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Biodegradable and electroconductive poly(3,4-ethylenedioxythiophene)/carboxymethyl chitosan hydrogels for neural tissue engineering

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ABSTRACT

Electroconductive hydrogels with excellent electromechanical properties have become crucial for biomedical applications. In this study, we developed a conductive composite hydrogel *via in-situ* chemical polymerization based on carboxymethyl chitosan (CMCS), as a biodegradable base macromolecular network, and poly(3,4-ethylenedioxythiophene) (PEDOT), as a conductive polymer layer. The physicochemical and electrochemical properties of conductive hydrogels (PEDOT/CMCS) with different contents of PEDOT polymer were analyzed. Cell viability and proliferation of neuron-like rat phaeochromocytoma (PC12) cells on these three-dimensional conductive hydrogels were evaluated *in vitro*. As results, the prepared semi-interpenetrating network hydrogels were shown to consist of up to 1825 ± 135 wt% of water with a compressive modulus of 9.59 ± 0.49 kPa, a porosity of 93.95 ± 1.03% and an electrical conductivity of (4.68 ± 0.28) × 10^{-3} S·cm^{-1}. Cell experiments confirmed that PEDOT/CMCS hydrogels not only had no cytotoxicity, but also supported cell adhesion, viability and proliferation. These results demonstrated that the incorporation of conductive PEDOT component into CMCS hydrogels endowed the hydrogels with enhanced mechanical strength, conductivity and kept the biocompatibility. Thus, the attractive performances of these composite hydrogels would make them suitable for further neural tissue engineering application, such as nerve regeneration scaffold materials.

1. Introduction

The nervous system has a finite ability to repair nerve damage by itself. It often requires external adjuvant therapy. Electrical signals, as one of the most important biological factors, can control cell metabolic activity, including cell adhesion, proliferation, differentiation and secretion of cytokines, especially at nerve and myocardial cells [1]. Therefore, an important aspect of well-functionalized scaffolds for nerve regeneration is their ability to conduct electricity. Hydrogels are 3D macromolecular polymers that crosslinked by hydrophilic chains, which typically have high water absorption ability and good biocompatibility, and the tunable mechanical and chemical properties make it possible to meet the requirements of various biomedical applications [2–4]. For example, numerous stimuli-responsive hydrogels based on temperature [5,6], mechanical [7,8] and electrical stimuli [9] have been developed by altering the chemical components of the matrix structure. Recently, hydrogels have been widely used for various biomedical applications such as drug delivery [10–12] and tissue engineering [13,14].

Electroconductive hydrogels are a class of composite materials with endowed electrical properties *via* blending, doping or chemical modification with electroactive materials. It has been reported to control the differentiation of nerve cells, enhance neurite outgrowth and peripheral nerve regeneration even in the absence of electrical stimulation [15–17]. Poly(3,4-ethylenedioxythiophene) (PEODT, a polythiophene derivative) is one of the most promising conductive polymers for biomedical applications because of its remarkable conductivity, electrical stability and good biocompatibility [18]. Unlike the undesirable *α–β* and *β–β′* couplings in polypyrrole (a most studied conductive polymer) and polythiophene backbone, the presence of 3,4-dioxy substitution in PEDOT blocks the undesirable couplings and leads to a more regionally regular structure with a coplanar heterocyclic backbone. It is known that PEDOT has a higher conductivity and stability under biological conditions due to the lack of *α–β* and *β–β′* couplings [19,20]. Another of most investigated conductive polymer named polyaniline may have contradictory biocompatibility because it has been noted to cause chronic inflammation once implanted [21]. One the other hand, the good biocompatibility of PEDOT and the ability to support nerve cells adhesion, proliferation and neuronal extension were reported in numerous studies [22–24]. With such properties, PEDOT may be expected

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to offer opportunities to develop well-functioned conductive scaffolds for nerve regeneration application. Unfortunately, the use of PEDOT polymer is limited due to its non-biodegradability and high stiffness. To overcome those disadvantages, several research groups have designed composite hydrogels to enhance their mechanical and biodegradable properties, through a combination of biodegradable flexible material and conductive PEDOT component. For example, Abidian et al. [25] found that the mechanically reinforced agarose nerve conduit with a thin coating layer of PEDOT supported superior axonal growth in a 10 mm nerve gaps in rats, compared to the plain agarose conduits. Sirivisoot et al. [26] reported the preparation of conductive type I collagen gels containing PEDOT nanofibres and living cells. The results showed that the conductive gels increased neurite outgrowth of PC12 cells after 7 days of culture when compared with the collagen positive controls. In our previous study [27], a conductive hydrogel was prepared by the blend of HA-doped PEDOT nanoparticles and chitosan/gelatin materials. The porous hydrogel could support PC12 cells adhesion and growth, especially expressed higher synapse growth genes of GAP43 and SYP compared with control group. From the above-mentioned reports, it is evident that conductive hydrogels may help to repair and regenerate degenerated nerve tissue.

Carboxymethyl chitosan (CMCS), an important water-soluble derivative of chitosan, has many outstanding properties including excellent water solubility, water retention properties, biodegradability, biocompatibility, antibacteria and antioxidant activity [28,29]. In addition, CMCS is an amphoteric polyelectrolyte, and can be easily modified owing to the functional –OH, $–NH_2$ and –COOH groups [30]. For these advantages, CMCS has received considerable attention in neural tissue engineering applications. For example, Lu et al. [31,32] demonstrated that 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)-crosslinked CMCS films enhanced the spread and provided a good proliferation substratum of Neuro-2a cells. The same research group [33] also reported that the CMCS films had no cytotoxicity to Schwann cells, and CMCS tubes had better nerve regeneration performance than that of chitosan tube and demonstrated equivalence to nerve autografts *in vivo* experiments. Moreover, He et al. [34] found that CMCS/DMEM solution stimulated the proliferation of Schwann cells *in vitro* by activating the intracellular signaling cascades of extracellular signal-regulated kinase (ERK1/2) and phosphatidylinositil-3 kinase (PI3K/Akt). Therefore, CMCS may be one of promising scaffold materials for nerve regeneration application.

In this study, we described the fabrication of conductive biodegradable hydrogels using pre-crosslinked CMCS hydrogel (as the first network) modified with PEDOT layer (as the second network), so as to significantly enhance PC12 cells proliferation. CMCS was chosen as the base material due to its flexible, biodegradable and biocompatible properties. PEDOT was used as conductive component due to its positive attributes such as remarkable conductivity, electrical stability and good biocompatibility. The conductive hydrogels were prepared *via in-situ* chemical polymerization method, resulting in a uniform coating of PEDOT layer on the inner and outer surfaces of pre-crosslinked porous CMCS hydrogels. The physicochemical and electrochemical properties of hydrogels including chemical structure, compressive modulus, electrical conductivity, surface morphology, water absorption ability and biodegradation were evaluated. PC12 cells were cultured on the conductive hydrogels, and the good biocompatibility of these hydrogels and their ability to promote PC12 cells adhesion and proliferation were successfully demonstrated even in the absence of electrical stimulation. This study also aimed to lay the foundation for future studies on the role of electrical stimulation in the survival and neurite outgrowth of PC12 cells, which may engender new applications such as nerve regeneration scaffold material.

2. Materials and methods

2.1. Materials

Chitosan (86% deacetylation degree, 50 cps viscosity) was supplied by Haidebei Marine Bioengineering Co., Ltd. (China). 3,4-ethylenedioxythiophene (EDOT) monomer (purity 99.98%) was supplied by Shanghai Kuanghong New Materials Technology Co., Ltd. (China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were supplied from GIBCO (USA). The cross-linkers 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxy-succinamide (NHS) were purchased from the Shanghai Medpep Co., Ltd. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Calcein-AM, and propidium iodide (PI) were supplied by Sigma-Aldrich Inc. (St. Louis, Mo, USA). All the other reagents in this study were supplied by Sinopharm Chemical Reagent Co., Ltd. (China) and were of analytical grade. Neuron-like rat phaeochromocytoma (PC12) cells were obtained from Institute of Biochemistry and Cell Biology, SIBS, CAS (Shanghai, China) and cultured in high-glucose DMEM medium supplemented with 10% FBS, 100 U/L streptomycin and 100 U/L penicillin in an incubator at 37 °C, 5% CO_2 and 95% humidity.

2.2. Preparation of carboxymethyl chitosan hydrogels

Carboxymethyl chitosan was prepared by a method of Chen et al. [35] with slightly modified. In brief, chitosan (10 g), sodium hydroxide (13.5 g), distilled water (20 mL) and isopropanol (80 mL) were added into a flask (250 mL) to swell and alkalize at 35 °C for 2 h. Then monochloroacetic acid (15 g) was dissolved in isopropanol (20 mL), and the mixture was added dropwise into the chitosan reaction solution for 30 min. Stirring was continued at 35 °C for another 6 h. Reaction mixture was then filtered and the filtered solid product (sodium salt of CMSC) was thoroughly rinsed with ethanol. Further, sodium salt CMCS (8 g) was converted to H-CMCS by immersing it in 80% ethanol (500 mL) and adding 37% HCl (50 mL) for acidification at room temperature (RT) for 2 h. The resultant suspension was filtered, thoroughly rinsed with ethanol, and finally dried in vacuum at 50 °C. The product was H-form of CMCS, and for next characterization and preparation of CMCS hydrogels. CMCS hydrogels were prepared by chemically crosslinking using EDC/NHS according to our previous study [36]. The obtained white porous CMCS hydrogel scaffolds were used for next preparation of PEDOT/CMCS conductive hydrogels.

2.3. Preparation of PEDOT/CMCS conductive hydrogels

PEDOT/CMCS hydrogels were synthesized by the introduction of PEDOT component into pre-crosslinked CMCS hydrogels using *in-situ* chemical polymerization. In a typical procedure, the pre-crosslinked CMCS hydrogels were immersed in aqueous solution containing ammonium persulphate (APS) (0.1 M, as oxidant) and sodium *p*-toluenesulfonate (pTS-Na) (0.1 M, as dopant) under vacuum, to assure the thorough and uniform dispersion of reagent in hydrogels. EDOT precursor solution was prepared by mixing different concentrations of EDOT monomer in n-hexane (0.1, 0.2 and 0.3 M). The hydrated hydrogels were transferred into EDOT/hexane solution to accomplish the *in-situ* polymerization of EDOT and the formation of PEDOT-pTS second network on the surface of hydrogels. The polymerization was carried out in vibrator with a slight vibration at RT for 72 h. Finally, as-prepared composite hydrogels were completely washed with 70% ethanol, and distill water, in order to wash out low molecular weight components. The control samples underwent the same procedure but without EDOT monomer.