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基于聚苹果酸的贻贝仿生粘合剂的制备及性能研究 *

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摘要 目的:以实验室制得的聚苹果酸(PMLA)为高分子骨架,通过酰胺化反应将多巴胺(DA)分子连接到聚苹果酸,制得既具有良好粘合性能,又有优异生物相容性的贻贝仿生粘合剂。**方法:**L-天冬氨酸通过内酯开环聚合合法合成高分子化合物聚苹果酸,将PMLA与DA加EDC/NHS反应得到粘合剂PMLA-DA,傅里叶变换红外光谱、核磁共振、紫外可见光谱等对其进行结构表征,标准曲线法测多巴胺取代度,采用搭接剪切测试法评估粘合剂对不锈钢、玻璃、猪皮三种材质的粘合强度,MTT法检测贻贝仿生粘合剂的细胞毒性,通过降解试验验证PMLA-DA的降解性能。**结果:**测得PMLA-DA中多巴胺的取代度能达到21.3%,搭接剪切试验测得粘合剂对猪皮的粘合强度为22.68 kPa,高于目前市场上常用的生物医用粘合剂纤维蛋白胶15.38 kPa的粘合强度,PMLA-DA对不锈钢与玻璃亦有很好的粘合性能。细胞毒性研究和体外降解试验显示PMLA-DA无细胞毒性,降解性能良好。**结论:**通过聚苹果酸与多巴胺反应制得贻贝仿生粘合剂PMLA-DA,该粘合剂对多种材质均具有良好的粘合力;无细胞毒性,降解性能良好,对皮肤组织的粘合强度优于目前商用的生物粘合剂纤维蛋白胶。无论是对无机材料间的粘接,或者是医学领域的伤口粘合,均具有良好的应用前景。

关键词:聚苹果酸;多巴胺;贻贝仿生;粘合剂

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Preparation and Properties of Mussel-Inspired Adhesive Based on Poly (β-malic acid) *

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ABSTRACT Objective: Using the laboratory made poly (β-malic acid) (PMLA) as a polymer backbone to attach dopamine (DA) by amidation reaction, obtain a mussel-inspired adhesive with good adhesion and excellent biocompatibility. **Methods:** The polymer compound PMLA was prepared from L-aspartic acid by lactone ring-opening polymerization method, then PMLA and DA react with EDC/NHS to get the adhesive PMLA-DA. It is characterized by FT-IR, ¹H-NMR and UV-Vis. Determination of dopamine substitution degree by standard curve method. The adhesion strength of PMLA-DA to stainless steel, glass and porcine skin are evaluated by lap shear strength test. The cytotoxicity of the mussel-inspired adhesive is detected by MTT experiment. Using *in-vitro* experiment to verify the degradation performance of PMLA-DA. **Results:** The dopamine substitution degree in PMLA-DA could reach 21.3%, and the adhesion strength to porcine skin was 22.68 kPa, which was higher than the clinically used fibrin glue's 15.38 kPa, PMLA-DA also has good adhesion to stainless steel and glass. Cytotoxicity experiments and *in vitro* degradation tests show that PMLA-DA is non-cytotoxic and has good degradation performance. **Conclusions:** A mussel-inspired adhesive PMLA-DA was prepared from poly (β-malic acid) and dopamine, this adhesive has good adhesion to a variety of materials with no cytotoxicity, and good degradation performance. The adhesion strength to skin tissue is superior to the current commercial bio-adhesive fibrin glue. Its biocompatibility is better than cyanoacrylate adhesive. Whether it is bonding between inorganic materials or wound adhesion in the medical field, it has a good application prospect.

Key words: Poly (β-malic acid); Dopamine; Mussel-inspired; Adhesive

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前言

贻贝仿生粘合剂是目前人们对粘合剂研究中的一个新热点^[1]。贻贝能够在水中粘附于各种非特异性的界面,贻贝的强粘

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附能力在于其足分泌的粘附蛋白中存在一种叫 L-3,4- 二羟基苯丙氨酸(L-DOPA)的含邻苯二酚结构的氨基酸^[2-4]。在氧化或碱性条件下,DOPA 上的邻苯二酚羟基被氧化成邻醌促使足部粘合蛋白发生交联反应,随后触发分子间交联,使蛋白质网络具有内聚力和体积弹性的性质^[5,6]。

聚苹果酸(Poly β -malic acid)是以苹果酸为唯一单体相互通过酯键连接而成的一种脂肪族聚酯,在体内酶的作用下最终降解为水和二氧化碳排出体外,不会有蓄积毒性^[7]。本文受贻贝的粘附性质启发,以聚苹果酸作为聚合物骨架,与多巴胺直接缩合连接,以期得到反应更加简单,结构更加简洁,粘合性能更强的类贻贝粘附生物粘合剂,摸索了合成反应的最佳条件与路径,并研究了该粘合剂对不锈钢、玻璃与猪皮三种不同材质的粘合强度^[8,9]。

1 仪器与试剂

1.1 仪器

Advance DMX500 核磁共振波谱仪(德国 Bruker 公司);FD5-2.5E 冷冻干燥机(美国 GOLD-SIM 公司);IRAFFINITY-1S 傅里叶变换红外光谱仪(日本 shimadzu 公司);UV-6100S 紫外分光光度计(上海美谱达公司);EZ-TEST 台式电子试验机(日本 shimadzu 公司)。

1.2 试剂

多巴胺(DA)(华夏试剂);N- 羟基丁二酰亚胺(NHS)(罗恩试剂);1- (3- 二甲氨基丙基)-3- 乙基碳二亚胺盐酸盐 (EDC·HCl)(国药化学试剂);透析袋购自科昊生物工程有限公司;不锈钢板及猪皮购自建材市场与超市。

2 方法与结果

2.1 PMLA-DA 的制备

以 L- 天冬氨酸为初始原料,参照本实验室现有的 PMLA 合成工艺,通过内酯开环聚合法合成得到 PMLA^[10],GPC 测定分子量。取 0.2 g 制得的 PMLA (相对分子质量 12 kDa,含 -COOH 1.7 mmol)、0.39 g NHS(3.4 mmol)和 0.65 g EDC·HCl(3.4 mmol)置于 25 mL 烧瓶中,加 10 mL 水使其溶解,避光搅拌反应 6 h,按不同比例加入一定量的 DA 试剂,抽真空、通氮气,通过反应条件筛选,当温度为 25 °C,反应时间为 3 d 时,产物产率最高,达 96.13%。经过透析,冻干得到 PMLA-DA(图1)。

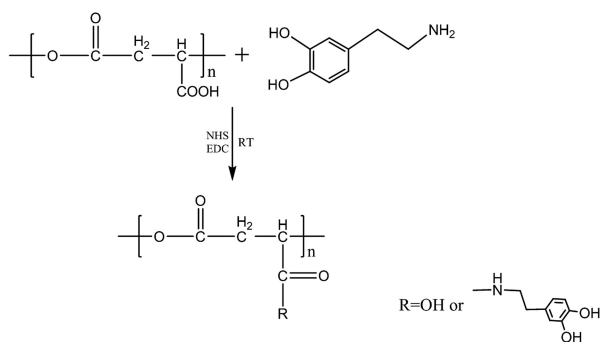


图 1 PMLA-DA 的合成路线

Fig. 1 Synthetic route of PMLA-DA

2.2 PMLA-DA 的结构表征

红外图谱和核磁共振谱对产物 PMLA-DA 进行表征。红外图谱中 PMLA-DA 的 1527 cm^{-1} 为酰胺键的特征峰,表明多巴胺成功连接到聚苹果酸分子中^[11],3433 cm^{-1} 处 O-H 的伸缩振动峰发生红移,而且宽度增加,是由于多巴胺的引入增加分子内的氢键所致(图 2A)。核磁共振谱 2.8-3.1 ppm 为 PMLA 主链上亚甲基质子峰,5.2-5.4 ppm 为主链上次甲基质子峰;8.15 ppm 为 DA 的氨基质子峰,PMLA-DA 在 6.4-6.7 ppm 出现的苯环质子峰来自多巴胺的邻苯二酚结构,而氨基质子峰在 PMLA-DA 上消失,进一步证实了多巴胺通过酰胺反应成功连接到聚苹果酸分子中^[12](图 2B)。

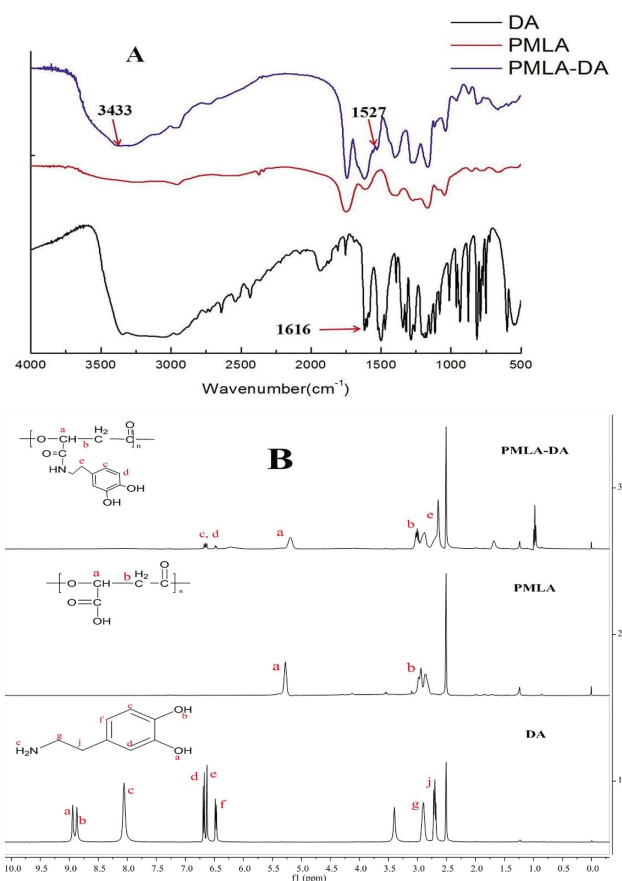


图 2 PMLA-DA 的结构表征

(A. PMLA-DA,PMLA 与 DA 的红外图谱 B. PMLA-DA,PMLA 与 DA 的核磁共振谱, DMSO-d₆)

Fig. 2 Structural characterization of PMLA-DA

(A.FT-IR spectrum of PMLA-DA , PMLA and DA B. 1H-NMR of PMLA-DA, PMLA and DA, DMSO-d₆)

2.3 DA 取代度的测定

用蒸馏水配置 0.01、0.02、0.04、0.06、0.08、0.10 mg/mL 等一系列浓度梯度的 DA 溶液,通过紫外分光光度计记录其不同浓度的 DA 在 280 nm 的吸光度的值,通过线性拟合建立标准曲线: $y=13.43x-0.0032$ $R^2=0.9992$ (y 代表 280 nm 处吸光度, x 代表多巴胺浓度 mg/mL)。按多巴胺与聚苹果酸羧基投料摩尔比分别为 25 %、50 %、75 %、100 %、125 %反应,得到的产物配成 1.00 mg/mL 的溶液进行 UV-Vis 测试,记录 280 nm 处的吸光度,通过标准曲线计算得到各投料比反应所得产物中多巴胺

的取代度(表 1)。当 DA 与 PMLA 的 -COOH 投料摩尔比为 1:1 时,DA 在产物中的取代度达到 21.3%, 继续增加多巴胺的用量,取代度不再增加。由于多巴胺对粘合剂的粘合性质起到关键性作用,因此选用 P-D 1.00 组产物进行粘合强度测试。

表 1 PMLA-DA 中多巴胺的取代度

Table 1 Degree of substitution of dopamine in PMLA-DA

粘合剂名称	DA: -COOH (摩尔比)	取代度(wt%)
P-D 0.25	25%	8.9
P-D 0.50	50%	14.3
P-D 0.75	75%	20.6
P-D 1.00	100%	21.3
P-D 1.25	125%	19.2

2.4 PMLA-DA 对不同基材粘合强度的测定

用 Shimadzu 台式电子试验机,按照 ASTM D1002-02 标准测试搭接剪切强度^[13],拉伸速度为 5 mm/min,基材分别为 7.6 cm × 2.5 cm 的载玻片、不锈钢和猪皮。将 20 μL PMLA-DA 的 PBS 溶液(50wt%) 均匀涂抹于基材一端,将另一块同类基材粘附其上,搭接面积为 1.5 cm × 2.5 cm,置于室内环境 2 h,接着

进行拉伸剪切强度测试,每组基材测试 3 次,取平均值(图 3)。结果显示,PMLA-DA 对三种不同基材均具有粘合性,其中在玻璃界面的粘合度最大,达到 34.12 kPa;对不锈钢界面的粘合度 12.05 kPa,在类似人体皮肤组织的猪皮的粘合强度可以达到 22.68 kPa,高于目前已上市用于临床的纤维蛋白胶对猪皮 15.38 kPa 的粘合强度^[8]。

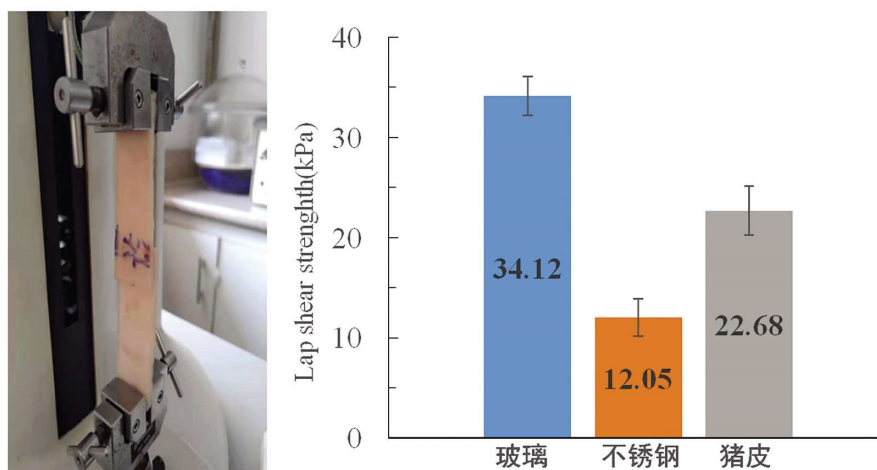


图 3 PMLA-DA 在不同基材上的粘合强度

Fig. 3 Adhesion strength of PMLA-DA on different substrates

2.5 细胞毒性研究

以培养到对数期的成纤维细胞(L929)为模型细胞,采用噻唑蓝 (MTT) 法对粘合剂 P-D 1.00 的细胞毒性进行检测^[14](图

4)。结果显示,PMLA-DA 的浓度增大到 2 mg/mL,亦没有明显的细胞毒性($P>0.05$),表明其可用于人体组织的粘合。

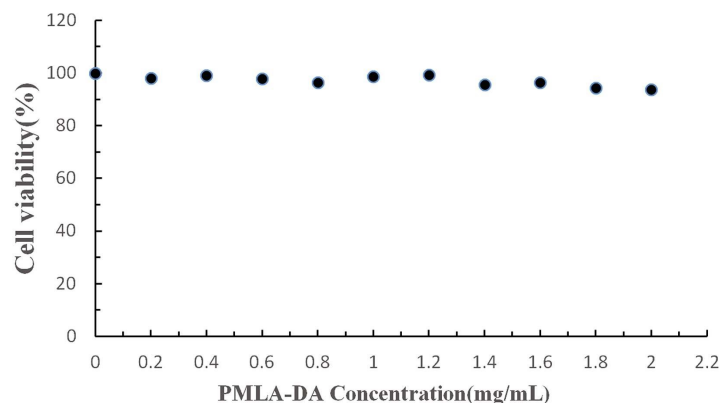


图 4 PMLA-DA 的细胞毒性结果

Fig. 4 Cytotoxicity results of PMLA-DA

2.6 PMLA-DA 的体外降解研究

取三组 PMLA-DA 样品各 10 mg, 使其完全浸没在 10 mL 的 PBS 缓冲液(pH=7.4)中。将含有样品的缓冲液的烧杯放在 37℃ 振荡培养箱中。每隔一段时间取出样品, 透析、冻干后称重。记录样品质量, 重复三次, 取其平均值(图 5)。

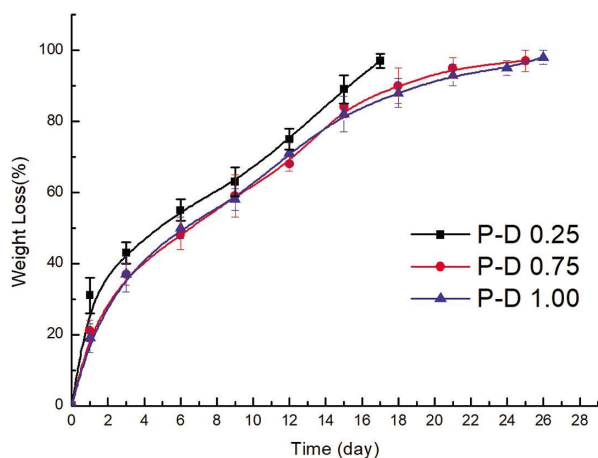


图 5 PMLA-DA 的体外降解结果

Fig. 5 In vitro degradation results of PMLA-DA

从三组不同组分的 PMLA-DA 的体外降解曲线可以看出, PMLA-DA 的质量均随着降解时间的延长而减小。对比三条曲线可以看出, P-D 0.25 的降解速度最快, 第 17 天降解 95% 以上; 而 P-D 0.75 和 P-D 1.00 的降解速率相当, 第 25 天能够完成降解 95% 以上。这是由于与 P-D 0.75 和 P-D 1.00 相比, P-D 0.25 中接入的多巴胺量较少, 导致体系交联程度也较小, 表现为其降解速度最快。

3 讨论

Mehdizadeh 等人合成的仿贻贝生物粘合剂的反应中, 柠檬酸首先与聚乙二醇发生酯化反应, 多巴胺利用氨基与柠檬酸上的羧基反应, 连接到聚乙二醇骨架中去^[9]。但是, 聚乙二醇仅两端含有可反应的羟基, 导致合成的产物中多巴胺的含量较少, 限制了它的粘附强度。为使制得的贻贝仿生粘合剂拥有更强的粘附能力, 应该尽可能地在产物中增加多巴胺的含量。本试验以化学开环聚合合法合成的 PMLA 作为高分子骨架, 通过多巴胺进行酰胺反应得到粘合剂 PMLA-DA。反应所用试剂少, 安全系数高, 原料聚苹果酸与多巴胺均具有良好的生物相容性和可降解性; 反应过程安全可控, 简单易行, 得到的产物化学结构规整, 生物相容性良好。通过 UV-Vis 测试 PMLA-DA 在 280 nm 处的吸光度值, 计算得到粘合剂中多巴胺的取代度可以达到 21.3 wt%。

通过搭接剪切试验测试粘合剂对三种不同基材的粘附强度, 结果表明 PMLA-DA 对玻璃的粘附性能最强, 猪皮次之, 对不锈钢的粘合力最差。我们推测这与材料和 PMLA-DA 之间形成的螯合作用、氢键作用、共价键作用以及介质的表面能等因素有关^[15,16]。不锈钢界面的官能团较少, 不能与粘合剂形成氢键, 而且不锈钢质地坚硬, 表面能小, 不利于粘附, 导致粘合力较小; 与猪皮的粘附机制可能是酚羟基氧化后形成的邻苯醌与

猪皮组织中的氨基通过席夫碱反应或迈克尔加成反应形成的共价键作用, 但是猪皮的表面不像玻璃那么平滑均匀, 导致粘附性能有所下降^[17-20]; 而玻璃表面存在大量的硅羟基, 这能够和粘合剂形成氢键, 加之玻璃界面光滑, 表面能大, 有利于粘合剂的涂覆以及与表面的紧密接触, 可能是玻璃界面粘附力最大的原因^[21-23]。本试验制得一种可降解、无细胞毒性的粘合剂, 可对多种基材进行粘附连接, 对猪皮的粘附性能高于市场上的纤维蛋白胶粘合剂, 具有较高的应用前景。

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