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Preparation and *in vitro* Degradation of Methoxy Poly (Ethylene Glycol)-*b*-Poly (D, L-Lactide) Tubes for Nerve Tissue Engineering

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Abstract: This study has proposed novel biodegradable nerve guide tubes. Nerve guide tubes were made of a methoxy poly (ethylene glycol)-*b*-poly (D, L-lactide) diblock copolymer. The diblock copolymer was completely amorphous. The nerve guide tubes were prepared by a dip-coating technique. Three types of nerve guides with inner diameter of approximately 790 μm were constructed by variation of the number of dip-coated layers. Wall thicknesses of the tubes were 186, 217 and 278 μm for three, five and seven dip-coating steps, respectively with smooth in surface appearance. All tubes showed good transparent and flexibility that can be used for nerve guide regeneration. Glass transition temperatures of diblock copolymer tubes increased after degradation. From *in vitro* degradation test, it was demonstrated that the tubes with 5 and 7 layers were still undamaged after 3 weeks degradation period. The tubes maintained gross structural integrity in the first 3 weeks except the 3 layer tube, followed by sharp weight loss and most complete disappearance at about 5 weeks degradation period. Porous structures were occurred throughout the tube surfaces and matrices after degradation for a week.

Key words: Biodegradable polymers, diblock copolymer, nerve guide tubes, morphology

INTRODUCTION

Usually an autologous nerve graft is used to bridge the defect when a part of a peripheral nerve is lost. Biodegradable nerve guide tubes are advantageous over their non-degradable analogs, obviating the need for their removal after regeneration. Various biodegradable polyesters including polylactide (Yang *et al*., 2004), poly (L-lactide-*co*-glycolide) (Bini *et al*., 2006), poly (L-lactide-*co*-ϵ-caprolactone) (Aldini *et al*., 1996), poly (D, L-lactide-*co*-glycolide) (Wen and Tresco, 2006) and trimethylene carbonate-caprolactone block copolymer (Lietz *et al*., 2006). However, the preparation of nerve guide tubes from methoxy poly (ethylene glycol)-*b*-poly (D, L-lactide) diblock copolymer (MPEG-*b*-PDLL) has not been reported.

MPEG-*b*-PDLL is a biodegradable polymer which has been widely investigated for using in biomedical and pharmaceutical applications (Lucke *et al*., 2000; Kim *et al*., 2005). Its films prepared by solvent evaporation technique show very good flexibility as reported in the earlier study (Morakot *et al.,* 2008).

In this research project, the nerve guide tubes of MPEG-*b*-PDLL were prepared by dip-coating method. *In vitro* degradation of the tubes was investigated from their percentages of weight losses with degradation time. Morphology changes of the tubes during degradation were also determined from SEM micrographs. Influence of tube wall thickness on the degradation behaviours was evaluated and discussed.

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MATERIALS AND METHODS

Materials

Methoxy poly (ethylene glycol)-*b*-poly (D, L-lactide) diblock copolymer (MPEG-*b*-PDLL) with number-average molecular weight and polydispersity index of 73,600 g mol^{-1} and 1.88, respectively was synthesized as previously described (Baimark *et al*., 2007). The MPEG with molecular weight of 5,000 g mol^{-1} and stannous octoate were used as the initiating system. The obtained diblock copolymer was completely amorphous state. All solvents in analytical grade were used.

Methods

Preparation of Nerve Guide Tubes

Nerve guide tubes of diblock copolymer were prepared by dip-coating method described as follows. A mandrel of diameter 1 mm was vertically dipped into the 20% w/v diblock copolymer solution in anhydrous ethyl acetate at rotation speed of 150 rpm for 5 min. The mandrel was subsequently rotated horizontally for 15 min to reduce variation in the wall thickness along the axis of the tube and at the same time, to facilitate the process of air drying. Three, five and seven coating steps were used to obtain three tube types with different wall thicknesses, called as nerve guide tubes with 3, 5 and 7 layers, respectively. The tubes were slipped off the mandrel after storage in desiccator at room temperature for 2 weeks.

Morphology Study of Nerve Guide Tubes

The morphology and wall thickness of nerve guide tubes were determined by Scanning Electron Microscopy (SEM) using a JEOL JSM-6460LV SEM. Before SEM measurement, the films were sputter coated with gold for enhancing the surface conductivity. The wall thicknesses were measured from SEM micrographs using smile view software (version 1.02).

In vitro Degradation Test

In vitro degradation test of the tubes was evaluated under static culture condition in 0.1 M Phosphate Buffer Solution (PBS) pH 7.4 at 37°C to measure weight loss changes. The 0.5 % (w/v) sodium azide was added for preventing microorganism growth. At selected time points, the tubes were removed and dried to constant weight under vacuum before weighing. The PBS solution was replaced every week. The percentage of Weight Loss (WL) was calculated according to the following equation. The WL values are obtained from average values of three independent samples.

$$\text{WL}(\%) = [(W_0 - W_r)/W_0] \times 100$$

where, W_0 and W_r are the dry initial and remaining weights of the tubes, respectively.

The morphology and glass transition temperature (T_g) of degraded tubes was investigated by SEM and Differential Scanning Calorimetry (DSC) using a Perki -Elmer Pyris Diamond DSC, respectively. For DSC, approximately 10 mg of the sample were placed in a sealed aluminium pan and heat at the rate of 10°C min^{-1} under helium flow to measure the T_g.

RESULTS AND DISCUSSION

This study has provided the details on the preparation of MPEG-*b*-PDLL hollow tubes for using as nerve guide tubes by the dip-coating method. The chemical composition of MEPG-*b*-PDLL was determined from the ^{1}H-NMR spectrum by rationing the integral peak areas corresponding to the Ethylene Oxide (EO, repeating units of MPEG) methylene protons at chemical shift is 3.4-3.6 ppm

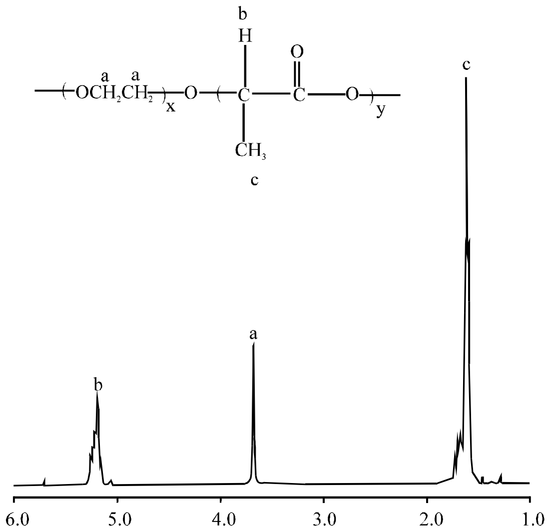

Fig. 1: ^{1}H-NMR spectrum of MPEG-*b*-PDLL (peaks assignment as shown)

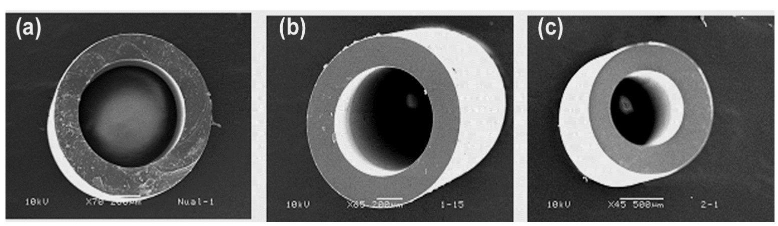

Fig. 2: Morphology of nerve guide tubes with (a) 3, (b) 5 and (c) 7 layers

and the DLL methane protons at chemical shift is 5.0-5.3 ppm. The ^{1}H-NMR spectrum of MPEG-*b*-PDLL is shown in Fig. 1. From the peak area integrations of the peaks a and b in Fig. 1, the copolymer composition can be determined as EO:DLL = 21:79 mol% corresponding to the MPEG:DLL mole ratio of 1:429. As would be expected, this copolymer composition is very similar to the MPEG:DLL feed mole ratio (1:416). Therefore, the synthesis reaction was taken to quantitative conversion.

The numbers of dip-coating steps (3. 5 and 7 dipping times) were varied to change their wall thicknesses. The wall thickness of nerve guide tubes has effect to nerve guide application as follows. When the wall thickness of the nerve guide is made too thick, the nerve guides will become occluded after swelling. On the contrary, when the wall thickness of the nerve guide is made too thin, the nerve guides will collapse unless a stent. Figure 2 shows SEM micrographs of the cross-sections of nerve guide tubes with similar 800 μm in inside diameter. The tube inner surfaces, outer surfaces and matrices were smooth in surface appearance. Voids did not detect throughout the tube matrices. The wall thicknesses increased with the number of dip-coating process. The average wall thicknesses were 186, 217 and 278 μm for the nerve guide tubes with 3, 5 and 7 layers, respectively.

The tubes can be bended in various shapes without deflated surfaces and de-stability of tube dimensions as example of which is shown in Fig. 3 for the nerve guide tube with 7 layers. The pliability of MPEG-*b*-PDLL tubes was consistently better than those made of homopolymers of polylactides. The attachment of MPEG blocks increased the flexibility of the tubes. This indicates the soft and flexible MPEG-*b*-PDLL tubes show potential for use as nerve guide tubes without addition any plasticizers.

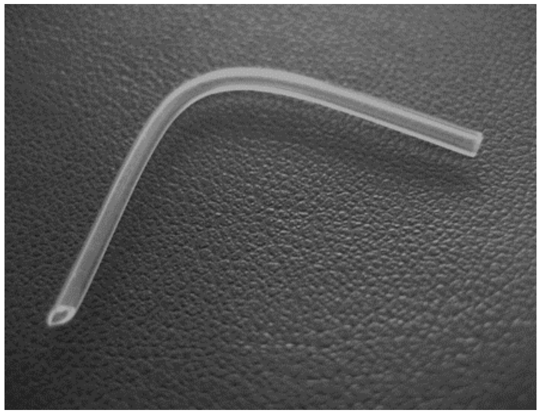

Fig. 3: Pliability of nerve guide tube with 7 layers

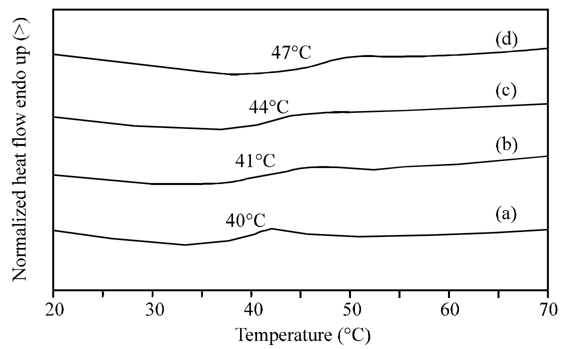

Fig. 4: DSC thermograms of nerve guides with degradation times of (a) 0, (b) 1, (c) 3 and (d) 5 weeks

Table 1: Glass transition temperatures (Tg) of MPEG-*b*-PDLL tubes with different degradation times

	Degradation time (weeks)			
Tube layers	0	1	3	5
3	40	46	49	53
5	40	45	48	51
7	40	41	44	47

Tg Changes of Degraded Nerve Guide Tubes

The T_g changes of MPEG-*b*-PDLL tubes after degradation were measured from DSC thermograms, as example of which is shown in Fig. 4 for the tubes with 7 layers. The results of T_g changes are summarized in Table 1. It was found that the T_g increased with degradation time. This can be explained that the MPEG block is first degraded and released out. The MPEG block act as an internal plasticizer to decrease the T_g of the MPEG-*b*-PDLL. Then, the T_g of MPEG-*b*-PDLL tube would be increased when some MPEG block was degraded according to the literature (Morakot *et al.*, 2008).

Weight Losses of Degraded Nerve Guide Tubes

The percentages of weight losses of all tubes increased with degradation time with the nerve guide tube with 3 layers showing the fastest degradation followed by the nerve guide tubes with 5 and then 7 layers which had the slowest degradation behaviour as shown in Fig. 5. The all tubes show slowly

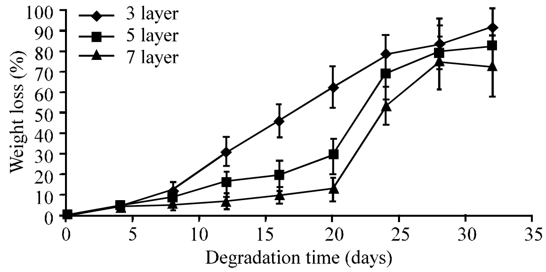

Fig. 5: Weight losses at 37°C in PBS with degradation time of nerve guide tubes with 3, 5 and 7 layers

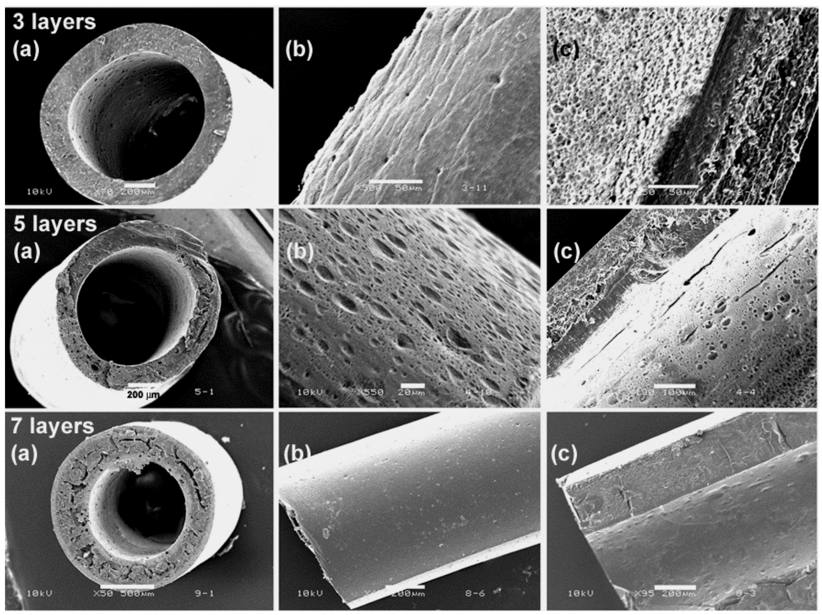

Fig. 6: SEM micrographs of MPEG-*b*-PDLL nerve guide tubes with different layers after a week degradation period; (a) cross-sections (b) outer surfaces and (c) inner surfaces

degradation in the first week due to the water is diffusing through the tube surfaces into tube matrices before bulk degradation and rapidly dry weight decreased. Using the longer time for water diffusion in thicker wall thickness was observed. Then, tubes with 5 and 7 layers showed fast degradation after 3 weeks.

Morphology of Degraded Nerve Guide Tubes

The tubes with 3 layers showed dimensional stability only in the first a week of degradation, while the both tubes with 5 and 7 layers can stable until the third week of degradation. The morphology changes of degraded tubes are investigated from SEM micrographs as shown in Fig. 6 and 7 for the first and third weeks of degradation, respectively. It was interesting to note that the porous structures were observed on the both tube surfaces and matrices. These porous structures can be detected after a week degradation period especially the tubes with 3 and 5 layers as shown in Fig. 6. These porous structures increased with the degradation time as shown in Fig. 7.

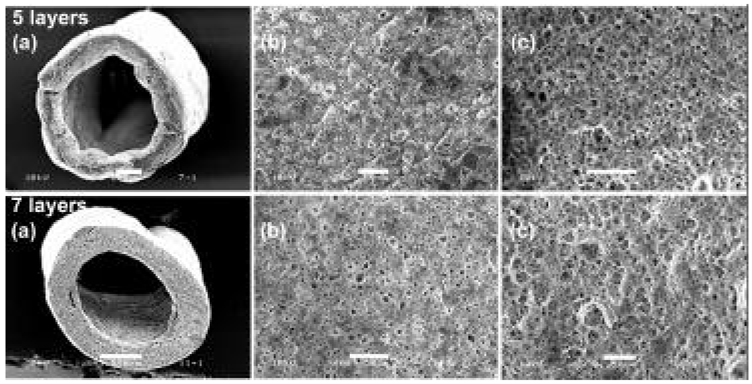

Fig. 7: SEM micrographs of MPEG-*b*-PDLL nerve guide tubes with different layers after 3 weeks degradation period; (a) cross-sections (bars = 200 and 500 μm for 5 and 7 layers, respectively), (b) outer surfaces (bar = 50 μm) and (c) inner surfaces (bar = 50 μm)

These porous structures could be interesting for nerve cell attachment and proliferation throughout the tube matrices and sufficient nutrient permeation. In addition, dimensional stability of these nerves guide tubes on degradation time can be adjusted from number of tube layers which may be used as the choice for using in different sites and types of nerve tissue engineering.

CONCLUSION

The advantages of dip-coating method for preparing the nerve guide tubes are fast preparation, simple, low cost apparatus and no risk of thermal degradation because the polymer is not melt. Different wall thicknesses can be controlled by the number of coating step. The tubes of MPEG-*b*-PDLL prepared from the dip-coating method are transparent, soft and flexible. The T_g of MPEG-*b*-PDLL tubes were increased as the degradation time increased. Their tubes contained porous structures on the both outer and inner surfaces after a week of degradation. These porous tubes might be a valuable modality in nerve tissue engineering. Further *in vitro* degradation test of the MPEG-*b*-PDLL nerve guide tubes with different MPEG and PDLL block lengths are currently being measured.

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