

Biodegradation and mechanical characteristics of poly (lactic-co-glycolic acid) biliary stents *in vitro**☆

Han Bing, Yu Liang, Miao Shan, Liu Xue-min

Abstract

BACKGROUND: The biliary stents commonly used in clinic are made of plastics and metal, both of which have some irresolvable defects. New type of material characterizing by great biocompatibility and degradation for the making of biliary stents needs to be found and studied.

OBJECTIVE: To evaluate the biodegradation law and the change in radical force of five different mole-ratios of biliary stents made of poly (lactic-co-glycolic acid) (PLGA) in human bile *in vitro*.

DESIGN, TIME AND SETTING: A Completely random design was performed at the Laboratory of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University between July and December 2006.

MATERIALS: Five different mole-ratios of PLGA biliary stents (90L/10G, 80L/20G, 70L/30G, 60L/40G, and 50L/50G) were provided by Daigang Biological Science and Technology Co., Ltd., Jinan; Human fresh bile was obtained from the patients who underwent external drainage of bile in the Department of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University.

METHODS: Thirty-two PLGA biliary stents of each proportion were respectively divided into eight groups, with four stents in each group. All of them were independently submerged into test tubes with 10 mL fresh human bile which were then placed in constant temperature oscillator. The temperature was set at 37 °C. The bile was changed every day. After incubated for one week to eight weeks, the PLGA biliary stents were respectively taken out of test tubes from the first to the eighth group each week. They were washed with distilled water and dried in calorstat for 24 hours.

MAIN OUTCOME MEASURES: ① Gross morphology, color and integrality of PLGA stents; ② Scanning electron microscope; ③ the mass and the radical force of PLGA stents.

RESULTS: ① Five different mole-ratios of PLGA stents followed a similar changing law as to gross morphology: stents remained intact → stents became soft → the surface of stents became coarse and cracked → stents collapsed → stents were degraded into pieces → stents were completely degraded. ② Before degradation: limpid stereo reticular structure of PLGA; One week after degradation: PLGA was corroded and the reticular structure was decreased; Three weeks after degradation: airspace was gradually disappeared and the reticular structure was further decreased; Five weeks after degradation: airspace and the reticular structure were completely damaged, which was in an indefinite form. ③ The mass loss of each proportion of PLGA stents gradually speeded up along with the time extension. Furthermore, with the content of PGA increasing, the mass loss of the stents became faster. The initial radical force was different among five different mole-ratios of biliary stents. With the content of PLA increasing, the radical force of the stents became stronger.

CONCLUSION: The material of PLGA possesses adjustable biodegradation and good mechanical feature, so it is suitable for preparation of biliary stents.

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INTRODUCTION

Biliary stents are now widely used in the operations of bile duct, relieving obstruction and retaining the fine drainage of bile. They have played an important part in the prevention and cure of benign or malignant biliary stricture^[1]. The biliary stents commonly used in clinic are made of plastics and metal. However, both of them have some defects caused by the material itself^[2-4]. Poly (lactic-co-glycolic acid) (PLGA) is a new type of high polymer material which consists of PLA and PGA copolymerized in certain proportion^[5]. It is a biodegradable material and possesses good compatibility.

Reportedly, PLGA has an extensive application prospect in the areas of orthopedics, stomatology and urinary surgery^[6-10] and it can also be made into microparticles or nanoparticles to be the carrier of delivering drugs^[11-13]. Chepurov *et al*^[14] used biodegradable SR-PLGA ureteral stents in treating patients with infravesical obstruction. Introduction of this kind of stents provided adequate spontaneous urination, allowed avoiding external drainage and lowered the number of inflammatory complications.

It has also been confirmed by domestic scholar that PLGA ureteral stents possess good biocompatibility and ideal support performance^[15-16]. However, the research on PLGA stents in the treatment of biliary diseases is still less at present. In this study, self-made PLGA biliary stents of different proportions were put in human bile duct *in vitro* and the biodegradation law and the change in radical force were measured, aiming at investigating the feasibility of clinical use.

MATERIALS AND METHODS

Design

A randomized study.

Time and setting

This study was performed at the Laboratory of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University between July and December 2006.

Materials

Human fresh bile was obtained from the patients who underwent external drainage of bile in the Department of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University. All the

patients have signed the informed consent form for the research.

Main material	Source
Five different mole-ratios of PLGA biliary stents (90L/10G, 80L/20G, 70L/30G, 60L/40G, 50L/50G)	Daigang Biological Science and Technology Co., Ltd., Jinan
Constant temperature shaker	LKB Company, Sweden
Asepsis drying oven	No.4 Medical Equipment Factory, Shanghai
TMC-2 electronic balance	Western Suburbs Medical Equipment Factory, Beijing
S-450 scanning electron microscope	Olympus Corporation, Japan

Methods

Preparation of PLGA biliary stents

Five different mole-ratios (90L/10G, 80L/20G, 70L/30G, 60L/40G, and 50L/50G) of PLGA materials (molecular weight 100 000) were dissolved in dichloromethane, and then the solution was cast into a ring-shaped mould. After volatilizing at room temperature, PLGA biliary stents were made (1.2 cm length, 0.6 cm external diameter, and 0.1 cm thickness).

Grouping and treatment

Thirty-two PLGA biliary stents of each proportion were respectively divided into eight groups, with four stents in each group. All of them were independently submerged into test tubes with 10 mL fresh human bile which were then placed in constant temperature oscillator. The temperature was set at 37 °C. The bile was changed every day. After incubated for one week to eight weeks, the PLGA biliary stents were respectively taken out of test tubes from the first to the eighth group each week. They were washed with distilled water and dried in calorstat for 24 hours.

Main outcome measures

① Gross morphology, color and integrality of PLGA stents; ② Scanning electron microscope: the surface of four 90L/10G PLGA stents (untreated and treated for 1 week, 3 weeks, and 5 weeks) was observed by scanning electron microscope; ③ To measure the mass of PLGA stents and calculate the remaining mass rate: initial mass was the original mass of PLGA stents which were untreated; remaining mass was the mass of PLGA stents which were incubated in human bile for different time; remaining mass rate (%) = initial mass/remaining mass × 100%; ④ To measure the radical force of PLGA stents: After dilated sufficiently, PLGA stents were placed in two blocks for measurement. Each block had an arcuated groove whose diameter was same to the stent. Weights were put on the block gradually and the radical force could be read when the two blocks just touched.

Design, enforcement and evaluation

This study was designed and conducted by the first and third authors and evaluated by the second and fourth authors.

Statistical analysis

SPSS 13.0 software was used by the first author in this study. The remaining mass rate and radical force of PLGA stents were presented as Mean ±SD.

RESULTS

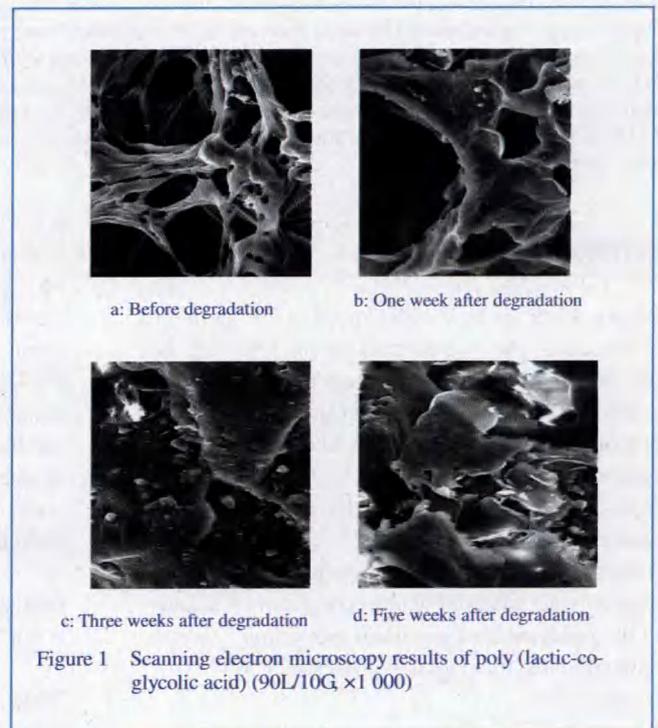
Gross morphology of PLGA stents

Five different mole-ratios of PLGA stents followed a similar changing law as to gross morphology: stents remained intact → stents became soft → the surface of stents became coarse and cracked → stents collapsed → stents were degraded into pieces → stents were completely degraded.

With the content of PLA decreasing, the degradation time of the stents became shorter. The collapse time of five different mole-ratios of biliary stents was weeks 6, 5, 4, 3 and 2, which were degraded into small pieces two weeks thereafter. The degradation peak time of five different mole-ratios of biliary stents was 35–42 days, 28–35 days, 21–28 days, 14–18 days, and 8–15 days, respectively.

Observation by scanning electron microscope

Before degradation: limpid stereo reticular structure of PLGA. One week after degradation: PLGA was corroded and the reticular structure was decreased. Three weeks after degradation: Airspace was gradually disappeared and the reticular structure was further decreased. Five weeks after degradation: Airspace and the reticular structure were completely damaged, which was in an indefinite form (Figure 1).



Change of mass

The mass loss of each proportion of PLGA stents gradually speeded up along with the time extension. Furthermore, with the content of PGA increasing, the mass loss of the stents became faster. As for 90L/10G, 80L/20G and 70L/30G PLGA stents, the mass loss was not significant in week 1 after degradation. It speeded up gradually since week two and the mass decreased by 50% in weeks 6, 5, and 4, respectively. As for 60L/40G and 50L/50G PLGA stents, the mass loss was

significant in week 1 after degradation ($\geq 10\%$). It decreased by 50% in week 3 (Figure 2).

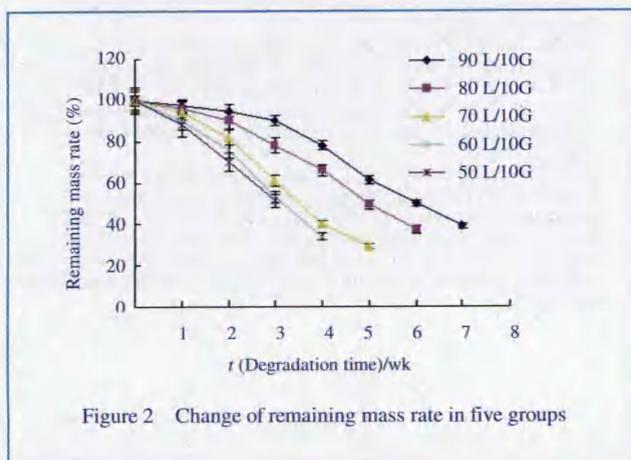


Figure 2 Change of remaining mass rate in five groups

Change of radical force

The initial radical force was different among five different mole-ratios of biliary stents. With the content of PLA increasing, the radical force of the stents became stronger. The radical force of each proportion of PLGA stents gradually fell down along with the time extension. When the stents collapsed, the radical force became zero at once (Figure 3).

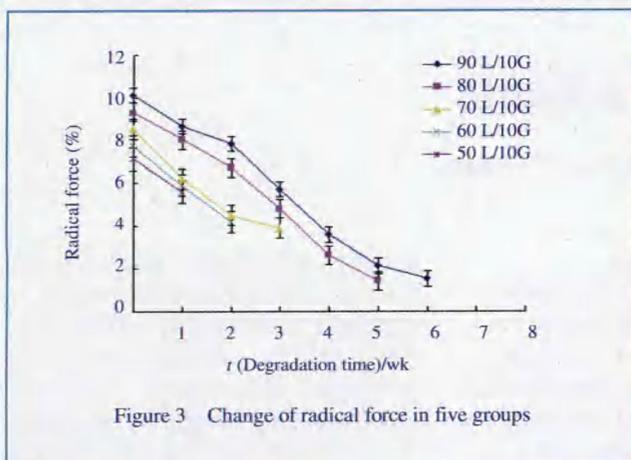


Figure 3 Change of radical force in five groups

DISCUSSION

The biodegradation of PLGA is affected by many factors, including the proportion of PLA and PGA, molecular mass, interval porosity, temperature, PH, and so on, among which the proportion of PLA and PGA is the most important factor^[17-18]. Lactic acid is poorer in hydrophilicity than glycolic acid, so the hydrophilicity of PLGA abundant in PLA is poorer than that of PLGA abundant in PGA, which results in a higher crystallinity and a longer degradation time of the former. The degradation time of PLGA could be effectively regulated by adjusting the proportion of PLA and PGA. In this study, with the content of PLA decreasing, the degradation time of the stent became shorter. The degradation peak time of five different mole-ratios of biliary stents was 35–42 days, 28–35 days, 21–28 days, 14–18 days, and 8–15 days, respectively, which was consistent with the law above.

Although the degradation time of five different mole-ratios of PLGA stents was different, they followed a similar changing law in gross morphology (described in results). It was shown clearly by scanning electron microscope that PLGA with a stereo reticular structure was gradually corroded by human bile.

It was found in this study that with the content of PLA decreasing, the mass loss of the stents became faster, which was in accordance with the changing law in gross morphology. The time when the mass of five different mole-ratios of biliary stents decreased by 50% was weeks 6, 5, 4, 3, and 2, respectively. There was also some regulation in mass change in each proportion of stents. The mass loss speeded up gradually along with the time extension. The chain of PLGA was cut off randomly by means of hydrolyzation which firstly caused the decrease of molecular mass (not mass). Only when the hydrolyzed products were small enough to go out of the polymer, conspicuous mass loss could take place. So the mass loss in early stage was slower than that in later stage.

Radical force, which determines whether stents can attach bile duct wall firmly and provide enough holding power, is one of the most important technical indexes that affect their clinical application. In this study, radical force was measured easily by self-made blocks both of which had an arcuated groove whose diameter was same to the stents. It was reported by Toouli^[19] that choledochal inner pressure in human was 1.20–2.27 kPa in normal and conscious status. It was shown in this study that with the content of PLA increasing, the radical force of the stents became stronger. The time for which five different mole-ratios of biliary stents might sustain the support in the bile duct was 5 weeks, 4 weeks, 3 weeks, 2 weeks, and 1 week, respectively. The stents were degraded into small pieces three weeks thereafter and could be discharged with human bile. At present, T tube is generally pulled out 14 days after the exploration of common bile duct. The time could be conditionally prolonged for aged and patients in bad nutrition. According to this study, we believe that 90L/10G, 80L/20G, and 70L/30G PLGA stents can meet the requirement of T tube and are hopeful to replace T tube. Reportedly, the degradation peak time of 80L/20G PLGA biliary stents in human bile was 17–18 days^[20]. The time was shorter than that in our study because the molecular mass of 80L/20G PLGA biliary stents in the two studies was different (the former: less than 50 000; the latter: 100 000).

According to the data in our study, PLGA biliary stents have the potential to be used in patients who need short-term drainage of bile. A further study *in vivo* needs to be taken to confirm their practical effect.

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Topic background: The biliary stents commonly used in clinic were made of plastics and metal, both of which had some irresolvable defects. Poly (lactic-co-glycolic acid) (PLGA) was a new type of high polymer material, characterizing by good compatibility and degradation. This study was supported by the grants from the First Affiliated Hospital of Xi'an Jiaotong University (No. 2006 YK.6) and designed to

evaluate the biodegradation law and the change in radical force of five different mole-ratios of biliary stents made of PLGA in human bile *in vitro*.

Clinical application: ① In this study, radical force was measured easily by self-made blocks both of which had an arcuated groove whose diameter was same to the stents. ② According to the data in

our study, PLGA biliary stents had the potential to be used in patients who needed short-term drainage of bile.

Subject concept: Radical force, which determines whether stents can attach bile duct wall firmly and provide enough holding power, is one of the most important technical indexes that affect their clinical application.

聚丙交酯/乙交酯胆道支架的体外降解规律及力学特性*☆

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摘要

背景: 临床上使用的胆道塑料支架和金属支架存在胆泥形成、支架嵌入胆道壁等难以克服的缺陷。生物可降解胆道支架因其良好的生物相容性和可降解性逐渐受到重视, 但目前的研究尚处于初级阶段, 缺乏系统的研究结果。

目的: 评价 5 种不同摩尔比例的聚丙交酯-乙交酯共聚物 [Poly(lactide-co-glycolide), PLGA] 胆道支架在体外胆汁中的降解规律及其径向支撑力的变化规律。

设计和地点: 完全随机设计, 于 2006-07/12 在西安交通大学医学院第一附属医院肝胆外科实验室完成。

材料: 5 种比例的 PLGA 支架为济南岱罡生物科技有限公司产品; 人体新鲜胆汁取自实验期间于西安交通大学医学院第一附属医院

肝胆外科行胆汁外引流的患者。

方法: 取 5 种比例 (丙交酯和乙交酯的摩尔比分别为 90:10, 80:20, 70:30, 60:40, 50:50) 的 PLGA 支架各 32 个, 将各支架独立浸泡于装有 10 mL 新鲜人体胆汁的平底试管内, 并置于恒温振荡器中, 调节温度至 37 °C, 摇动速度恒定, 胆汁每天更换 1 次。浸泡 1, 2, 3, 4, 5, 6, 7, 8 周后各比例支架分别取出试件各 4 个, 蒸馏水洗涤后 25 °C 恒温箱干燥 24 h。

主要观察指标: ① 大体形态、色泽及完整性。② 扫描电镜观察降解前, 降解 1, 3, 5 周 PLGA (丙交酯和乙交酯的摩尔比为 90:10) 支架的表面形态。③ PLGA 支架的质量变化及径向支撑力变化。

结果: ① 5 种比例 PLGA 支架的大体形态变化遵循同一规律: 外形保持完整 → 质地变软 → 支架表面粗糙, 出现裂痕 → 管壁塌陷 → 支架破碎为大块, 并进一步解离为小碎片 → 支架完全降解。② 降解前 PLGA (丙交酯和乙交酯的摩尔比为 90:10) 支架材料呈清晰的三维立体网状空隙结构; 降解 1 周后, 材料表

面受侵蚀, 网状结构减少, 孔洞灶性连接呈片状; 3 周时材料表面受侵蚀现象加重, 空隙逐渐消失, 网状结构进一步减少, 聚合物融合呈大片状; 5 周后材料空隙、网状结构完全消失, 呈不定型均质状。③ 各种比例 PLGA 支架的质量损失随时间的延长逐渐加快, 且乙交酯所占比例越大, 支架的质量损失速度越快。初始径向支撑力随丙交酯含量的增多, 支撑性能逐渐增强。

结论: PLGA 材料具有可调节的生物降解性和良好的力学特性, 适宜制成胆道支架。

关键词: 聚丙交酯/乙交酯; 生物降解; 力学特性; 胆道支架

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