

## Covalently Conjugation of Genistein with Biodegradable Poly L-Lactide

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*A novel drug (Genistein) conjugated biodegradable polymer (PLLA) was synthesized by the direct coupling of Genistein with Poly L-Lactide (GEN-PLLA) and was characterized by FTIR, HPLC and GPC study. These results indicate that the unique property of bound Genistein has an inhibiting influence on the coagulation, plasma protein adsorption, and subsequent platelet adhesion systems. This novel GEN-PLLA conjugate could be applied as blood/tissue compatible biodegradable materials for implantable medical devices and tissue engineering, especially to medical devices like coronary stents. © Society for Biomaterials and Artificial Organs (India), 20090220-38.*

### Introduction

Drug delivery plays an important role in the development of pharmaceutical dosage forms for the animal health care industry because often the duration of drug release needs to be extended over days up to several months. The application of polymeric materials for medical purpose is growing very fast. The biodegradable polymers have found applications in such diverse biomedical fields as tissue engineering, implantation of medical devices and artificial organs, prostheses, ophthalmology, dentistry, bone repair, and many other medical fields. All these applications benefit from the fact that the polymer "disappears" after providing a desired function.

Biodegradable polymers are widely used as drug carriers, wound dressing [1], medical devices [2] and scaffolds in tissue engineering [3]. Aliphatic polyester contains flexible ester bonds and easily degrades in to nontoxic matter in the body. Using PLLA directly as drug carrier, burst release or biphasic release occurred [4]. Covalently attachment of the drug to lactic acid oligomers with the aim of achieving temporary hydrophobization and slower release controlled by the separation of the drug from the

degradable link within the polymer matrix [5] can be a promising option to the present drug delivery stent base systems.

By reviewing the field of oncology, conjugation method of Drug-Polymer is an important aspect to control the cell growth by target site drug delivery. Conjugated polymer gives pro-long release of drug at the local site.

Polymer-drug conjugation is one of the major strategies for drug modifications, which manipulates therapeutic agents at molecular level to increase their solubility, permeability and stability, and thus biological activity.

The most attractive and commonly used biodegradable polymers are polyesters such as poly-L-lactide, poly (lactide-co-glycolide) (PLGA) and poly caprolactone (PCL). These materials are commercially available in different compositions and molecular weights which allow control degradation of the polymer [6,7].

Genistein (5,7,4'-trihydroxy isoflavone) are synthesized by a wide variety of dietary plant species such as grapes, mulberries and

peanuts [8] and in dietary isoflavones such as beans and legumes [9]. Phenolic rings in Genistein are responsible for estrogen receptor binding [10]. The hydroxyl group at the C-4' position enhanced the antioxidant properties of Genistein drug. The structure of Genistein consisting of two benzyl rings by a three- carbon bridge, which is simplified as C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>. It is also classified as a phytoestrogens due to its weak estrogenic activity in mammalian systems [11].

Genistein is a potential flavonoid which possesses anti-thrombotic and anti-proliferative properties. Data suggest that Genistein may exert a strong anti-carcinogenic effect, and that this effect possibly involves an induction of p21, which inhibits the threshold kinase activities of Cdk's and associated cyclins, leading to a G2/M arrest in the cell cycle progression [12, 13]. It inhibited collagen induced human platelet aggregation in a dose-dependent manner [14,15]. It has been shown to enhance NO production from the endothelium. NO is a potent inhibitor of platelet adhesion, aggregation and thrombosis. Impaired platelet production of NO has been associated with acute coronary syndromes. Thus Genistein may affect platelet aggregation via an NO-dependant signal transduction pathway, a potential mechanism. The antitumor effect of Genistein has been reported through the inhibition of protein kinase pathways leading to gene expression modification of many proteins, including VEGF.

Novel drug-biodegradable polymer conjugates can serve the purpose of sustained drug delivery simultaneously with the polymer degradation period. In the present study, Genistein was conjugated with biodegradable PLLA and was performed as coating on coronary stent.

#### Materials and methods

The Stainless steel 316L electropolished stents (16mm length) were used in the present study.

L-Lactide (Boehringer-Ingelheim, Germany) was purified by recrystallization from dry ethyl acetate and dried in vacuo at room temperature. Genistein was obtained from Ren Young Pharmaceutical Co Ltd, China and used without

further purification process. Stannous octoate (Sn-oct, Sigma), Dicyclohexylcarbodiimide (DCC, FLUCA), 4-(dimethyl amino) pyridine (DMAP, FALUCA), dichloromethane (DCM), Dimethyl Sulphoxide (DMSO) and other chemicals used in the current investigation were of HPLC grade procured from Ranbaxy Fine Chemicals Ltd, India.

#### Methods

PLLA was synthesized by the reported method [16]. Briefly, L-lactide (10 g, 0.07 mol) was melted at 180 °C for 30min in a nitrogen atmosphere. Stannous octoate (0.1 wt % of L-lactide) was added to the melted L-lactide solution. The mixture was degassed and stirred at 150 °C for 30 min. The product was dissolved in chloroform and then precipitated in excess methanol. The precipitate was filtered and dried overnight under vacuum.

A DCC, DMAP chemistry was used to synthesize the PLLA-Genistein. Genistein C<sub>15</sub>H<sub>10</sub>O<sub>5</sub> (0.02702 gm 1x10<sup>-4</sup> mol) and PLLA (0.1g, 2.0x10<sup>-4</sup> mol) was dissolved in the DMSO (50 ml), N, N-dimethyl formamide (DMF, 50 ml) respectively. DCC (2x10<sup>-4</sup>mol) and DMAP (2x10<sup>-4</sup>mol) were added to drop by drop PLLA solution in DCM was added to Genistein solution with stirring for 10 min at constant 50°C. The reaction was further carried out for 18 hours under nitrogen atmosphere. After the coupling reaction, the reaction solution was precipitated in excess methanol. The precipitate was dissolved in chloroform and the solution was precipitated in excess methanol. After filtering, the precipitate was dried at 35°C for 24 h in vacuum to eliminate the residual solvent.

HPLC analysis of Genistein were performed on HPLC-LC-2010 AHT [Shimadzu (Asia Pacific) Pvt. Ltd.] consisting of X-Terra RP-18 (250\*4.6 mm) analytical column having particle size 5µm. The drug content was analyzed using mobile phase consisting of acetonitrile: methanol: water (45:35:20 v/v) at flow rate of 1.2 ml/min and oven temperature 40°C. The retention time for Genistein was kept at 2.9 minute and was detected at 254 nm by UV absorption.

This analysis was performed on mother liquor to set molar ratio of genistein and PLLA. Liquor was taken from the reaction mixture at each 18





the figures that the coating surface is free from lumps, crack, and webbing or bridging. It is proved the feasibility of Genistein-PLLA conjugation on stent surface.

### Conclusion

From FTIR analysis Genistein was successfully conjugated with PLLA. The GEN-PLLA can be applied to stent coating, which may release genistein from the stent surface after stent implantation, which may reduce the risk of restenosis and other implications which are challenges of post implantation of stent after angioplasty.

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