragments→completely degraded. (2) The degradation speeds of the stents in the experimental group were obviously slower than those in the control group. The fracture of the connecting rods in control group and experimental group appeared 20 days and 40 days later, respectively. The peak time of degradation in the control group and experimental group were 30 days and 50 days, respectively. The stents were fully biodegraded within 70 days in the control group while within 90 days in the experimental group (Figure 2). (3) Scanning electron microscope observation: after drying, the stent was amplified locally by the scanning electron microscope to understand the changes in the stent structure. Although the sediments and corrosion products on the stent surface had been removed, there was still a small amount of impurities on the specimen surface. The roughness, etch pit density, area and depth on the stent surface reflected the degree of stent degradation. With time, the stent surface became more rough, and the density, area and depth of etch pits were all increased in the two groups. At the same stage, the degradation speed of the control group was more rapid than that in the experimental group.
Figure 1  The schematic diagram and physical map of the dynamic numerical simulation system of the magnesium alloy biliary stent degrading in vitro in human bile

Note: (A) schematic diagram, (B) physical map. (1) Dynamic numerical simulation system consists of three parts, namely, peristaltic pump, stent container, connecting pipe. The peristaltic pump maintained the circulation of bile in the system to simulate the dynamic process of human bile duct. The temperature of water bath maintained at 37 ℃ to simulate the body temperature. The bile was exchanged daily, and the stent container was sterilized weekly to reduce the impact on biliary stent degradation caused by the bacteria reproduction and pH variation. (2) In the dynamic system, the bile was harvested from the drainage tube after laparoscopic choledochofiberscopic hepatocholangiolithotomy T-tube drainage; according to ASTM Standard G31-72, the ratio of the solution volume and surface area of the sample should be 20-40 mL/cm²[15]. During the experiment, the ratio was approximately 20 mL/cm², meeting the ASTM Standard G31-72. The bile in the dynamic system was required to be exchanged daily to ensure the fresh bile in the system.

Figure 2  General observation of magnesium alloy biliary stents treated with or without micro-arc oxidation that were degraded in human bile at different periods

Note: A-D represent the general morphology of magnesium alloy biliary stents with micro-arc oxidation treatment degraded at days 0, 20, 40, 60; E-H represent magnesium alloy biliary stents without micro-arc oxidation treatment degraded at days 0, 20, 40, 60. (1) The variation in the general morphology of the stent in the two groups followed the same pattern: intact shape→connecting rod fracture→softened→tube wall collapse→cleaved into big fragments→cleaved into smaller fragments→completely degraded. (2) The degradation speeds of the stents in the experimental group were obviously slower than those in the control group. The fracture of the connecting rods in control group and experimental group appeared 20 days and 40 days later, respectively. The peak time of degradation in the control group and experimental group were 30 days and 50 days, respectively. The stents were fully biodegraded within 70 days in the control group while within 90 days in the experimental group.
The corrosion resistance in the experimental group was better than that in the control group. The stent in the experimental group was more likely to be a biodegradable biliary stent (Figure 3).

Quality changes of the stents in the two groups
Over time, the quality of stents was reduced in both groups. There was no difference in mass loss in the two groups within 20 days, but after 20 days, the quality of the stents in the experimental groups was reduced slower than that in the control group. At 40 days of degradation, the qualities of stents in the experimental and control groups were reduced to 77.9% and 49.7% of the original quality. The stents were completely degraded within 70 days in the control group, while within 90 days in the experimental group (Figure 4).

Figure 3  Surface observation of magnesium alloy biliary stents treated with or without micro-arc oxidation that were degraded in human bile at different periods (× 1 000)
Note: A–D represent the partial enlarged view of magnesium alloy biliary stents with micro-arc oxidation treatment degraded at days 0, 20, 40, 60; E–H represent the partial enlarged view of magnesium alloy biliary stents without micro-arc oxidation treatment degraded at days 0, 20, 40, 60. With time, the stent surface became more rough, and the density, area and depth of etch pits were all increased in the two groups. At the same stage, the degradation speed of the control group was more rapid than that in the experimental group.

Figure 4  Quality changes of the magnesium alloy biliary stents treated with or without micro-arc oxidation that were degraded in human bile at different periods
Note: Over time, the quality of stents was reduced in both groups. There was no difference in mass loss in the two groups within 20 days, but after 20 days, the quality of the stents in the experimental groups was reduced slower than that in the control group. At 40 days of degradation, the qualities of stents in the experimental and control groups were reduced to 77.9% and 49.7% of the original quality. The stents were completely degraded within 70 days in the control group, while within 90 days in the experimental group.

Figure 5  Radial force changes of the magnesium alloy biliary stents treated with or without micro-arc oxidation that were degraded in human bile at different periods
Note: The radial force was reduced gradually with time in both groups, but faster for magnesium alloy biliary stents without micro-arc oxidation treatment. For the magnesium alloy biliary stents without micro-arc oxidation treatment, the radial force showed a rapid downtrend at early stage, the connecting rods were fractured at about 20 days, the wall was collapsed within 40 days, and the radial force was reduced to zero at 40 days. For the magnesium alloy biliary stents with micro-arc oxidation treatment, the connecting rods were fractured at about 40 days, the wall was collapsed within 70 days, and meanwhile, the radial force was reduced to zero.
Changes in the radial force in the two groups

The radial force was reduced gradually with time in both groups, but faster in the control group. In the control group, the radial force showed a rapid downtrend at early stage, the connecting rods were fractured at about 20 days, the wall was collapsed within 40 days, and the radial force was reduced to zero at 40 days. In the experimental group, the connecting rods were fractured at about 40 days, the wall was collapsed within 70 days, and meanwhile, the radial force was reduced to zero (Figure 5).

DISCUSSION

Magnesium is considered to be a green engineering material in the 21st century, and magnesium alloys are mixtures of magnesium with other metals. Because of excellent biocompatibility, good biodegradability, good mechanical support performance, easily obtained, and low prices, magnesium alloys have the potential to become a biodegradable material. However, the rapid degradation in vitro limits the clinical application of magnesium alloys. The corrosion resistance of magnesium alloys have been greatly improved by the development, alloying, rapid solidification technology, surface treatment (chemical conversion coating and organic layer) of high-purity magnesium. In this experiment, we improved the corrosion resistance of magnesium alloys by the surface micro-arc oxidation method. A large amount of studies have been done worldwide in the medical application of magnesium alloys as degradable biomaterials that have been widely used in cardiovascular medicine, orthopedics, stomatology and other fields. A foreign clinical study has shown that after magnesium alloy stent implantation, coronary angiography and intravascular ultrasound in patients with coronary artery stenosis showed that the incidence of vascular stenosis was significantly reduced, some narrow vessels were increased in diameter, and the blood supply was good, indicating the magnesium alloy stent implantation achieves good clinical efficacy. Zhang et al. implanted the magnesium alloy rods into the femur of New Zealand rabbits, and these researchers found that magnesium alloys can be used as biodegradable bone fixation materials with good biocompatibility and excellent mechanical properties. However, the magnesium alloys used in the biliary tract are rarely reported. Our experiment for the first time introduced magnesium alloys to the biliary tract, which was designed to observe the degradation rule of magnesium alloy stents in the bile and to assess the possibility of magnesium alloys used in the biliary tract.

*In vitro* experimental studies on the biliary stents previously reported mostly observed the stents in a static environment, and the dynamic environment of human biliary tract could not be properly simulated, leading to the deviation of the results of *in vitro* experiments. We adopted a dynamic numerical simulation system that could better simulate the biliary environment, and our experimental results were closer to the stent variation in the body. Currently, the biliary stents made of biodegradable polymer materials are only tubular stents. Here, we prepare the hollowed-out balloon-expandable biliary stents engraved by laser, which have good elastic memory function, is fitted well with the bile duct, and not prone to loss and displacement. The biliary stents *in vitro* can be compressed that are more easy to pass through the narrow part of the biliary tract, which is more convenient and safer.

Radial force of the stent refers to the radial resistance to external pressure or the strain force to external forces, which determines whether the stent has sufficient supporting role. The radial force is one of the most important indicators of the stents, directly affecting their clinical application. In this study, the universal testing machine for biomaterials was employed to measure the radial force using planar compression method, and the radial force was recorded when the stent displaced 1 mm. This method is simple, easy, and has small error. Our experimental findings showed that the radial force was decreased with degradation time, which was decreased slower in the experimental group than the control group, indicating the magnesium alloy biliary stents with micro-arc oxidation treatment are ongoing to maintain an effective support to meet the clinical requirements of the supporting force of biliary stents.

Compared with previous experimental studies, there is a lot of improvements in the present study. But further studies are required for the clinical application of magnesium alloys (AZ 31B) biliary stents. We used the dynamic numerical simulation system to simulate the human bile environment as far as possible in the following aspects, including biliary internal temperature, pressure, pH, flow rate and other physical and chemical factors. However, there is still a difference from the real human bile environment; to some extent, these differences affect the degradation of magnesium alloy stents. Therefore, animal experiments and clinical trials are necessary to learn more about the degradation of the stent, and improve the experimental data. Previous studies have evaluated the biological safety of magnesium alloys used in cardiovascular medicine and orthopedics, showing excellent biocompatibility. But there are no studies on the biocompatibility of magnesium alloy used in the biliary tract. During the gradual degradation of magnesium alloys in the biliary tract, how the degraded products are excreted out of the body and whether it leads to increased blood magnesium concentration and magnesium ion deposition in the local tissue is not clear. Magnesium alloy stents are firstly degraded into large fragments and further degraded into small fragments. Whether these small fragments become the core of biliary stones and then lead to the formation of stones should be further validated by *in vivo* experiments. Therefore, the further analysis of biological safety of magnesium alloys as biliary stents is necessary, which can provide further experimental basis for clinical application. In this study, the biliary stents were
30 mm in length, 5 mm in outer diameter, 0.3 mm in thickness, which show some differences from the biliary stents used in clinic. The stent size, mechanical properties and degradation law may have a significant impact, therefore which need further research. In this study, the balloon-expandable biliary stents were employed. The stents can be clinically implanted through retrograde cholangiopancreatography under the endoscope, which should be compressed first and open by balloon dilatation. During the implantation process, the stents can be damaged, resulting in changes in their mechanical properties, thereby affecting their function of the stent. Consequently, it should be further verified by clinical trials.

These results suggest that the magnesium alloy stents with micro-arc oxidation treatment can be degraded slower than the bare stents, to achieve the basic clinical requirements of biliary stent degradation time. Magnesium alloys are expected to become an ideal material for novel biodegradable biliary stents.

REFERENCES
新型可降解镁合金胆道支架的体外降解规律及力学性能

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目的：评估 AZ31B 镁合金胆道支架在体外胆汁中的降解过程及力学性能变化规律。

方法：将 AZ31B 镁合金激光镂空雕刻成囊球状可膨胀胆道支架，其中表面经微弧氧化处理的胆道支架和裸胆道支架各 15 枚，分别作为实验组和对照组。体外建立动态数值化模拟系统，模拟人体胆道系统，将两组支架置于模拟系统内，每 20 d 为一个观察单位，观察支架的形态、质量及径向支撑力，扫描电镜观察支架表面形态。

结果与结论：① 实验组较对照组降解速度明显减慢，对照组 20 d 开始出现连杆断裂，降解高峰在 30 d 左右，70 d 内完全降解；实验组 40 d 开始出现连杆断裂，降解高峰在 50 d 左右，90 d 内完全降解。两组随时间延长，支架表面变得粗糙，蚀坑密度增加，蚀坑面积增大。实验组相对对照组降解程度明显降低。② 实验组与对照组支架的质及径向支撑力随降解时间的延长逐渐下降，实验组较对照组下降速度明显减慢。表明 AZ31B 镁合金胆道支架经过表面微弧氧化处理后降解速度适宜，能够达到临床对胆道支架降解时间的要求。

关键词：生物材料；材料相容性；镁合金；胆道支架；生物可降解；径向支撑力。

主题词：生物相容性材料；镁；胆道；组织相容性。

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摘要：大量文献表明镁合金可在体内降解，具有优良的机械支撑性能，但未见其应用于胆道支架的报道。目的：评估 AZ31B 镁合金胆道支架在体外胆汁中的降解过程及力学性能变化规律。

方法：将 AZ31B 镁合金激光镂空雕刻成囊球状可膨胀胆道支架，其中表面经微弧氧化处理的胆道支架和裸胆道支架各 15 枚，分别作为实验组和对照组。体外建立动态数值化模拟系统，模拟人体胆道系统，将两组支架置于模拟系统内，每 20 d 为一个观察单位，观察支架的形态、质量及径向支撑力，扫描电镜观察支架表面形态。

结果与结论：① 实验组较对照组降解速度明显减慢，对照组 20 d 开始出现连杆断裂，降解高峰在 30 d 左右，70 d 内完全降解；实验组 40 d 开始出现连杆断裂，降解高峰在 50 d 左右，90 d 内完全降解。两组随时间延长，支架表面变得粗糙，蚀坑密度增加，蚀坑面积增大。实验组相对对照组降解程度明显降低。② 实验组与对照组支架的质及径向支撑力随降解时间的延长逐渐下降，实验组较对照组下降速度明显减慢。表明 AZ31B 镁合金胆道支架经过表面微弧氧化处理后降解速度适宜，能够达到临床对胆道支架降解时间的要求。

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学术术语：支架的径向支撑力是指支架对于其径向外压的抗力或对用于其外力的应变力，此特性决定支架是否具有足够的支撑作用，是支架最重要的指标之一。

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