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Preparation and evaluation of chitosan biocompatible electronic skin

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ABSTRACT

Polydimethylsiloxane (PDMS) is often used in flexible electronic skin, due to their non toxic and tough. However, the lack of degradation and cell biocompatibility of PDMS would limit the potential use in the biological field. To overcome the limitation, PDMS was modified by surface treatment for enhancing cell adhesion and proliferation. This research is focused on preparation and evaluation of novel chitosan biocompatible electronic skin. Chitosan electronic skin was prepared by a thermal induced phase separation method, following treatment with NaOH gelating agent, followed by analyzing the surface morphology, swelling behavior, mechanical strength, degradation rate. The electrode was produced by screen printing technique, followed by analyzing the adhesion between the ink and cell biocompatibility. From scanning electron microscope (SEM) observations, NaOH gelated chitosan skin had the smooth surface morphology. The swelling ratio of all chitosan skin gelated by NaOH were less than 5%. The chitosan skin, prepared by 300k kDa chitosan gelated by NaOH for 3hr, had the smallest swelling ratio. Furthermore, its mechanical strength showed the highest value of Young's modulus (~151 kPa). It indicated that 300 kD chitosan skin gelated by NaOH for 3hr had better size stability. All chitosan skins degraded about 20% of initial weight after 40 days in vitro shaking test. The adhesion strength of the silver inks on the surface of chitosan was better than carbon ink, and the electrical characteristic is almost the same with commercial polycarbonate (PC) substrate. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and SEM observation indicated that chitosan skin with silver electrode showed the cell biocompatibility. By further increasing the application of both screen imprinting technique and biopolymer, the idea of interdisciplinary can be achieved and it would be a creative revolution to the field of wearable device into tissue engineering.

1. Introduction

PDMS is often used in flexible electronic skin, due to their optical transparency, light weight, tough, non toxic, relatively inert in biochemical reaction and heat resistance [1–3]. However, the surface of PDMS is hydrophobic and poor adhesion with different kind of materials. Therefore, surface fabrications of PDMS with bioactive molecules have been markedly studies to tailor its properties to satisfy the requirements for particular application, especially in biological field [4,5]. Many researches attempted to modify the surface for enhancing cell adhesion and proliferation. Sung Hee Chung and Junhog Min directly treated the PDMS surface by 3-aminopropyltrimethoxysilane (APTES) and diethylene- triamine (DETA) [6]. Lung-Jieh Yang and Yu-Cheng Ou fabricated micro patterns of glutaraldehyde (GA)-crosslinked gelatin on the glass to regulate the cell grow [7]. Nevertheless, being unable to degrade is still the major drawback in the development of biomedical field. Therefore, the utilization of the natural polymer directly as a substrate for cell chip could overcome the lack of degradation and cell biocompatibility. Therefore, this research employs a natural polysaccharide, chitosan as the base material. Chitosan is a linear polysaccharide produced via deacetylation of chitin. Chitosan is composed of randomly distributed β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine and it is one of the few cationic polysaccharides found in nature. Chitosan has many advantages such as biocompatibility, biodegradability, non toxic, low cost, rich in resource and resemblance with the environment of the extracellular matrix [8–10].

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Moreover, scaffold made from chitosan has well mechanical strength of flexibility, which able to support cells growth as well as being use as substrate in electronic skin. Besides, chitosan is consisting of many functional groups (−OH and $-NH_2$) which also allow surface modification to increase its application in biosensor and tissue engineering [11–14].

For electronic skin fabrication is usually using photolithography by photomask, a photoresist, and by exposing to a specific wavelength of light source, and then a designed pattern is transferred into the underlying substrate. This conventional printing process includes etching, physical or chemical deposition and plating of integrated circuit. Physical deposition such as sputtering and evaporation need to be performed in a vacuum environment and thus limiting the continuous production. On the contrary, screen printing technology is another method often used in the fabrication of electrodes for the development of biosensor, being oriented for short production run that is the whole process includes printing a designed circuit onto the substrate, and then the printed circuit was solidified via heating or UV irradiation. Therefore, screen printing technique is more likely to replace conventional lithographic technique in this project due to simplicity, ease of operation, short production run, and production with high stability, modest cost and mass production capabilities [15–17].

Natural biopolymers show several unique properties, such as biodegradation, easy modification and biocompatibility. Consequently, natural biopolymers are widely used for biomedical applications. However, the instability of size of natural biopolymers in aqueous solution would limit the application on the biosensor. Based on our previous studies, we found that chitosan film would not significantly change the size under aqueous solution. It would be great potential for use on the electrical skin substrate. The aim of this project was to evaluate chitosan as a novel electronic skin substrate. Base on the theory of tissue engineering, screen printing technique and chitosan can be combined to produce a novel flexible electronic skin. The investigations of chitosan electronic skin are including physical and chemical properties, electrochemical properties and focus on biocompatibility.

2. Materials and methods

2.1. Materials

Chitosan of two different molecular weights, 70 kDa and 300 kDa, acetic acid and sodium hydroxide were purchased from Sigma (St. Louis, MO). All chemicals used in this study were of reagent grade.

2.2. Preparation of chitosan electronic skin film

Chitosan powder (70 kDa and 300 kDa) was dissolved in 0.1 M acetic acid to prepare 2% (w/v) chitosan solution. Chitosan was completely dissolved aided with a stirrer and a magnetic bar. Adequate amount of chitosan solution was loaded into petri dish, and dried in oven for overnight. The film was then immersed in sodium hydroxide to induce gelation. Finally, these resultant chitosan electronic skin film were repeatedly washed with distilled water to remove any traces of reacting agents and then dried in oven for overnight. All prepared chitosan electronic skin films were stored in desiccator at room temperature.

2.3. Characterization of chitosan electronic skin film

2.3.1. Swelling test

The degree of chitosan electronic skin film swelling was measured by the following procedure. The chitosan film was immersed in phosphate *buffered* saline (PBS). At predetermined time intervals (5, 10, 15, 30 and 60 min), the film was removed from PBS and the film area was immediately measured. The swelling ratio is defined in the following equation. A0 and A1 are the areas of dry and swollen film, respectively. Each measurement experiment was repeated 3 times and expressed as average ± S.D.

2.3.2. Mechanical strength test

A chitosan electronic skin film was cut into a rectangular shape of 1 cm × 6 cm, and then immersed in 0.1 M PBS (pH7.4) for 24 h. The mechanical properties of these chitosan electronic skin films were calculated and recorded automatically by using a MTS Systems at a crosshead speed of 10 mm/min.

2.3.3. Degradation test

The initial weight of chitosan electronic skin film was measured before immersed in PBS (pH7.4) on a shaker set at 40 rpm at 37 °C. Every five days the film was taken out and washed with distilled water, dried and the weight of this film was measured. The degradation profile was obtained as the accumulated weight losses of the film. Each measurement experiment was repeated 3 times.

2.4. Fabrication of chitosan electronic skin with electrode by screen printing technique

Carbon ink (SC-1010, ITK) and silver ink (NT-6307-2, PERM TOP) were chosen for electrode printing by screen printing machine (NSP-1A, Yulishih Intdustrial), equipped with a 200 threads per inch polyester screen and PU squeegee. Chitosan film printed with carbon and silver ink was dried at 60 °C for 30 min and at 120 °C for 60 min, respectively. The thickness of electrode was about 8 μm.

2.5. Characterization of chitosan electronic skin with electrode

2.5.1. Adhesion test of the electrode

The portable sensors made by screen printing method have been already extensively applied in biomedical field. However, there are only a few researches of using nature polymers as screen printing substrate. Therefore, adhesion property of either carbon or silver ink on chitosan film and PC substrate was measured by cross-cut method. Pressure-controlled tape was applied to the cut and then pulling out to assess the adhesion characteristics according to ASTM D 3359-95 standard.

2.5.2. Electrical characteristic

All electrochemical experiments were conducted with a ZAHNER Zennium IM6 Electrochemical Workstation (ZAHNER-elektrik GmbH & Co. KG, Kronach, Germany) with three-electrode configuration using an Ag/AgCl electrode as reference electrode and platinum wire as counter electrode. The cyclic voltammetric measurements of electrodes were carried out using 0.1 mM potassium ferri(III) cyanide ($K_3[Fe(CN)_6]$) in PBS solution at scan rate of 100 mV s^{-1}.

2.5.3. Cell biocompatibility assay

Cell viability was evaluated using MTT assay and was based on the mitochondrial conversion of the tetrazolium salt into a purple colored formazan product with an absorbance at 570 nm. The mouse fibroblast cell line L929 was cultured in DMEM medium supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. L292 in an initial concentration of 2×10^4 cells/ml were seed in each well with test sample. At 3 days of culture, the original medium in each well was replaced with 100 μl MTT solution (5 mg/ml), and the wells were incubated at 37 °C for 4 h to enable the formation of formazan crystals. Then, the solution was removed and DMSO was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes to ensure that all crystals were dissolved, the plates were read on a multi-well scanning ELISA reader. SEM was used to observe the surface of chitosan with Ag-SPE (screen-printed silver electrode) as well as cellular morphology. Fixation and dehydration of sample was performed using glutaraldehyde, osmium acid and ethanol (30%, 50%,