Materials Science & Engineering C 90 (2018) 16–26

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Preparation, characterization and application research of a sustained dexamethasone releasing electrode coating for cochlear implantation

Muqing Xu[a], Dong Ma[b], Dongxiu Chen[a], Jieqing Cai[a], Qiaofang He[c], Fan Shu[a], Jie Tang[d], Hongzheng Zhang[a,*]

[a] Department of Otolaryngology Head & Neck Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China
[b] Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Department of Biomedical Engineering, Jinan University, Guangzhou 510632, China
[c] Operating Room of Anesthesiology Department, Zhujiang Hospital of Southern Medical University, Guangzhou 510282, China
[d] Department of Physiology, School of Basic Medical Sciences, Institute of Mental Health, Southern Medical University, Guangzhou, 510515, China

ARTICLE INFO

Keywords:
Cochlear implantation
Hydrogel
Electrode coating process
Drug delivery
Biocompatibility

ABSTRACT

Cochlear inflammatory response after cochlear implantation (CI) is an important mechanism for implantation trauma and hearing loss. The hearing loss was also caused by damage to auditory hair cells (HCs), whereas ion homeostasis within the cochlea can ensure survival of HCs. In our study, pure hyaluronic acid (HA) was crosslinked with 1, 4-butanediol diglycidyl ether (BDDE) and the successful preparation of the cross-linked hydrogel (CHA) was confirmed by rheological characteristics and FTIR spectra. Artificial perilymph (APL) was prepared to simulate the ion homeostasis microenvironment within scala tympani of human cochlear, and served as the major component of artificial perilymph soaked CHA (APL-CHA). The conductivity experiment indicated that APL-CHA is more suitable to the requirements of the electrical conductivity in scala tympani. The electrode coating process found that the extrusion coating method have advantages of controllable adhesive capacity of APL-CHA, uniform coating thickness and smooth surface as compared to common method. Due to CI surgery application requirement, optimization of coating process was selected as follows: extrusion coating method, degree of 3.6 vol%, pinhole diameter of 32G (110 μm), pressure of 200 ± 15.81 Psi. Controlled dexamethasone 21-phosphate sodium salt (DSP) release of 20 days could be demonstrated using the hydrogel filled reservoir via a validated HPLC method. The morphological structure of CHA showed different sizes of porous structure among APL-CHA provided structural basis for drug delivery. L929 fibroblasts culture and Spiral Ganglion Neuron Explants culture results revealed that APL-CHA possesses fine biological compatibility. APL-CHA shows a promising application in CI surgery and has great potential in preventing hearing loss with well simulation of ion homeostasis within the cochlear, local DSP delivery for target anti-inflammatory, approximate conductivity within the scala tympani and optimization of electrode coating process.

1. Introduction

Cochlear implantation (CI) has become the standard procedure of treatment for patients suffering from profound sensorineural hearing loss [1–3]. However, substantial acoustic hearing loss has occurred in 24% of the patients after CI surgery, among them, 13% sustained a total hearing loss base on the data from a previous article [4–6]. A recent retrospective review showed that 38% of 85 individuals receiving CI surgery encountered delayed-onset hearing loss of various degrees and rates of change [7]. Surgically induced cochlear trauma from insertion of the electrode into the scala tympani and delayed host responses induced from the alien electrodes were considered to be the major contributors to hearing loss [4,8]. Oxidative stress and inflammation responses to the former cochlear damage during insertion of electrodes can lead to loss of auditory hair cells (HCs) [9,10]. The latter tissue reaction, consists of inflammation and fibrosis, increase electrode impedance and decrease the residual hearing [8].

Various drug delivery systems have been developed to preserve residual hearing. Drug delivery from the systemic circulation delivery into the cochlea have been proved to be disadvantages of insufficient drug concentration, poor therapeutic efficacy as well as severe side effects due to the blood-cochlea-barrier [11]. Local delivery to the inner ear, including approaches of the round window membrane (RWM) diffusion, micropump via cochleostomy, electrode substrate silicone or electrode carrier sustained-release system, is expected to overcome these bottlenecks. The most common approach of RWM diffusion is to

* Corresponding author.
E-mail address: zhanghzh@outlook.com (H. Zhang).

https://doi.org/10.1016/j.msec.2018.04.033
Received 27 August 2017; Received in revised form 24 March 2018; Accepted 15 April 2018
Available online 16 April 2018
0928-4931/

inject drug delivery system, such as hydrogel, onto the RWM through tympanum. As passive transport depends on the concentration gradient across the membrane, the kinetics of drug is complex and often unpredictable, and the drug is more likely to distribute near the cochleostomy [12]. The micropump may get blocked and increase the risk of cochlea infection, which may lead to totally deaf [11]. Properties of sustained release have been demonstrated in the drug loaded electrode substrate silicone. After the drug was dissolved in the perilymph, however, small voids might appear on silicone gradually [13], which would result in compromising the integrity of the electrode, and finally, the electrode conductivity damage.

In recent years, the advantages of the electrode coating of cochlea, such as biocompatible 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer [4], protein repelling hydrogel (*star*-shaped polyethylene glycol prepolymer, sPEG) [14,15], collagen/fibrin hydrogel compositions [16] were studied. This delivery mode can not only carry the drugs into the inner ear directly, bypass the blood labyrinth barrier, and avoid severe side effects of systemic medication, but also overcome the shortcomings of the uncontrollable pharmacokinetics of round window membrane method and the increased infection of the micro pump infusion. In addition, the integrity and performance of the electrode can also be guaranteed. Therefore, it is superior to other drug delivery methods mentioned above. However, scala tympani micro environment of cochlea, including ion species, ion concentration, conductivity and other compositions of human perilymph, have never been taken into account. More importantly, the process of electrode coating, which better control the loading amounts of drug and hydrogel, has never been investigated either. Looking into the lack of electrode coating research as well as the reasons of hearing loss, including inflammation, fibrosis and oxidative stress, we developed an electrode coating of cross-linked sodium hyaluronate hydrogel (CHA), which is much more suitable for cochlear environment, and served as controlled drug delivery system to embed dexamethasone 21- phosphate sodium salt (DSP) for the purpose of sustained release and target anti-inflammatory effect within the cochlea.

Dexamethasone has been confirmed to be beneficial in preserving residual hearing on recent researches [17]. As the most potent glucocorticoid for anti-inflammatory effects, dexamethasone can reduce cochlear inflammation, regulate expression levels of apoptotic genes and pro-inflammatory gene, and protect against TNF α-induced apoptosis of HCs [18,19]. It can also reduce oxidative stress level and protects against cisplatin-induced loss of OHCs [20]. Moreover, fibrous tissue growth after cochlear implantation can be reduced by application of the glucocorticoid DSP [14,15,21]. The reduction of cochlear fibrosis would contribute to reimplantation surgery, and therefore provide opportunity for further treatment technologies.

As a natural linear polysaccharide, hyaluronic acid (HA), consists of alternating units of a repeating disaccharide (b-1, 4-Dglucuronic acid-b-1, 3-*N*-acetyl-D-glucosamine). HA generally distributes in extracellular matrix (ECM) [22]. HA hydrogel possesses many advantages, including biocompatibility, biodegradability, bioactivity, non-immunogenicity, unique viscoelastic nature, wound healing, fine anti-inflammatory ability and promoting intracellular signaling, and have been applied in the biomedical field widely [23,24]. More importantly, HA has better protective effect on hearing associated with dexamethasone when compared with topical dexamethasone alone or the control electrode group [25].

However, HA chains (polysaccharides) would usually be dissociated by enzymes or radicals, and eliminated by the liver/kidneys. Therefore, 1, 4-butanediol diglycidyl ether (BDDE) was chose to crosslink with HA to increase the longevity. The crosslinking agent, BDDE, was used as market-leading HA fillers and properties of stability, biodegradability, and long safety were supported by clinical and biocompatibility data for > 15 years [26,27].

The degeneration of HCs may ultimately lead to cell death, was associated with progressive hearing loss, whereas ion homeostasis can ensure survival of HCs [28]. These data demonstrate the significance of electrode coating component for simulating the ionic microenvironment of the perilymph around the basolateral membranes of HCs. The perilymph contains extraordinarily high concentrations of Na^+ (148 mM), low concentrations of K^+ (4.2 mM), and variety of ions, including Ca^+ (1.3 mM), Cl^- (119 mM), as well as Glucose (3.6 mM) [29–31], while endolymph has low concentration of sodium ions and high concentration of potassiums (K^+ 157 mM). Artificial perilymph was prepared based on the above ion and its concentration [32], and served as the major component of cross-linked sodium hyaluronate hydrogel (CHA), which would make it much more suitable for perilymph microenvironment, maintain the ion balance of the inner ear, and improve the survival of hair cells, and protect residual hearing. In the current study, we investigated the characteristics of electrode coating of CHA, and the technical process of electrode coating for better targeted drug delivery.

2. Experiment

2.1. Materials

Hyaluronic acid sodium salt (HA, also known as hyaluronan or sodium hyaluronate) with an average molecular weight of 1.5×10^6 Da, was supplied by Meilun Biotech Co. Ltd. (Dalian, China) as dry powders. 1, 4-butanediol diglycidyl ether (BDDE) and dexamethasone 21-phosphate sodium salt was purchased from Aladdin Industrial Co. Ltd. (Shanghai, China). HEPES was provided by Amresco (Texas, USA). Glucose, Calcium chloride, magnesium chloride, potassium chloride, and sodium chloride with analytical reagent were obtained from Dingguo Biotech Co. Ltd. (Guangzhou, China). Dulbecco's Modified Eagle Medium(DMEM), Neurobasal medium, Polylysine(PLL), PBS, B27, TritonX-100, Anti-fluorescent sealant, 488-conjugated Goat anti-rabbit IgG, β-tubulin isotype III antibody were purchased from Yongjin Biotech Co. Ltd.

2.2. Preparation of CHA via crosslinking and the artificial perilymph

20 mg of NaOH was firstly dissolved in 2.0 mL Ultra-pure water (UP), after which 200 mg of HA and then different amount of BDDE were added to the above 1% NaOH solution with vigorous stirring for 20 min. The final concentrations of BDDE were 2.0 vol%, 2.4 vol%, 2.8 vol%, 3.2 vol% and 3.6 vol%, respectively. After that, the above solutions were transfer to a little flat-bottomed bottle with the inner diameter of 23 mm and kept in a 40 °C water bath for 5 h (Scheme 1).

Artificial perilymph, an aqueous solution of 1.3 mmol/L calcium chloride, 1.8 mmol/L magnesium chloride, 5.4 mmol/L potassium chloride, 137 mmol/L sodium chloride, 5 mmol/L glucose, and 5 mmol/L HEPES, was prepared to simulate the microenvironment within the scala tympani of the cochlea [32].

2.3. Characterization

The rheological properties of HA and CHA were analyzed by a strain-controlled Pneumatics ARES rheometer fitted with a parallel plate geometry whose diameter is 20 mm and the gap is 2.0 mm. To ensure the rheological measurements within linear viscoelastic regions, the viscoelastic parameters were measured as a function of strain at a constant frequency of 1.0 rad/s at 25 °C. Strain applied to hydrogel samples increased from 0.5 to 100%.

The hydrogel adhered to the surface of the cochlea electrode is not expected to continue to swell which led the scala tympani tissue to be pressed and caused a second damage. Therefore, it is necessary to examine the swelling behaviors. CHA of different crosslinking degree were immersed, respectively, in containers containing artificial perilymph of 100 mL at constant temperature (37 °C) until swelling equilibrium had been reached. A precision measuring cylinder of 50 mL was