Degradation and biocompatibility behaviors of fully covered biodegradable polydioxanone biliary stent for human body

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LIU YANHUI
JIANG RUITAO
WANG MIAN
FU SHAOJU
ZHANG PEIHUA

ABSTRACT – REZUMAT

Degradation and biocompatibility behaviors of fully covered biodegradable polydioxanone biliary stent for human body

This paper presents a study of the degradation and biocompatibility behaviors of fully covered biodegradable polydioxanone biliary stents (FCBPBSs) developed for human body. To investigate the relationship between the mechanical property of FCBPBSs and degradation time, nine FCBPBSs were prepared both by braid-hand method using a self-made cylinder of copper pipe mold and electrospinning. Meanwhile, the response of quality of FCBPBSs with increasing of degradation time was investigated. Futhermore, FCBPBSs and cells were cultured together to study the biocompatibility of FCBPBs indicating good biocompatibility.

Keywords: *fully covered biodegradable polydioxanone biliary stents (FCBPBSs); degradation behaviors; biocompatibility; cell culture*

Comportamentul de degradare și biocompatibilitate al stentului biliar biodegradabil complet acoperit din polidioxanonă pentru corpul uman

Această lucrare prezintă studiul asupra comportamentului de degradare si biocompatibilitate al stenturilor biliare biodegradabile complet acoperite din polidioxanonă (FCBPBS-uri), dezvoltate pentru organismul uman. Pentru a investiga relația dintre caracteristicile mecanice ale FCBPBS și timpul de degradare, nouă tipuri de FCBPBS au fost realizate, atât prin metoda de împletire manuală, folosind un cilindru din matriță de țeavă de cupru, cât și prin electrofilare. În paralel, a fost studiat nivelul de calitate al FCBPBS-urilor funcţie de timpului de degradare. În plus, FCBPBS-urile și celulele au fost cultivate împreună pentru a studia biocompatibilitatea acestora, obţinându-se rezultate satisfăcătoare.

Cuvinte-cheie*: stenturi biliare biodegradabile complet acoperite din polidioxanonă (FCBPBS-uri); comportament de degradare; biocompatibilitate; cultură celulară*

INTRODUCTION

Sung [1] studied that endoscopic biliary stenting has become a standard palliative treatment for obstructive jaundice due to malignancies of the pancreas and the hepatobiliary system. Since the late 1970s, despite the high initial success rate in achieving biliary drainage, endoscopic stenting therapy has been limited by the clogging of biliary stents, usually after four to five months, due to formation of adherent bacterial biofilm and accumulation of biliary sludge. In 1979, Soehendra [2] firstly described an endoscopic method for placing an internal drain in the bile-duct. Compared to the nasobiliary suction-tube, this method can guarantees the physiological flow of the bile into the duodenum, avoiding the inconvenience brought to patients, especially, for high-risk or inoperable cases, the method can be considered as a desirable alternative to choledochoduodenostomy. To date there are three types of biliary plastic stents applied in the biliary field, including polythylene biliary stent, polyurethane biliary stent and teflon biliary stent, respectively. Van Berkel [3] described a total of 9 different types of unused 10F endoprostheses were examined by scanning electron microscopy (SEM): polyethylene Amsterdam-type, polyurethane Amsterdam-type, Teflon Amsterdam-type, and pointed out stents also had multiple particles protruding into the stent lumen with adjacent holes in the wall of the stent comparing other two type stent to explain the controversial results of clinical studies.

In 1985, Carrasco et al. [4] firstly pointed out expandable stents constructed of stainless-steel wire were inserted in the extrahepatic bile ducts of five animals to determine the effect of the endoprosthesis on the ductal wall, and found that expandable wire stents could be safely used to relieve biliary obstruction. Foerster [5] implanted Wallstent, a new self-expanding mesh stent, in seven patients for bridging choledochal stenosis, and on the basis of the current data endoscopic reconstructive splinting of benign choledochal stenosis would appear to be a promising technique.

Lammer [6] compared treatment with plastic versus metal stent at common bile duct obstruction due to malignancy, the mortality rate and obstruction rate were both significantly lower for metal stents than plastic stents. Restricture of bile duct was formed

because pathological tissues grow through the mesh of stents, covered biliary stent was invented duo to this disadvantage above. Severini [7] investigated Prototypes of Gianturco-Rosch Z-stents coated with polycarbonate urethane (PCU) in the biliary tree of pigs, and demonstrated the biocompatibility, efficacy, and stability of PCU-coated Gianturco-Rosch stents in the biliary environment.
Gregory [8] placed a new bioabsorbable biliary stent made by polylactic acid (PLA) monofilament into a porcine model and concluded that the bioabsorbable biliary stent, can be effectively deployed endoscopically, is self-expanding, is visualized radiographically, and remains patent up to 6 months. Giovanni et al. [9] have evaluated feasibility and safety of patients treated with biodegradable polydioxanone (PDO) biliary stents. The biodegradable biliary stent above mentioned is uncovered, however few fully covered biodegradable polydioxanone biliary stents (FCBPBSs) were studied based on their advantages that they can be degradable and as an obstacle which prevent the tissue from growing into the cavity of stents through the mesh.
In this paper, FCBPBSs were firstly fabricated by polydioxanone (PDO) monofilament, and secondly covered by electrospinning with polycaprolactone (PCL) material. PDO material, as the more widely used biodegradable material, is degraded with low-toxicity to CO_2 and H_2O, and has good biocompatibility and safety [10–11]. The monofilament with smooth surface and good mechanical property can be obtained by melt spinning processes [12]. PDO monofilament used for human body had further obtained the certification of FDA (U.S. Food and Drug Administration) [13]. Meanwhile, biodegradation and biocompatibility of FCBPBS were investigated.

EXPERIMENTAL WORK

Specifications of FCBPBSs

In this paper, FCBPBSs were fabricated by handing braid method. Firstly, non-covered biodegradable biliary stents (NBPBSs) were fabricated with PDO monofilament using a self-made cylinder of copper pipe mold. After finishing the braiding processes of the non-covered biliary stent, the heat setting process was carried out for the mold and NBPBSs in an environment of 75°C for about 15 min [14]. The detailed parameters of the PDO monofilament used for NBPBSs are shown in table 1. The structure of NBPBS was provided in figure 1, *a*.
Second, FCBPBSs were manufactured with NBPBSs by the method of electrospinning [15].
Polycaprolactone (PCL, Density: 1.14 g/cm^3, Mn = 80 000) offered by Donghua University was used as film material on account of good property, including good biodegradation, good biocompatibility property. The organic solvent of PCL was mixed solution of dimethylformamide (DMF) and dichloromethane (DCM). Schematic representation of experimental facility of electrospinning was shown in figure 1, *b*. The positive electrode of high voltage power supply (HVPS) was connected with syringe needle, the size of syringe needle we used in experiment was 23G. The negative electrode of HVPS was connected with revolving reception facility on which NBPBS was put. Through the effect of voltage difference between the positive and negative electrode, film material in syringe was transported on the non-covered biodegradable Polydioxanone Biliary Stent to reach purpose of coating. To get nanofiber film, the pre-experiment of electrospinning coating was conducted in advance, so, experiment parameters of electrospinning were set as shown in table 2.
FCBPBSs we design as shown in figure 1, *c* were conducted the compression tests preciously conducted by Xu H.J. [16]. The stent specimens were compressed to a deformation about 50% of the initial

Table 1

THE FUNDAMENTAL PROPERTIES OF PDO MONOFILAMENT

Material	Number	Diameter (mm)	Crystallinity (%)	Tensile strength (N)	Linear density (Tex)
PDO	A	0.23	70.7	23.56	63.13
	B	0.29	70.4	34.81	95.83
	C	0.36	70.5	45.92	125.21

Table 2

EXPERIMENT PARAMETERS OF ELECTROSPINNING

Solute concentration of PCL (g/mL)	0.18
Volume fraction of DMF (%)	43
Voltage of HVPS (kV)	15
Distance between syringe needle and revolving reception facility (cm)	20
Reception time (h)	2
Rotating speed of revolving reception facility (rpm)	1000

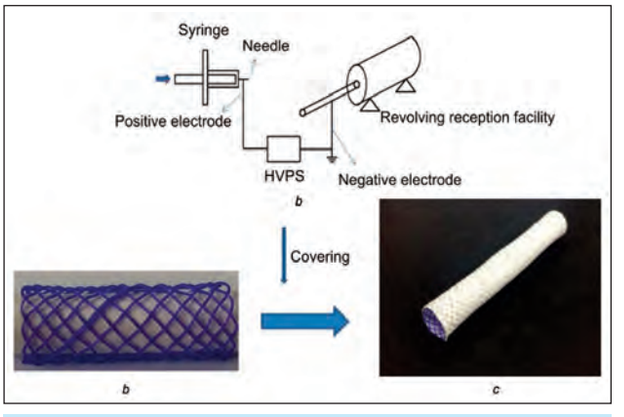


Fig. 1. *a* – the appearance of NBPBS; *b* – the appearance of NBPBS; *c* – experimental facility of electrospinning

Table 3

THE STRUCTURE PARAMETERS OF FCBPBS

Material	Number of specimens	Braiding number (pins)	External diameter (mm)	Thickness of film (mm)	Internal diameter (mm)
PDO	A2	8	8.83	0.07	8
	A3	10	8.90	0.069	8
	A4	12	8.92	0.068	8
	B2	8	9.15	0.072	8
	B3	10	9.17	0.07	8
	B4	12	9.21	0.073	8
	C2	8	9.52	0.068	8
	C3	10	9.56	0.069	8
	C4	12	9.67	0.071	8

biliary stent diameter which is the internal diameter of biliary stent at a load speed of 20 mm/min. The size of the compression platen is 100 mm in length and 24.5 mm in width. In the compression process, the compression's initial height is defined as the position in which the compression platen just makes contact with the biliary stents. When compression displacement reaches 50% of the initial biliary stent diameter, the compression platen was stopped and the current compression force was defined as maximum compression in the whole compression process. After staying 5 second, the compression platen was back to original position at a speed of 20 mm/min. The structure parameters of FCBPBS9 were shown in table 3.

Degradation and biocompatibility

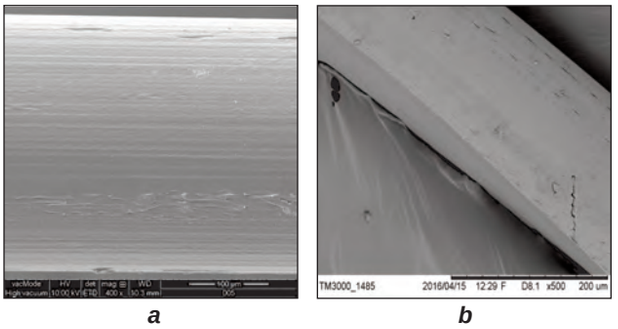
FCBPBSs we design were dipped into tube with human bile with pH 7.4, and incubated in surrounding of 5% CO_2 and 37°C. The human bile in the tube with FCBPBSs change one time every week and the FCBPBSs were taken out every two weeks, washed by deionized water and freeze-dried. The compression force and quality of FCBPBSs was investigated every two weeks to study relationship between mechanical performances above and degradation time until FCBPBSs were completely degraded same with the relationship between quality of FCBPBSs and degradation time.

In order to evaluate the interaction between cells and materials (PCL and PDO), the method of in vitro cellular evaluation was used in this work. Firstly, the appropriate size of PDO monofilament and PCL film was prepared to put them in one well of 48-well plates. Human umbilical vein endothelial cells (HUVECs) were seeded on the surfaces of PCL film and PDO monofilament in 48-well plates, and then incubated in the conditions of 5% CO_2, 95% humidity, and 37°C. On day 1 and day 7, F-actin/DAPI staining was conducted. The samples were observed using a Fluorescence Microscope (Carl Zeiss Inc., Oberkochen, Germany).

RESULTS

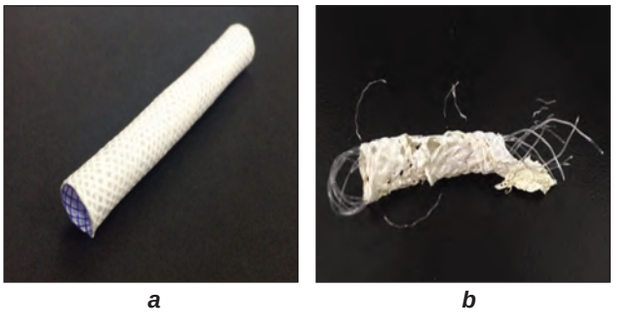
Surface morphology of PDO monofilament and FCBPBS after degradation

Based on the figures 2 and 3, surface morphology of PDO monofilament and FCBPBS after degradation was very different with that of them before degrading. Before degradation, morphology of PDO monofilament is smooth and intact in the figure 2, *a*. Refer to figure 2, *b*, the crack was generated on the surface of PDO monofilament after degradation. The surface of FCBPBS is intact, color of FCBPBS is vivid and the color of PDO monofilament is blue as depicted in figure 3, *a*. However, with degrading, the color of PDO monofilament was faded and PDO monofilament was fractured completely so that FCBPBSs were collapse indicating radial supporting force was lost in the last stage according to figure 3, *b*.



a *b*

Fig. 2. *a* – the surface morphology of PDO monofilament before degradation; *b* – the surface morphology of PDO monofilament after degradation



a *b*

Fig. 3. *a* – The surface morphology of FCBPBS before degradation; *b* – The surface morphology of FCBPBS after degradation

The mechanical property in the degradation process

In this work, the compression force value at the position that compression distance reaches 50% of the stent diameter is extracted and plotted into the compression force-degradation time curve of FCBPBSs in the compressing process. As depicted in the figure 4, the curves of all kinds of FCBPBSs we tested

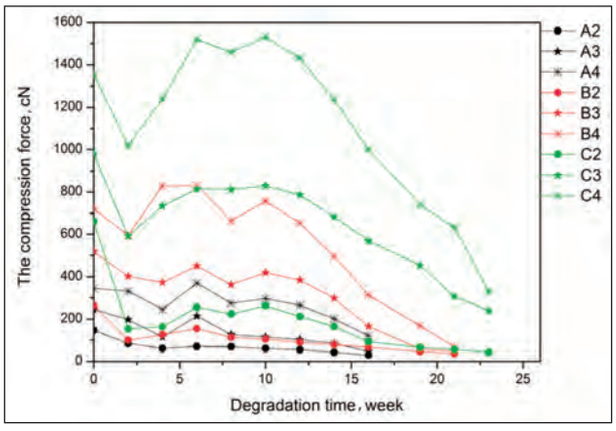


Fig. 4. The mechanical performance of different kinds of FCBPBSs in the degradation process

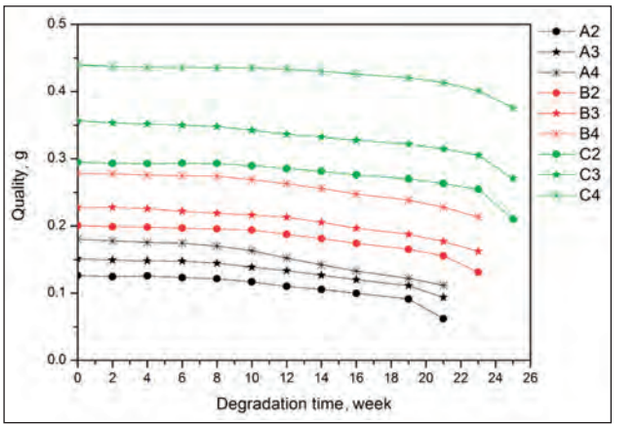


Fig. 5. The quality variation of different kinds of FCBPBSs in the degradation process

exhibited the same tendency, at second week of degradation process, the compression force decreased comparing with that of 0 week. With further degradation, the compression force began to increase and reach a peak about 10 weeks, at last, the compression force reduced until cannot be tested. It is also clear seen that FCBPBSs (C2, C3, C4) keep the most longest time of the compression force in the degradation process about 22 weeks, the middle is FCBPBSs (B2, B3, B4) about 20 weeks, and the shortest time is FCBPBSs (A2, A3, A4) about 16 weeks indicating that degradable time of FCBPBSs increased with growing of the PDO diameter of FCBPBSs under expected mechanical condition which can be tested.

The quality variation of FCBPBSs with degrading

To investigate relation between quality of FCBPBSs and degradation time, the quality of FCBPBSs were measured every two weeks and end to test when the FCBPBSs cannot be compressed. As shown in the figure 5, quality of FCBPBSs decreased with growing of the degradation time, different kinds of FCBPBSs have same variation tendency above. Before 8 weeks, the slope of quality-degradation time curves of different kinds of FCBPBSs were very slow to drop, indicating that quality loss of FCBPBSs was very small, however, quality loss of FCBPBSs began to quickly fall down after eighth week, and dramatically declined in the last four weeks.

Biocompatibility of FCBPBSs

In order to evaluate the interaction between cells and materials (PCL and PDO), HUVECs were seeded on the surfaces of PCL film and PDO monofilament in 48-well plates. As depicted in the figure 6, *a*, the most amount of HUVECs survived and grow on the surface of PCL after the one day culture, after cell culture of Day 7, cell cytoskeleton (green) and nuclei (blue) of HUVECs on the surface of PCL spread bigger than that of Day 1 indicating good biocompatibility between cell and PCL film. As shown in figure 6, *b*, comparing to Day 1, larger amount of HUVECs stretched on the surface of PDO suggesting that the high interaction between cell and PDO. Fluorescence images of HUVECs like fibroblast-like morphology indicated that materials of PDO and PCL dose not interfere with the adhesion properties of the cell, confirming strong affinity of materials towards HUVECs.

CONCLUSION

Degradation and biocompatibility Behaviors of FCBPBSs for Human Body was investigated in this study, the conclusions was drawn as follows:

- Through degradation, the crack was generated on the surface of PDO monofilament and the PDO monofilament of FCBPBSs was fractured completely so that FCBPBSs were collapse indicating radial supporting force was lost;
- All kinds of FCBPBSs exhibit the same tendency in the degradation process, FCBPBSs (C2, C3, C4) keep the most longest time of the compression force in the degradation process about 22 weeks,

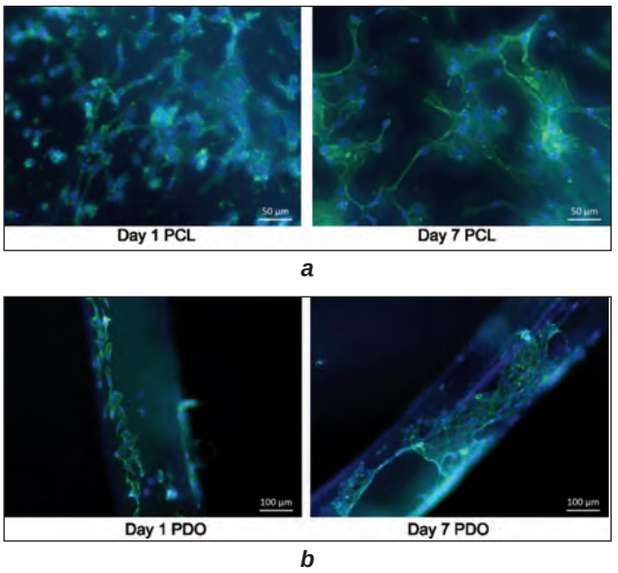


Fig. 6. Biology property of PCL and PDO: *a* – Morphology of HUVECs toward PCL; *b* – Morphology of HUVECs toward PDO. Cytoskeleton of HUVECs was stained for F-actin (green) and nuclei of HUVECs were tagged with DAPI (blue)

the middle is FCBPBSs (B2, B3, B4) about 20 weeks, and the shortest time is FCBPBSs (A2, A3, A4) about 16 weeks indicating that degradable time of FCBPBSs increased with growing of the PDO diameter of FCBPBSs;

- The quality loss of FCBPBSs was very little in front 8 weeks of whole degradation process, however, quality loss of FCBPBSs began to quickly fall down after eighth week, and dramatically declined in the last four weeks in the whole degradation process;
- By culturing the HUVECs with PCL film and PDO monofilament together, PCL film and PDO monofilament exhibit good biocompatibility.

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REFERENCES

[1] Sung, J.Y., Chung, S.C., *Endoscopic stenting for palliation of malignant biliary obstruction: a review of progress in the last 15 years*, In: Dig Dis Sci, 1995, 40, 6, 1167–1173
[2] Soehendra, N., Reynders-Fredeix, V., *Palliative bile duct drainage. A new endoscopic method of introducing a transpapillary drain*, In: Endoscopy, 1980, 12, 1, 8–11
[3] VanBerkel, A.M., VanMarle, J., VanVeen, H., *A scanning electron microscopic study of biliary stent materials*, In: Gastrointest Endosc, 2000, 51, 1, 19–22
[4] Carrasco, A.B., Wallace, N., Chamsangavej, H.P., *Expandablebiliary endoprosthesis: an experimental study,* In: AJR, 1985, 145, 1279–12
[5] Foerster, E.C., Hespffer, N., Domschke, W., *Bridging of benigncholedochal stenoses by endoscopic retrograde implantion of mesh stents*, In: Endoscopy, 1991, 23, 3, 133–137
[6] Lammer, J., Hausegger, K.A., Fluckiger, F., *Common bile duct obstruction due to malignancy: treatment with plastic versusmetal stents*, In: Radiology, 1996, 201, 167–172
[7] Severini, A., Mantero, S., Tanzi, M.C., *In vivo study of polyurethane-coated Gianturco-Rosch biliary Z-stent*, In: Cardiovasc Intervent Radiol, 1999, 22, 6, 510–514
[8] Gregory, G., Janak, S., Anthony, C.D., *Invivo evaluation of a new bioabsorbable self-expanding biliary stent*, In: Gastrointest Endosc, 2003, 58, 5, 777–784
[9] Mauri, G., Michelozzi, C., Melchiorre, F., *Biodegradable biliary stent implantation in the treatment of benign bilioplastic-refractory biliary strictures: preliminary experience*, In: European radiology, 2013, 23, 3304–10
[10] Zhai, W.Q., Zhen, H.Y., Xing, H.C., *The in vitro degradation study of two degradable intravascular stents materials*, In: Proceedings of 2011 International Forum Biomedical Textile Materials, Shanghai, China, 2011, 1
[11] Zhai, W.Q., Zhen, H.Y., Xing, H.C. *The in vitro degradation study of biodegradable intravascular stents of two different materials*, In: Proceedings of 2012 International Forum Biomedical Textile Materials, Shanghai, China, 2012, 1
[12] Wang, Y.H., Dou, H.J., *Dynamic mechanical properties of biodegradable stents*, In: Journal of Shanghai Jiao Tong University, 2010, 44, 11, 1605–1609
[13] Docob, D.F., Sacristan, P.C., Flor, C.B., *Randomized clinical study of polydioxanone and nylon sutures for Laparotomy closure in high-risk patients*, In: Cirugia Espanola, 2006, 79, 5, 305–309
[14] Liu, Y.H, Zhang, P.H., *Characterization and modeling of compression behaviors of biodegradable polydioxanone biliary stent for human body*, In: Industria Textila, 2016, 67, 1, 62–70
[15] Bing, J.Z, Yuan, Y.L., Yue, H. L., *The Biocompatibility Between Electrospinning Scaffold of PCL and PDLSCs* (in chinese), In: Journal of Clinical Stomatology, 2014, 30, 269–72
[16] Xu, H., Wang, C., Que, Z., Zhang, P., *Preparation and Radial Compression Performance of PDO Intravascular Stents*. In: Journal of Donghua University Natural Science Edition, 2014, 40, 418–23

Authors:

LIU YANHUI[1,2,3*], JIANG RUITAO[2,3*], WANG MIAN[4], FU SHAOJU[2,3], ZHANG PEIHUA[2,3]

[1]College of Textiles & Clothing, State Key Laboratory of Bio-Fibers and Eco-Textiles, Qingdao University
[2]College of Textile, Donghua University
[3]Key of Laboratory of Textile Science &Technology, Ministry of Education, China Technology, 2999 North Renmin Road, Shanghai, 201620, P.R.China
[4]School of Chemistry and Chemical Engineering, Guangxi University, Nanning 53004, China
*These authors contributed equally to this work and should be considered co-first authors
e-mail: chainliuyanhui1@163.com, phzh@dhu.edu.cn

Corresponding author:

ZHANG PEIHUA
e-mail: phzh@dhu.edu.cn