http://dx.doi.org/10.5012/bkcs.2012.33.12.4137

In-vitro and *in-vivo* Behaviors of Poly(glycolide-caprolactone) Copolymer for Bioabsorbable Suture Materials

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Received July 26, 2012, Accepted September 28, 2012

A novel bioabsorbable suture material, poly(glycolide-caprolactone) (PGLCL) monofilament, was prepared by spinning of the PGLCL copolymer. The physical properties, strength retention, biocompatibility, and organism resolvability of the PGLCL monofilament were investigated. The results showed that the knot pull strength of the monofilament was higher than that stated in European Pharmacopoeia. The *in vivo* retention strength following implantation was 64%, 23%, 7%, and 0% after one, two, three, and four weeks, respectively. Mortality, clinical signs, validation, and sterility tests indicated that all items had passed. Organism resolvability tests showed that the PGLCL monofilament, as a suture, was absorbed within 91 days.

Key Words : Poly(glycolide-caprolactone), Absorbable suture, Monofilament, Biocompatibility

Introduction

Sutures, which are used in surgery to close skin incision, can be absorptive or non-absorptive. Absorptive sutures can be classified into natural absorptive sutures, which comprise catgut, extracted from the intestines of cows and sheep, and synthetic absorptive sutures such as those fabricated from polyglycolic acid. Non-absorptive sutures, too, can be made of natural sutures, such as cotton and bast fibre, or synthetic materials such as polyester, nylon, polyethylene, and polypropylene.

In 1940, the United States Pharmacopoeia (USP) and European Pharmacopoeia (EP) proposed that sutures be classified on the basis of their thickness.

In recent years, poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(ε-caprolactone) (PCL) have been investigated widely for application in sutures owing to their excellent biodegradability, biocompatibility, and bioresorbability.[1-6]

PGA is a polymer of glycolic acid (or hydroxyacetic acid), which is the smallest α-hydroxy acid. PGA is a hard, tough, and crystalline polymer with a glass transition temperature of 36 °C.[7,8] PCL, which is produced by the ring-opening polymerization of ε-caprolactone, is a semicrystalline linear resorbable aliphatic polyester with a relatively low glass transition temperature (−60 °C).[9-11]

In a previous study, poly(glycolide-caprolactone) (PGLCL) copolymer, which has the advantages of both PGA and PCL, was synthesized by second step polymerization, and its chemical structure and thermal properties were determined.[12] In the present study, PGLCL monofilaments were prepared by spinning of the PGLCL copolymer. The tensile strength and knot strength of the PGLCL monofilament were investigated *in vitro* and *in vivo*. Biocompatibility tests, including mortality and clinical signs, validation tests, and sterility tests, were carried out on the monofilament. The organism resolvability of the monofilament after 2, 91 and 119 days was also measured.

Experimental

Materials. The PGLCL copolymer used in this study was synthesized according to a previously reported method.[12] The PGLCL monofilament was prepared by spinning the copolymer and used as an absorbable suture material. Figure 1 shows the chemical structure of the PGLCL copolymer.

Synthesis of the PGLCL Copolymer. Glycolide (1000 g, 8.62 mol) and ε-caprolactone (805 g, 7.06 mol) were placed in a pilot reactor. Lauryl alcohol (4.155 g) and the catalyst (33.4 mL) were then added. The solvent was removed by heating at 60 °C for 20 min, under reduced pressure. The resulting mixture was heated gradually to 200 °C and allowed to react for 5 h. The mixture was then re-heated to 220 °C, and glycolide (2276 g, 19.62 mol) was added. The resulting mixture was allowed to react at this temperature for 1 h. Finally, chip-type melting materials were obtained. FT-IR (KBr; *n*, cm^{-1}): 2970 (CH), 1725 (C=O), 1638 (C=C), 1620 (C=C), 1190 (C-O-C), 1062 (C-H), 988 (CH).

Spinning of PGLCL Copolymer. To achieve uniform pressure distribution during the spinning of the copolymer, the nozzle pack composed of metal particles was used as a filter medium, and the spinneret diameter was 1.8 mm. The

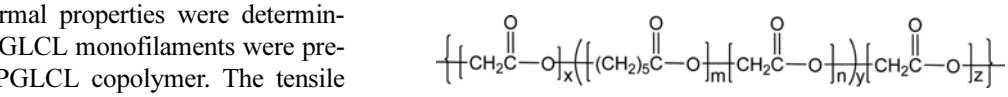

Figure 1. Chemical structure of PGLCL segmented block copolymer.

temperature ranges for the feeding, melting, and mixing sections of the barrel zone were 215-220, 220-225, and 225-230 °C, respectively. The temperature of the die head was 230-240 °C. The filament was prepared according to the USP 2-0 specification, and the diameter was 0.350-0.399 mm.

Characterization and Measurements. The tensile strength of the PGLCL monofilament (length: 200 mm) suture before and after implantation was measured using a universal tester (Instron Model 4014 mechanical tester) at a tensile rate of 200 mm/min. All the tensile properties were obtained as averages of seven experimental values.

In the validation test, badges with or without a black body were implanted in the strain. Fluid thioglycollate and soybean-casein digest badges were cultured at 30-35 °C and 20-25 °C for 7 days, respectively, and the level of fungus growth was examined. In the sterility test, fluid thioglycollate and soybean-casein digest badges were cultured at 30-35 °C and 20-25 °C for 14 days, respectively, and the level of fungus growth was investigated.

The internal muscle of a male rat was sutured using a 10 cm PGLCL monofilament. The local effects of the suture material on the rat muscle was examined by measuring the extent of inflammation, encapsulation, hemorrhage, necrosis, and discoloration.

Results and Discussion

Knot Strength. Table 1 lists the knot strength specifications of an absorbable monofilament in EP. The knot strength reported in this table is the minimum average value. The PGLCL monofilament was prepared by spinning of the PGLCL copolymer. Table 2 lists the physical properties of the PGLCL monofilament. The knot pull strength of the PGLCL monofilament was 3.0-3.1 kgf, which was higher than the minimum value (2.73 kgf) epecified in EP (USP 2-0), as mensioned in Table 1.[13]

Table 1. Knot Strength Specification of Absorbable Monofilament

USP size	EP metric	Knot pull strength (kgf)
7-0	0.5	0.14
6-0	0.7	0.26
5-0	1	0.63
4-0	1.5	0.97
3-0	2	1.80
2-0	3	2.73
0	3.5	3.98
1	4	5.18
2	5	6.46

Strength Retention. Generally, absorbable suture materials retain 50-60% of their initial strength after one week, 20-30% after two weeks, and zero after three to four weeks. The suture materials decompose completely after 90-120 days.[14]

The straight strength and knot strength of the PGLCL monofilament (USP size: 2-0) were measured, and the results are shown in Figure 2. Both the straight strength and knot strength decreased significantly with increasing immersion time. The straight strength was 8.5 kgf before immersion but decreased to 5.3 kgf and 1.7 kgf after one and two weeks of immersion, respectively. Absorption of the monofilament increased notably, which caused the straight strength to fall to zero after four weeks.[15]

Figure 3 shows the strength retention rate of the mono-

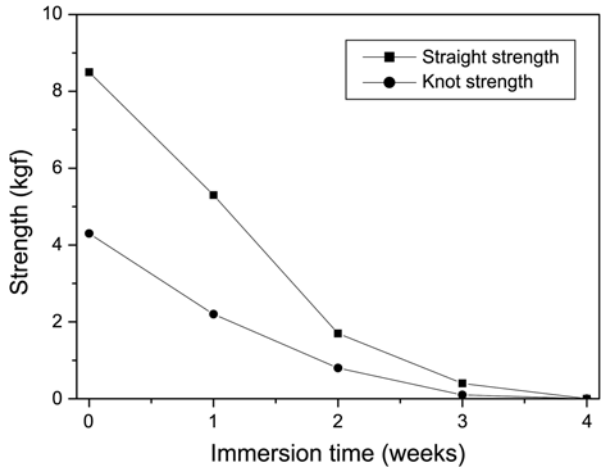

Figure 2. Strength retention of PGLCL monofilament under *in vitro*.

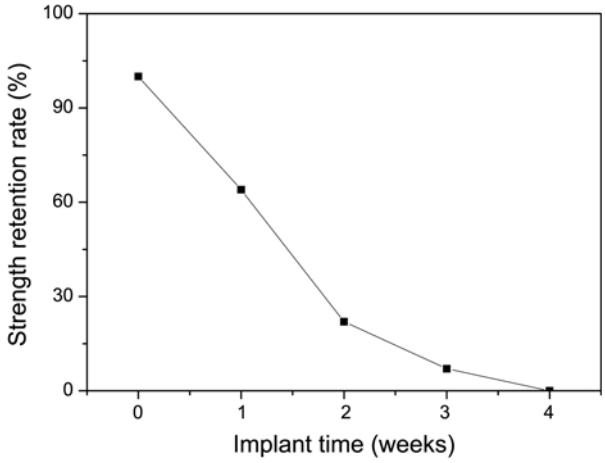

Figure 3. Strength retention rate of PGLCL monofilament under *in vivo*.

Table 2. Physical Properties of PGLCL Monofilament

Sample code	USP size	Tenacity (g/d)	Elongation (%)	Tenacity (Knot strength) (kgf)	Young's modulus (g/d)
1	2-0	6.0	50	3.0	7.8
2	2-0	6.1	48	3.1	8.0

Table 3. Mortality and Clinical Signs

Group/sex	Animal No.	Clinical signs (weeks)					Mortality (dead/total)
		0	1	2	3	4	
G20/male	2101	NAD	NAD	-	-	-	0%
	2102	NAD	NAD	-	-	-	
	2103	NAD	NAD	-	-	-	
	2104	NAD	NAD	-	-	-	
	2105	NAD	NAD	-	-	-	
	2106	NAD	NAD	-	-	-	
	2107	NAD	NAD	-	-	-	
	2108	NAD	NAD	-	-	-	
	2109	NAD	NAD	-	-	-	
	2110	NAD	NAD	-	-	-	
G21/male	2111	NAD	NAD	NAD	-	-	0%
	2112	NAD	NAD	NAD	-	-	
	2113	NAD	NAD	NAD	-	-	
	2114	NAD	NAD	NAD	-	-	
	2115	NAD	NAD	NAD	-	-	
	2116	NAD	NAD	NAD	-	-	
	2117	NAD	NAD	NAD	-	-	
	2118	NAD	NAD	NAD	-	-	
	2119	NAD	NAD	NAD	-	-	
	2120	NAD	NAD	NAD	-	-	
G22/male	2121	NAD	NAD	NAD	NAD	-	0%
	2122	NAD	NAD	NAD	NAD	-	
	2123	NAD	NAD	NAD	NAD	-	
	2124	NAD	NAD	NAD	NAD	-	
	2125	NAD	NAD	NAD	NAD	-	
	2126	NAD	NAD	NAD	NAD	-	
	2127	NAD	NAD	NAD	NAD	-	
	2128	NAD	NAD	NAD	NAD	-	
	2129	NAD	NAD	NAD	NAD	-	
	2130	NAD	NAD	NAD	NAD	-	
G23/male	2131	NAD	NAD	NAD	NAD	NAD	0%
	2132	NAD	NAD	NAD	NAD	NAD	
	2133	NAD	NAD	NAD	NAD	NAD	
	2134	NAD	NAD	NAD	NAD	NAD	
	2135	NAD	NAD	NAD	NAD	NAD	
	2136	NAD	NAD	NAD	NAD	NAD	
	2137	NAD	NAD	NAD	NAD	NAD	
	2138	NAD	NAD	NAD	NAD	NAD	
	2139	NAD	NAD	NAD	NAD	NAD	
	2140	NAD	NAD	NAD	NAD	NAD	

filament *in vivo*. The strength retention rate decreased significantly with increasing immersion time. The strength retention after one, two, three, and four weeks was 64%, 22%, 7%, and 0%, respectively.

Biocompatibility Tests. Mortality and clinical signs were investigated after suturing the rat internal muscle using the monofilament, and the results are listed in Table 3. No animal death or adverse effect of the filament on the rat was observed. During postmortem examination, the implantation region was analyzed using a magnifying glass, and few visible signs of inflammation and necrosis from the material were observed.

Table 4 lists the results of the validation test for bacteriostasis and fungistasis. The badge was compatible in this test, and the samples did not show any infection-resistant nature of the active material.[16]

A sterility test was carried out using the direct transfer method, and the results are listed in Table 5. No bacterial growth was observed, highlighting that the prepared monofilament material complies with the Korean Pharmacopoeia (8^{th} edition) sterility test.[17]

Organism Resolvability. The PGLCL monofilament was used for suturing the rat internal muscle, and the internal absorbability was measured on days 2, 91, and 119. The optical microscopy images in Figure 4 reveal initial the inflammatory responses after implantation and muscle fiber necrosis in some individuals 2 days after implantation. On the other hand, there was no such observation at 91 days, indicating that the PGLCL suture was absorbed completely within 91 days, with complete recovery from any inflammatory response.[18]

Conclusions

A new PGLCL monofilament was prepared for application as a bioabsorbable suture material. The knot pull strength of the monofilament was higher than that stated in EP. The *in vivo* retention strength was 64%, 23%, 7%, and 0% one, two, three, and four weeks after implantation, respectively. Mortality and clinical signs, validation, and sterility tests indicated that all items had passed. Organism resolvability tests showed that the PGLCL monofilament was absorbed within 91 days, with complete recovery from any inflammatory response.

Table 4. Results of Validation Test for Bacteriostasis and Funcistasis

Medium	Micro-organism	Inoculation quantity	Incubation	Test container	Control container	Interpretation
Fluid thioglycollate medium	*S. aureus*	10-100	35 °C, 7 days	Growth	Growth	Meet
	Soybean-casein digest medium	10-100	35 °C, 7 days	Growth	Growth	Meet
	C. sporogenes	10-100	35 °C, 7 days	Growth	Growth	Meet
Soybean-casein digest medium	*B. subtilis*	10-100	25 °C, 7 days	Growth	Growth	Meet
	C. albicans	10-100	25 °C, 7 days	Growth	Growth	Meet
	A. niger	10-100	25 °C, 7 days	Growth	Growth	Meet

Table 5. Results of Sterility Test

Medium	Incubation	2006-07-19	2006-07-26	2006-08-02	Result
Fluid thioglycollate medium	Specimen Negative control	Inoculation Inoculation	No growth No growth	No growth No growth	Pass
Soybean-casein digest medium	Specimen Negative control	Inoculation Inoculation	No growth No growth	No growth No growth	Pass

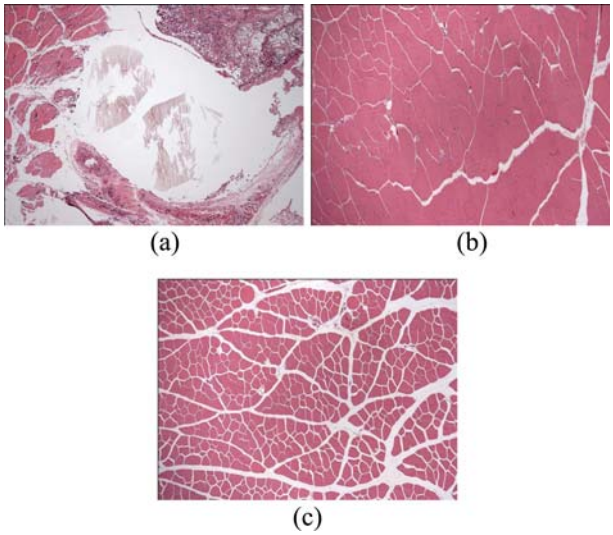

Figure 4. Optical micrographs after implantation: (a) after 2 days (200X); (b) after 91 days (50X); (c) after 119 days (50X).

Acknowledgments. This study was supported by the Dual Use Technology Development Project.

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