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Biodegradable Polyvinyl Alcohol Vascular Stents: Structural Model and Mechanical and Biological Property Evaluation

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ABSTRACT

This study proposes structural models of biodegradable vascular stents. One, two, or three plies of biodegradable polyvinyl alcohol (PVA) yarns are combined and twisted with twist factors of 2, 3, 4, 5, and 6 to form one-, two-, and three-ply PVA twisted yarns. The braided, warp-knitted, and weft-knitted PVA vascular stents are composed of PVA twisted yarns by using a braider, a warp knitting machine, and a weft knitting machine. The formation and mechanical properties of PVA vascular stents are evaluated, and the biological properties are examined in terms of biocompatibility through in vitro assay and subcutaneous embedding using in vivo assay. Test results indicate that the compression strength of PVA vascular stents is improved when using PVA twisted yarns containing a high number of plies and twist factor. Specifically, weft-knitted PVA vascular stents exhibit the optimal compression strength. PVA vascular stents treated with chemical cross-linking show weight loss lower than 3% after immersion in PBS solution for 30 days. Moreover, the antibacterial test and cell culture results suggest that PVA vascular stents are nontoxic and biocompatible. Subcutaneous embedding results show that PVA vascular stents retain intact formation when subcutaneously embedded in vivo for 28 days, indicating their good biological property. PVA vascular stents are suitable candidates for tissue engineering applications.

1. Introduction

Tissue engineering, which combines engineering science and life science, uses substitutes needed by organisms to heal wounds and maintain and improve the functions [1]. The substitutes of organisms should allow in vivo cells to attach, thereby promoting cell growth and proliferation. Therefore, substitutes are required to replace the tissues upon implantation in the organism. Vascular tissue engineering stents are primarily used as biomedical materials in the tissue engineering field. Stents should have a formation that fits the interior of blood vessels and provides the required biological and mechanical functions [2–4]. In addition, vascular stents should perform comparable functions with those of blood vessels, preventing differences in hemodynamics that cause thrombus formation and hyperplasia in the endomembrane [5].

To date, biodegradable biomaterials are usually synthetic polymers, including polyvinyl alcohol (PVA), polycaprolactone (PCL), polylactic acid (PLA), and other compounds [6–8]. Nevertheless, several biomaterials, such as silk fibroin, collagen, and chitosan (CS), which are obtained from natural polymers, exhibit biological activity and functions [9–13]. Polymers are produced in a micro-porous formation and allow the endothelial cells of blood vessels to grow through the micro-pores into the interior of the stent and then cover it. Vascular stents preserve the functions of original blood vessels until the cells completely grow and are decomposed and metabolized [7,11,12].

Numerous scholars are devoted to the development of biodegradable stents. Tumbic et al. developed composite stents by using PCL and PLA; PCL/PLA stents possess biocompatibility and rigidity suitable for

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the application of tissue engineering stents [14]. Punnakitikashem et al. used modified PCL to form vascular stents, which were loaded with drugs; the stents showed a stable drug release rate for 91 days, indicating their satisfactory biocompatibility [15]. Badhe et al. used gelatin and CS to form composite stents embedded with large-sized pores. The stent showed suitable elasticity and strength and enabled the cells to fully cover its surface, indicating good cell adhesion and proliferation [13]. Hence, biodegradable stents show considerable potential and are a major developed item of biomaterials [16–19].

In this study, biodegradable PVA yarns are formed into hollow tubes by braiding, warp knitting, and weft knitting to improve the mechanical properties of commercially available vascular stents and develop an ideal structural model. CS coating is used to secure the interlacing point of the braided PVA vascular stents. Chemical crosslinking with genipin (GP) is conducted to strengthen the mechanical properties of the vascular stents. Finally, braided, warp-knitted, and weft knitted PVA vascular stents are evaluated in terms of formation, mechanical properties, and biological properties. The influences of number of plies, twist factor of the PVA plied yarns, and chemical cross-linking are also determined.

2. Experimental

2.1. Materials

PVA with a specification 75 denier (D)/25f is obtained from Asiatic Fiber Corporation, Taiwan. CS with 85% deacetylation is acquired from Global Technology Co., Taiwan. GP, with molecular weight of 226.20 g/mol and concentration above 98%, is supplied by Challenge Bio, Taiwan. Phosphate-buffered saline (PBS) solution is purchased from Difco Laboratories Inc. USA. L929 fibroblast is provided by the Food Industry Research and Development Institute, Taiwan. Minimum essential medium (MEM) is supplied by Gibco Inc., USA. *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922) are obtained from the Food Industry Research and Development Institute, Taiwan. Dimethyl sulfoxide (DMSO) is acquired from Applichem Inc., USA.

2.2. Preparation of braided, warp-knitted, and weft-knitted PVA vascular stents

One, two, or three plies of 75 D PVA yarns are combined into PVA plied yarns. One-, two-, and three-ply PVA yarns are twisted with twist factors of 2, 3, 4, 5, and 6. The twisted yarns are braided, warp knitted, or weft knitted in a tubular manner by using a 16-spinning braider, a warp knitting machine, and a weft knitting machine, respectively. The PVA braids, warp knits, and weft knits are thermally set in an oven at 140 °C for 30 min. The tubes are immersed in 50 mL of 0.50% CS solution for 5 min; during which, the tubular braids, warp knits, or weft knits are separately affixed to an electric agitator that spins at a rate of 500 rpm and chemically cross-linked with 1.0% GP for 48 h. The samples are rinsed with ethanol to remove residual GP and obtain braided, warp-knitted, and weft-knitted PVA vascular stents. The specification of samples code is tabulated in Table 1.

2.3. Tests

2.3.1. Surface observation

A stereomicroscope (SMZ-10A, Nikon Instruments Inc., Japan) is used to observe the braided, warp-knitted, and weft-knitted PVA vascular stents. The samples mounted on the platform of the stereomicroscope are photographed and analyzed by Motic Images Plus 2.0 software (Motic Group Co., Ltd., USA).

2.3.2. Scanning electron microscopy (SEM)

The vascular stents are first coated with a thin layer of gold for 30 s by an ion sputter (E-1010, Hitachi, Japan) and observed using SEM (S3000, HITACHI, Japan) at an accelerating voltage of 15 kV.

Table 1
Specification of sample codes.

Structural models	Twist factors	Number of plies
I. Braided	2	1
II. Warp-knitted		2
III. Weft-knitted		3
	3	1
		2
		3
	4	1
		2
		3
	5	1
		2
		3
	6	1
		2
		3

2.3.3. Mesh size measurement

The mesh size of the vascular stents is measured using Image Pro Plus (Media Cybernetics, Inc., USA). The influences of the manufacturing parameters, namely, number of plies and twist factor, are examined. The number of samples for each specification is 10 pieces.

2.3.4. Bending property test

Ten vascular stents for each specification are cut to a length of 8 cm. The test samples are bent until both ends are 2 cm apart. The diameter is measured on the curve in the middle of the sample. The measured diameters are compared with the initial diameter of the sample by using the following equation:

$$\text{Diameter Variation Rate} = \frac{\text{Diameter of a lengthwise}-\text{bent sample}}{\text{Initial diameter of a sample}} \times 100\% \quad (1)$$

2.3.5. In vitro degradation test

PVA yarns dissolve and swell in water. The partial dissolution of the samples occurs when the solvent enters the amorphous region. Therefore, degradation test is performed to evaluate the in vivo preservation status and periods. Vascular stents are weighed as dry weight (W_0). The stents are then placed into a centrifuge tube filled with PBS and moved to a 37 °C thermostat shaking bath. PBS is changed every 12 h. The samples are removed using tweezers upon reaching the designed immersion times. The remaining solution is poured into a weighing paper. Along with the residual sample, the weighing paper is dried to ensure that the sample is completely removed from PBS. The dry fragments from the weighing paper are scratched using a spatula and weighed with the dried sample to obtain the degradation weight (W_t). Degradation rate (%) is computed using the following equation:

$$\text{Degradation Rate (\%)} = \frac{W_0 - W_t}{W_0} \times 100\% \quad (2)$$

2.3.6. Compression test

Nine samples for each specification are tested for compression strength by using an Instron 5566 universal tester (Instron, USA). Vascular stents are mounted on the testing plate and compressed by the clamp at a speed of 1 mm/min with a specified displacement of 2 mm.

2.3.7. Antibacterial property

The samples are placed into a sterile bottle and added with a diluted suspension of 5×10^8 colony-forming unit (CFU)/mL *S. aureus* or *E. coli*. The samples are cultured in an incubator at 37 °C for 24 h. The diluted suspension is evenly coated over agar plates by a stir rod and cultured in an incubator at 37 °C for 24 h. Finally, the CFU value of *S.*