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# Synthesis, characterization and antioxidant activity of a novel electroactive and biodegradable polyurethane for cardiac tissue engineering application

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ABSTRACT

There has been a growing trend towards applying conducting polymers for electrically excitable cells to increase electrical signal propagation within the cell-loaded substrates. A novel biodegradable electroactive polyurethane containing aniline pentamer (AP-PU) was synthesized and fully characterized by spectroscopic methods. To tune the physico-chemical properties and biocompatibility, the AP-PU was blended with polycaprolactone (PCL). The presence of electroactive moieties and the electroactivity behavior of the prepared films were confirmed by UV–visible spectroscopy and cyclic voltammetry. A conventional four probe analysis demonstrated the electrical conductivity of the films in the semiconductor range (~$10^{-5}$ S/cm). MTT assays using L929 mouse fibroblast and human umbilical vein endothelial cells (HUVECs) showed that the prepared blend (PB) displayed more cytocompatibility compared with AP-PU due to the introduction of a biocompatible PCL moiety. The in vitro cell culture also confirmed that PB was as supportive as tissue culture plate. The antioxidant activity of the AP-PU was proved using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay by employing UV–vis spectroscopy. In vitro degradation tests conducted in phosphate-buffered saline, pH 7.4 and pH 5.5, proved that the films were also biodegradable. The results of this study have highlighted the potential application of this bioelectroactive polyurethane as a platform substrate to study the effect of electrical signals on cell activities and to direct desirable cell function for tissue engineering applications.



## 1. Introduction

Myocardial infarction (MI), which often results from coronary artery blockage and ischemia, is a major cause of death worldwide. By surviving myocardial infarction, the patients are likely to confront necrotic myocardium which provokes inflammatory responses and recruitment of the local fibroblasts. Consequently, a large collagen-rich scar tissue forms in injured location in the following next few weeks. Although scar provides a rapid protection and keeps the organ intact from external trauma, it cannot reform the living tissues to their original state. It also weakens the heart while increasing susceptibility to compensatory pathology, aneurysm, additional MI events, and organ failure [1]. Besides, when the amount of myocardium loss is large, significant mechanical complications such as left ventricular dilatation, mitral regurgitation, and heart failure can occur [2]. One of the possible approaches for such ischemic events is cell transplantation to increase cardiac output and restore adequate cardiac function [3]. However, despite the potency of cell implantation in repairing infarction, lack of functional coupling of donor cells with the viable host tissues can hamper their electrical communications [4]. Also, the transplanted cells fail to show a satisfied engraftment rate [5] making significant challenges to increase the cell survival and function.

Another option is the tissue engineering strategies focusing on the use of pre-fabricated three-dimensional porous scaffolds or dense patches of synthetic and/or natural polymers to support the diseased region of the heart and help in the transfer of exogenous cells into it [6]. In fact, heart patches serve two functions: cell delivery and left ventricular restraint [7]. They could provide mechanical support and have the potential advantage of immediate functionality over the transplantation of cells alone [8]. Patches can either replace scar tissue or, after grafting to the scar, improve cardiac function by supporting and thickening the infarcted zone [9]. A tissue-engineered "cardiac patch" should predominantly be stable but flexible and mechanically strong to support the cardiac tissue. It should also function similar to the native heart in order to improve the performance of the infarct myocardium [10]. Therefore, the selection of material for construction of scaffold matrix is an important issue.

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Various synthetic and natural polymers have been studied as a cardiac patch for myocardial repair. Among synthetic polymeric materials, polycaprolactone (PCL) is a bioresorbable and biocompatible polymer [11] with good mechanical properties that holds a great potential for various tissue engineering applications such as vascular graft [12], musculoskeletal [13], nerve [14], skin [15], cardiac muscle [16] and so forth. For example, Shin et al. reported that rat cardiomyocytes can be successfully seeded on a PCL mesh [17]. The cardiomyocytes attach well on the PCL meshes to express cardiac-specific proteins.

Polyurethane-based materials have also been studied for cardiovascular tissue engineering application due to the elastic mechanical properties, biodegradability, processability and biocompatibility [3,18,19]. Biodegradable poly(ester-urethane-urea) (PEUU) was synthesized as a cardiac patch and has been implanted onto the ventricular region of a sub-acute myocardial infarct in the rat model. This patch was considered as a therapeutic option against post-infarct cardiac failure due to the effect of improved cardiac remodeling and contractile function [20].

Electromechanical coupling of myocytes is crucial for their synchronous response to electrical pacing signals [21]. Poor conductivity could limit the ability of the patch to contract effectively as a unit. Different materials such as gold nanowires [22], gold nanoparticles [23], carbon nanofibers (CNF) [24] and carbon nanotubes [25,26] have been incorporated throughout scaffolds to increase electrical signal propagation within the cell seeded scaffold. Increased expression of the proteins involved in muscle contraction and electrical coupling was detected in the composite matrices emphasizing that conductive scaffolds could facilitate cardiomyocyte function.

Particularly, conducting polymers (CPs) such as polypyrrole (PPy), polyaniline (PANi) and polythiophene (PT) have been investigated as electroactive substrates for the culture of especially electrically excitable cells. They have been proved to play an important role in stimulating proliferation, adhesion or differentiation of various cell types including Schwann cells [27], human umbilical vein endothelial cells (HUVECs) [28–30], L-929 fibroblasts [31], PC-12 [32], cardiomyocytes [33], human mesenchymal stem cells [30], human dermal fibroblasts [34] NIH-3T3 fibroblasts [34], C2C12 myoblasts [34], and H9c2 cardiac myoblast [35,36]. Also, different tissues including, cartilage, bone, spinal nerves, peripheral nerves, skin and cardiac muscle respond enthusiastically to electric impulses, leading to the consideration that designed conducting scaffolds could play a role in tissue engineering [28,30].

Polyaniline is one of the most promising conducting polymers demonstrating the potential to be used as an electroactive scaffold for cardiac patches [16,35,33–36], nerve repair [32] and culture of electrically excitable cells [31,35]. It has received increased attention due to its ease of synthesis, diversity of structural forms, highly environmental stability [6,33], easy to acquire raw materials, high electrical conductivity of conductive polymer [37], good biocompatibility and low cost [31]. Besides, the number of electrons associated with the polymer backbone does not change during the process, so it can be considered as the only non-redox doping conductive polymer [37]. PANi has been shown to modulate cellular activities, including cell adhesion and migration, DNA synthesis and protein secretion [6,16,34,38,39]. It has been reported that both conductive (E-PANi, emeraldine salt) and nonconductive (PANi, emeraldine base) forms of polyaniline do not provoke inflammatory responses in rodent model, suggesting its good tolerance and biocompatibility [40,41]. Also, PANi has been validated for the ability to scavenge harmful free radicals from the environment [42] which makes it a good candidate in incidents where tissues experience high oxidative stress especially post infarction.

Although degradability is generally a desired characteristic in tissue engineering substrates, the key limitation factor is non-degradability of CPs in vivo [27], bringing the inflammation and contributing to the second surgery for removal [32]. To tackle this issue, preparation of low molecular weight oligoanilines has been examined by different researchers to exhibit improved processability and biodegradability [43–50]. These compounds show promising conductivity of high molecular weight analogous, while their low molecular weight nature helps direct digestion by macrophages and subsequent kidney clearance [51,52,47]. This, in turn, reduces the chance of harmful foreign body response. Therefore, introducing these moieties into the backbone of inherently biodegradable material can be considered as a promising way for the construction of conductive biodegradable scaffolds. Various studies have also investigated this issue by combining oligoanilines with materials having biodegradable segments like ester linkages [32,43], fast degradable polymers like poly(lactic acid) (PLA) [43,46,47], or blending with natural polymers [45,53].

The purpose of this study was to fabricate novel conductive, biodegradable polyurethane containing aniline pentamer moieties and its blend with polycaprolactone. The behavior of the prepared samples against L929 mouse fibroblast and human umbilical vein endothelial cells (HUVECs) was evaluated. The effective contribution of conducting segments in final materials regarding cell functions was compared with corresponding non-conductive material. Meanwhile, all prepared samples were fully characterized by spectroscopic methods and some of their important physical and thermomechanical biodegradability were evaluated.

The DPPH free radical assay was used for the evaluation of antioxidant property of the electroactive oligoaniline-embedded polyurethane.

## 2. Materials and methods

### 2.1. Materials

Poly(ethylene glycol) (PEG, MW = 1000) and polycaprolactone (PCL, MW = 1000) were obtained from Aldrich and pre-dried at 90 °C under vacuum for 48 h. Then, these polyols were subjected to an azeotropic distillation with toluene to remove the residual water before being used. *N,N*-dimethylformamide (DMF) and toluene from Merck were dried by distillation over calcium hydride and sodium wire, respectively. Isophorone diisocyanate (IPDI) from Merck was purified via vacuum distillation. *N*-phenyl-1,4-phenylenediamine, ammonium persulfate, hydrochloric acid (37%), camphor sulfonic acid (racemic mixture, CSA), high molecular weight PCL (MW = 110000) and *N*-methyl-2-pyrrolidone (NMP) were supplied by Aldrich and used as received. A thermoplastic polyurethane elastomer (TPU, Desmopan 5377 A) from Bayer was used as received. Fetal bovine serum (FBS), Dulbecco's modified eagle's medium (DMEM), phosphate buffered saline (PBS), trypsin-EDTA, and penicillin streptomycin were purchased from Gibco, Germany. Isopropyl alcohol and 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma, USA. 1,4-Diaminobenzene from Merck was used as received. L929 mouse fibroblast and human umbilical vein endothelial cells (HUVECs) were received from Pasteur Institute, Iran and used as obtained.

### 2.2. Polymer synthesis and casting

#### 2.2.1. Synthesis of NCO-terminated prepolymer (NCO-PU)

Three-necked polymerization reactor equipped with a mechanical stirrer, condenser, dropping funnel and a nitrogen inlet was charged with PEG (22.6 g), PCL (22.6 g) and DMF (50 ml). A solution of IPDI (22.437 g) in DMF (20 ml) was slowly dropped into the reactor during 30 min under ambient temperature. While stirring, the temperature was increased to 85 °C. The reaction was continued until the free NCO content (NCO %) of the reaction mixture reached its half initial NCO% using back titration by the di-n-butyl amine method (ASTM D-2579).

#### 2.2.2. Synthesis of aniline-dimer end-functionalized polyurethane macromonomer (AD-PU)

The obtained solution of NCO-PU was dropped slowly into the stirred solution of *N*-phenyl-1,4-phenylenediamine(18.62 g) in DMF (25 ml) at ambient temperature during 0.5 h. The reaction was followed