Chemical Synthesis of Amine and Carboxyl Functionalized Polypyrrole Nanoparticles and Covalent Attachment of Antibody for Biosensing

Jifu Mao, Ze Zhang
Axe médecine régénératrice, Centre de recherche du CHU de Québec; Département de chirurgie, Faculté de médecine, Université Laval, Québec (QC), Canada

Introduction: N-functionalized polypyrrole (NPPy) has gained significant attention in biosensors and tissue engineering due to the unique combination of its properties including conductivity, biocompatibility and biofunctionality [1, 2]. In PPy-based biosensing, specific biomolecules are immobilized to PPy substrate to form recognition center. Among various immobilization methods, covalent grafting is considered the most effective approach because of the stability and reliability. Therefore, NPPy, with a variety of functional groups, has been extensively investigated for biosensing. However, to our knowledge, most NPPy polymers are synthesized through electropolymerization with limitations such as small size and low productivity. Consequently, the objective of this study was to chemically synthesize the NPPy nanoparticles suitable for the covalent immobilization of biomolecules used for biosensor. In this study, via emulsion polymerization, poly(pyrrole-co-(1-(2-carboxyethyl)pyrrole)) (PPy-COOH) and poly(N-3-aminopropylpyrrole-co-pyrrole) (PPy-NH_2) nanoparticles were prepared for the first time. Human serum albumin antibody (Anti-HSA) as a model sensing molecule was successfully immobilized onto the surface of particles.

Materials and Methods: Py-COOH and Py-NH_2 monomers were synthesized following the procedures in literature [2, 3]. PPy-COOH and PPy-NH_2 nanoparticles were synthesized through w/o and o/w emulsion systems using $FeCl_3$ as oxidant, chlorine anions (Cl^-) as dopant in the presence of emulsifiers. With the help of NHS-EDC chemistry, FITC conjugated Anti-HSA was grafted onto the surface of the nanoparticles. The specimens were characterized by SEM, FTIR, XPS and four-point probe resistivity measurement. To verify the activity of the grafted Anti-HSA, the Anti-HSA grafted specimens were firstly treated with BSA to block non-specific absorption then incubated in the rhodamine conjugated HSA/PBS solution. The specimens were observed under a fluorescence microscope to identify the grafted antibody and the antibody-antigen interaction.

Results and Discussions: The conductivity of the PPy nanoparticles measured in the order of 10 S/cm, while that of the PPy-COOH and PPy-NH_2 nanoparticles measured in the order of 10^{-3} S/cm. The decline in conductivity was expected because it is well known that the polymers of the N-derivatives of pyrrole have lower conductivity than that of the pure PPy. The morphology of the nanoparticles is showed in Figure 1. The mean diameters of all the polymer particles were in nano-scale (Fig. 1A-C), and the diameter of the PPy-NH_2 particles was around 150 nm, which was almost half of the other two types of particles. The FTIR spectra (Fig. 1D) confirmed the chemical structures of copolymers, including the characteristic C=O stretch vibration of PPy-COOH at 1715 cm^{-1} and the absorptions around 3250 cm^{-1} corresponding to the -NH_2 groups in PPy-NH_2. The XPS survey scans (Fig. 1E) showed remarkably increased oxygen content of the PPy-COOH particles compared with that of the pure PPy, meanwhile the nitrogen content of the PPy-NH_2 increased slightly. Altogether, the analyses demonstrated that both PPy-COOH and PPy-NH_2 particles had suitable electrical conductivity and chemical reactivity.

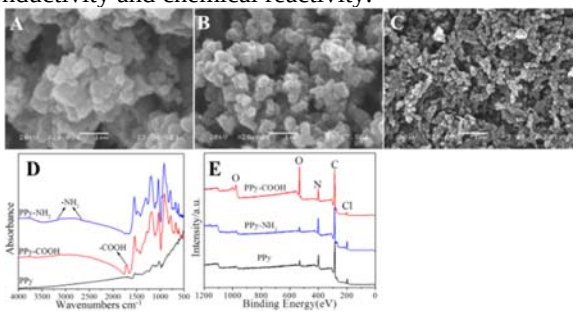

Figure 1. SEM photomicrographs of the PPy (A), PPy-COOH (B), and PPy-NH_2 (C) nanoparticles; and their FTIR (D) and XPS (E) spectra.

The results of fluorescence microscopy (Fig. 2) showed that the sample of PPy-NHS grafted with Anti-HSA has much stronger fluorescence intensity compared to unactivated PPy-COOH. And also the Anti-HSA grafted sample indicated more fluorescence intensity when reacted with HSA. This demonstrated that active Anti-HSA has been immobilized onto the PPy-COOH particles.

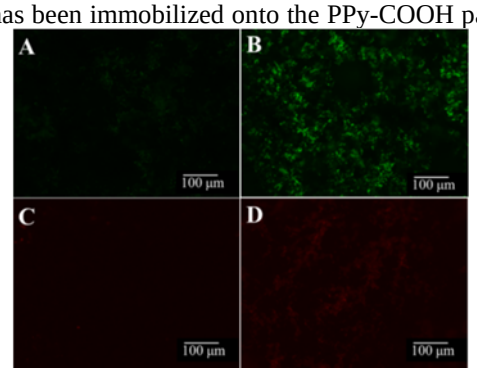

Figure 2. Fluorescence photomicrographs of PPy-COOH grafted by Anti-HSA (FITC) and detected by HAS (rhodamine): PPy-COOH (A) and PPy-NHS (B) incubated in Anti-HSA solution; sample A (C) and sample B (D) incubated in HSA solution.

Conclusions: Through emulsion polymerization, PPy-COOH and PPy-NH_2 nanoparticles were synthesized for the first time. Through NHS-EDC chemistry, the Anti-HSA antibody was successfully immobilized onto the surface of the PPy-COOH nanoparticles. These functional conducting PPy nanoparticles could be used in biosensor and other biomedical applications.

References:

[1] Shi, W. Chem. Phys. 2009;210:1379-1386.
[2] Lee JY. J Biomed Mater Res A.2014;00A:000-000.
[3] Ammar A. Lungmuir. 2004;20:3350-3356