

·快报·

GRGDS 修饰 D,L-丙交酯- β -苹果酸共聚物的生物相容性研究^{*}

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摘 要 聚(D,L-丙交酯- β -苹果酸)(P-COOH)进行改性制得 GRGDS 修饰聚合物(P-GS5),利用红外光谱、元素分析和 X 射线光电子能谱确定了材料的结构和组成.通过内皮细胞粘附实验发现 P-GS5 可明显提高内皮细胞粘附(PDLLA,45% P-GS5,109%),改善材料的细胞亲和性.同时血小板粘附实验结果也表明 P-GS5 可有效抑制血小板粘附(PDLLA,47% P-GS5,18%),降低血栓形成的几率.

关键词 抗血小板粘附, GRGDS, D,L-丙交酯- β -苹果酸共聚物

近年来,合成高分子材料广泛应用于生物医学领域^[1],但临床使用的生物材料与血液接触时,表面常会发生血小板粘附、活化、聚集等问题,限制了其使用效果^[2].为了改善材料的血液相容性,人们曾进行了多方面尝试,如合成新材料、对材料进行表面改性等,但均未达到理想效果.随着研究的深入,人们发现在材料表面种植内皮细胞可能是一种改善材料血液相容性的重要方法^[3].为提高材料对内皮细胞的粘附,研究者模拟天然细胞外基质,在材料表面涂敷各种细胞粘附蛋白,如胶原、纤粘连蛋白、层粘连蛋白等并取得了一定成果^[4].随后发现多种粘附蛋白结构中都含有 Arg-Gly-Asp(RGD)序列,该序列与粘附活动密切相关,可促进内皮细胞的粘附和生长,但其同时也可以识别血小板膜糖蛋白 IIb/IIIa(整合素 α IIb β 3),进而引发血小板聚集^[5].目前对 RGD 修饰材料的血液相容性还存在争议^[6,7].因此,对其血小板粘附性质的研究具有重要意义.

本文以聚(D,L-丙交酯- β -苹果酸)(P-COOH)为基质材料,接枝修饰 Gly-Arg-Gly-Asp-Ser(GRGDS),得到活性多肽修饰材料 P-GS5,并研究了内皮细胞和血小板在材料上的粘附情况.结果表明,P-GS5 不仅能有效提高内皮细胞的粘附,而且可在一定程度上抑制血小板的聚集,有望成为一种新型的抗凝血材料.

1 试剂与仪器

二环己基碳二亚胺(DCC)购于国药集团化学试剂有限公司;N-羟基琥珀酰亚胺(NHS)购于 Acros 公司;NH₂-GR(PdF)GD(otBu)S(tBu)-COOH(GS5)合成于吉尔生化有限公司;苯甲硫醚、乙二硫醇购于 Alfa Aesar 公司.其他化学试剂均为分析纯.元素分析仪(EA)Hemeus Vanio-EL;红外光谱仪(IR)MAGNA-560;全自动血细胞分析仪 Sysmex SE-9000;X 射线光电子能谱仪(XPS)Kratos AXIS Ultra DLD;接触角测定仪 Kruss G1023;扫描电镜(SEM)HITACHI S-3500N.

2 材料的合成

聚乳酸(PDLLA)和聚(D,L-丙交酯- β -苹果酸)(苹果酸内酯含量 8 mol%, $M_w = 4.5 \times 10^4$,PDI = 1.6)的合成参考文献报道^[8,9].将聚合物 P-COOH 溶于无水二氯甲烷中,氮气保护下加入 DCC 和 NHS 活化(DCC:NHS:COOH = 2:2:1,摩尔比),室温反应 24 h.过滤除去沉淀,滤液用乙醚沉淀.将沉淀重新溶于无水 DMF 中,与 GS5、三乙胺(TEA)室温反应 4 天(GS5:TEA = 1:2,摩尔比).除去溶剂后加入 K 试剂(三氟乙酸:水:苯酚:苯甲硫醚:乙二硫醇 = 82.5:5:5:5:2.5,体积比)室温反应 2 h,乙醚沉淀.所得产品 30℃真空干燥 1 天,得 P-GS5.合成路线如图 1 所示.聚合物 P-GS5 结构与组成通过 IR、EA 和 XPS 分析检测.

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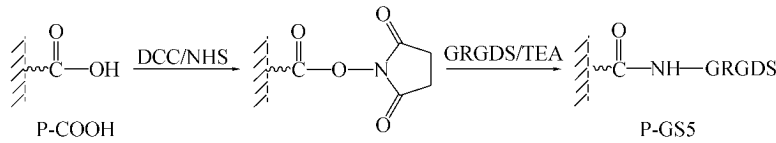


Table 2 Water contact angle of polymers

	PDLLA	P-COOH	P-GS5
Water contact angle(°)	84.51 ± 2.68	76.11 ± 1.73	58.40 ± 0.85

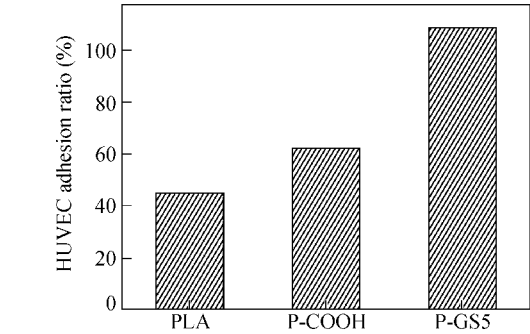


Fig. 3 HUVEC adhesion ratio on polymer films (24 h)

减少了血小板在聚合物上的粘附^[12]。从血小板粘附形貌的扫描电镜图中也可看出粘附数量的差异(图略)。PDLLA材料上血小板数

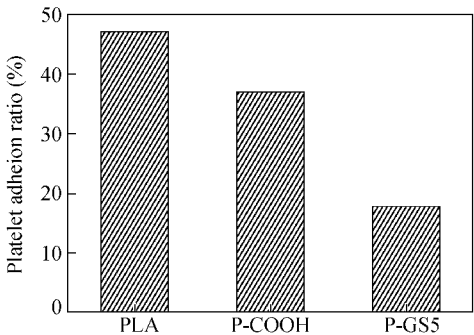


Fig. 4 Platelet adhesion ratio on polymer films (30 min)

量较多并有大量聚集,且有明显伪足形成;P-COOH上的血小板聚集现象较少;P-GS5上血小板则基本无聚集,形态正常。综上所述,与PDLLA、P-COOH相比,材料P-GS5同时具有较好的血液相容性和优越的细胞亲和性,可有效提高内皮细胞粘附,降低血栓形成的几率。

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BIOCOMPATIBILITY STUDIES ON GRGDS SEQUENCE MODIFIED POLY(D,L-LACTIDE-*co*- β -MALIC ACID)

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Abstract A novel peptide-grafted polymer P-GS5 was synthesized by modification of poly(D,L-lactide-*co*- β -malic acid) (P-COOH) with pentapeptides Gly-Arg-Gly-Asp-Ser (GRGDS), and its biocompatibility was investigated. Infrared spectroscopy (IR), element analysis (EA) and X-ray photoelectron spectroscopy (XPS) were used to characterize the synthesized materials (poly(D,L-lactide) (PDLLA), P-COOH, P-GS5). Hydrophilic properties of these polymers were investigated by contact angle measurements (CA). Subsequently, biocompatibility experiments were conducted by testing human umbilical cord vein endothelial cell (HUVEC) (24 h) and platelet (30 min) adhesion on polymer surfaces. The number of endothelial cell and platelet adhesion was counted by cytometer and automatic hematological analyzer, respectively. It has been shown that the hydrophilicity of P-GS5 was greatly improved. Water contact angle was 84° for PDLLA, 78° for P-COOH and 58° for P-GS5. Cell adhesion data indicated that P-GS5 could promote endothelial cell attachments. The adhesion ratio of PDLLA, P-COOH and P-GS5 was 45%, 62% and 109%, respectively. According to the platelet adhesion results, GRGDS on P-GS5 did not induce platelet aggregation. The platelet adhesion capacity of P-GS5 was much lower than that of the other two polymers, while the adhesion ratio of PDLLA, P-COOH and P-GS5 was 47%, 37% and 18%, respectively. In conclusion, P-GS5 displayed improved hydrophilicity, higher affinity to endothelial cell and better ability of anti-platelet adhesion compared with PDLLA and P-COOH. It could be a potential biomedical polymer.

Keywords Anti-platelet adhesion, GRGDS, Poly(D,L-lactide-*co*- β -malic acid)