Novel Biodegradable Copoly(amino acid)s Based on 6-Aminocaproic Acid and L-Proline

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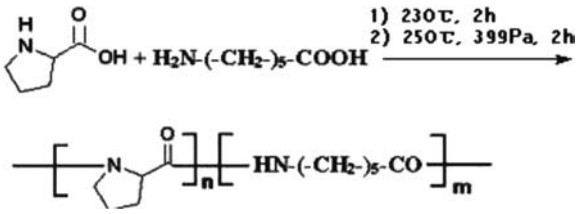
Novel biodegradable copoly(amino acid)s based on 6-aminocaproic acid and L-proline were prepared by melt polycondensation and characterized by IR and ^{1}H NMR. They showed high mechanical properties with high viscosity ranging from 0.72 to 1.70 dL/g. Enzymatic degradation showed that with increasing proline content the enzymatic degradation rate of the copolymers increased. The cellular interaction of copolymer films and HEK 293 cells was observed using SEM; cells adhered and spread well on the material surfaces.

α-L-Amino acids and glycine are basic units composing proteins. Poly(amino acid)s and random copolymers based on nonbiodegradable polymers and an appropriate amount of α-L-amino acid(s) are biodegradable through enzymolysis,[1–4] which can be degraded to amino acids. The amino acids are biocompatible, less toxic and can be absorbed and metabolized as nutriments. The polymers are favorably to be used as tissue engineer materials, drug releasing materials, or environment-friendly package materials and have attracted much attention.

However, poly(amino acid)s and other copolymers based on α-L-amino acids generally display poor thermal stability and low mechanical properties. These drawbacks limit their further application in many fields.

ω-Amino acids possess similar structures to α-L-amino acids. Some ω-amino acids are beneficial to organism.[5–7] It is known that widely used poly(ω-amino acid)s, called nylon in commerce, have excellent mechanical properties. It is expected that poly(amino acid)s based on α-L-amino acids and ω-amino acids should have prosperous fields. Some results showed that the enzymatic degradation of this kind of copolymer could be controlled according to the content of α-L-amino acids in copolymer.[8]

L-Proline, a kind of natural amino acid found abundantly in collagen, has a long methylene linkage with ring structure. It is well known that polymers with such structure generally have good stiffness and toughness. Based on the above considerations, a novel kind of poly(α-L-amino acid) derived from 6-aminocaproic acid and L-proline was synthesized and characterized by IR and ^{1}H NMR. 6-Aminocaproic acid and L-proline were added into a flask under nitrogen flow. The reaction was carried out at 230 °C for 2 h, and 250 °C, 399 Pa for another 2 h, and target polymers were acquired (Scheme 1). N6 indicates pure polyamide 6, and N6P*x* indicates copolymers made from *x* mol % of L-proline in comonomer. IR showed typical polyamide peaks at 3307 cm^{-1} (N–H stretching), 1640 cm^{-1} (C=O stretching, amide I) and 1541 cm^{-1} (N–H bending, amide II), proving the successful target products. Intrinsic viscosity was determined in solutions of the copolymers ($c = 0.4$ g/100 mL in 96% H_2SO_4) with an Ubbelohde viscometer at 25 °C. The properties of poly(amino acid)s are listed in Table 1.



Scheme 1. Synthesis of copoly(amino acid)s.

Table 1. Properties of poly(L-amino acid)s

Code	Molar ratio[a]	Molar content /%[b]	Intrinsic viscosity [η] /dL g^{-1}
N6	100:0	0	1.70
N6P10	90:10	10.1	1.48
N6P20	80:20	20.6	1.10
N6P30	70:30	30.7	0.87
N6P40	60:40	40.5	0.72

[a]Molar ratio in feed, 6-aminocaproic acid to L-proline.
[b]Derived from ^{1}H NMR results.

For copolymers are elastic, mechanical properties were measured on a series IX automated materials testing system (Instron Corporation, UK) at ISO527:3-1995 standard, specimens were pressed in a press machine close to melting point of polymers and then cut by a standard knife. Thickness of specimen was 2 mm. Speed of testing was 50 mm/min. Average value of five measurements was recorded for each sample. Tensile strength and break elongation are shown in Figure 1. With increasing proline content in comonomers, the tensile strength and break elongation of copolymers decrease. When increasing L-proline content in comonomers, molecular weight of copolymers decreases, which decreases mechanic properties of copolymer. The tensile strength and break elongation of copolymers are still high compared with other biodegradable polymers in general. High tensile strength was attributed to the ring structure of L-proline and hydrogen bonds between linkages, and large break elongation was attributed to the methylene linkage of L-proline and 6-aminocaproic acid.

Papain was used to perform the enzymatic degradation tests according to a method in the literature.[9] The polymer films, which were obtained by casting of formic acid solution of polymers on glass, were 10 mm in width, 10 mm in length, and 0.1 mm in thickness. Solution with papain was renewed per week. The results are shown in Figure 2. With increasing L-proline content in copolymer, degradataion rates increase. Copolymers with specified degradation rate can be acquired by adjusting the ratio of proline in comonomers. Andreoni et al.[10] report-

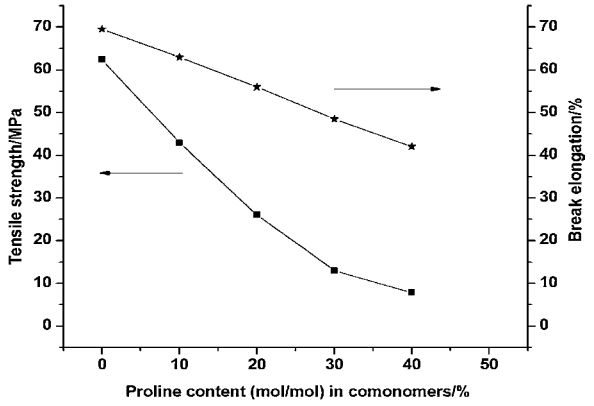


Figure 1. Tensile strength and break elongation of polymers with different proline content.

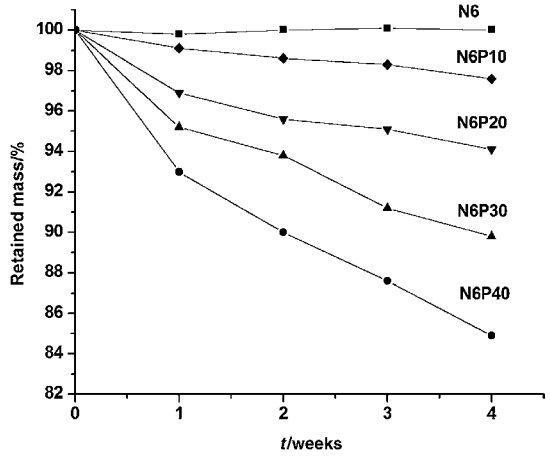


Figure 2. Retained mass of polymers with different proline content after enzymatic degradation.

ed that aerobic bacteria were able to grow on polyamides of low molecular weight (1400, 2100, and 6800) made of 6-aminocaproic acid as the only carbon and energy source, and the growth was accompanied by the utilization of oligomers of up to eight monomeric units present in the polymeric matrix. The extent of the growth was higher on polyamides of lower molecular weight and depended on the amount of oligomers utilized. It could be speculated that when the molar ratio of L-proline:6-aminocaproic acid is above 1:8, copolymers could be biodegradable if copolymer is highly random.

Cell behavior on the material surface is very complicated, involving in cells adhesion, growth, differentiation, and propagation, and relating to the surface characteristics of the material, such as roughness, surface charges, surface energy, hydrophilicity, and so on. Human embryonic kidney cells, also known as HEK 293, are widely used in cell biology research, such as evaluating cytotoxicy of materials.[11] Interactions of HEK 293 cells and copolymers were observed by a scanning electron microscope (SEM). The polymer films were located in a 24-bore culture plate. 2ML HEK 293 cell suspension in RPMI 1640 containing 5% calf serum with cell concentration of 5×10^4/mL was added to the each hole of the culture plate. The cultures were

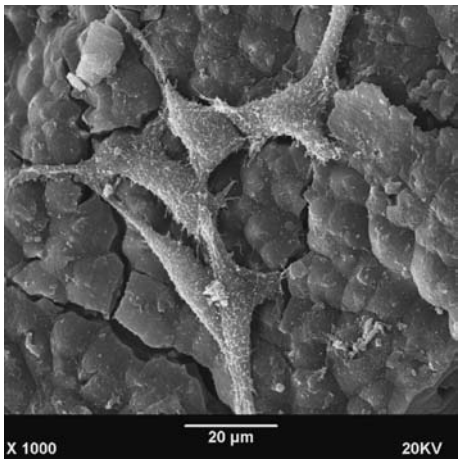


Figure 3. SEM micrograph showing HEK 293 cells adhering to the surface of N6P40 at 2 days of culture.

maintained at 37 °C and humidified atmosphere containing 5% CO_2 for 48 h. The samples were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer for 2 h at 4 °C, and dehydrated with a graded ethanol series and air dried at room temperature. After gold coating by ion sputtering, the samples were examined by SEM. Cells on every sample adhered and spread well. Figure 3 is a SEM micrograph of HEK 293 cell adhering to the surface of N6P40 film after 2 days of culture. The results showed that the obtained copolymers were suitable for cell adhesion and proliferation.

In summary, novel copoly(amino acid)s based on 6-aminocaproic acid and L-proline possess high mechanical properties. Moreover, these copolymers are enzymatically degradable. HEK 293 cells adhered and spread well on the material surfaces. The copolymers are hoped to be new biomedical materials. Detailed study is in progress.

References

1 T. Akagi, M. Higashi, T. Kaneko, T. Kida, M. Akashi, *Biomacromolecules* **2006**, *7*, 297.
2 N. Masateru, T. Naoki, W. Katuya, K. Hiroshi, *Polym. Prepr., Japan* **2005**, *54*, 5357.
3 F. Rypacek, *Polym. Degrad. Stab.* **1998**, *59*, 345.
4 S.-I. Han, B.-S. Kim, S.-W. Kang, H. Shirai, S. S. Im, *Biomaterials* **2003**, *24*, 3453.
5 I. Florentino-Pineda, L. Blakemore, G. Thompson, C. Poe-Kochert, P. Adler, P. Tripi, *Spine* **2001**, *26*, 1147.
6 S. Kalmadi, R. Tiu, C. Lowe, T. Jin, M. Kakaycio, *Cancer* **2006**, *107*, 136.
7 L. E. M. Padovan, T. Okamoto, M. C. R. A. Rezende, V. P. Curvello, D. Nicolielo, M. A. Matsumoto, *J. Biomed. Mater. Res. B: Appl. Biomater.* **2005**, *73B*, 209.
8 I. Arvanitoyannis, E. Psomiadou, N. Yamamoto, E. Nikolaou, J. M. V. Blanshard, *Polymer* **1995**, *36*, 2957.
9 A. Rodriguez-Galan, M. Pelfort, J. E. Aceituno, J. Puiggli, *J. Appl. Polym. Sci.* **1999**, *74*, 2312.
10 V. Andreoni, G. Baggi, C. Guaita, P. Manfrin, *Int. Biodeterior. Biodegrad.* **1993**, *31*, 41.
11 A. Basarkar, D. Devineni, R. Palaniappan, J. Singh, *Int. J. Pharm.* **2007**, *343*, 247.

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