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A facile synthesis method of hydroxyethyl cellulose-silver nanoparticle scaffolds for skin tissue engineering applications

Farah Hanani Zulkifli [a,*], Fathima Shahitha Jahir Hussain [a], Senait Sileshi Zeyohannes [a], Mohammad Syaiful Bahari Abdull Rasad [b], Mashitah M. Yusuff [a]

[a] Faculty of Industrial Sciences & Technology, University Malaysia Pahang, Lebuhraya Tun Razak, Gambang, 26300 Kuantan, Pahang, Malaysia
[b] Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Bandar Indera Mahkota Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia

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ABSTRACT

Green porous and ecofriendly scaffolds have been considered as one of the potent candidates for tissue engineering substitutes. The objective of this study is to investigate the biocompatibility of hydroxyethyl cellulose (HEC)/silver nanoparticles (AgNPs), prepared by the green synthesis method as a potential host material for skin tissue applications. The substrates which contained varied concentrations of $AgNO_3$ (0.4%–1.6%) were formed in the presence of HEC, were dissolved in a single step in water. The presence of AgNPs was confirmed visually by the change of color from colorless to dark brown, and was fabricated *via* freeze-drying technique. The outcomes exhibited significant porosity of >80%, moderate degradation rate, and tremendous value of water absorption up to 1163% in all samples. These scaffolds of HEC/AgNPs were further characterized by SEM, UV–Vis, ATR-FTIR, TGA, and DSC. All scaffolds possessed open interconnected pore size in the range of 50–150 μm. The characteristic peaks of Ag in the UV–Vis spectra (417–421 nm) revealed the formation of AgNPs in the blend composite. ATR-FTIR curve showed new existing peak, which implies the oxidation of HEC in the cellulose derivatives. The DSC thermogram showed augmentation in T_g with increased $AgNO_3$ concentration. Preliminary studies of cytotoxicity were carried out *in vitro* by implementation of the hFB cells on the scaffolds. The results substantiated low toxicity of HEC/AgNPs scaffolds, thus exhibiting an ideal characteristic in skin tissue engineering applications.

1. Introduction

Skin defects caused by skin or cutaneous wounds may interrupt skin functions at various stages leading to permanent disability or even death depending on the severity of the injury [1]. The emergence of tissue engineered skin replacements have conquered several limitations over conventional tissue transplants of autograft, allograft and xenograft such as preventing additional surgical procedures, eliminating the chance of graft rejection or the transmission of infection diseases [2–4]. For these reasons, the use of artificial skin equivalents becomes great and extensively grown in skin tissue markets. Moreover, since skin transplant becomes clinically practicable, the demand for safe, affordable and stable product always exceeds the available supply. Three main factors that contribute to the success of tissue engineering are cells, scaffolds and cell-scaffold interactions that play a major role in organizing and assembling subsequent function into particular tissues [5]. Nowadays, research using nanobiomaterials as scaffold in skin tissue engineering is tremendously increasing as these biomaterials mimic the structure of extracellular matrices and provide a platform for cell attachment, differentiation and proliferation [6,7]. The scaffold will provide structural support for the cellular organization *via* integrin that modulate intracellular signaling actions [8].

Nobel metal nanoparticles have great advantage in today's world due to its superior size-related physical and chemical properties that differed from large matters [9,10]. Recent research have been focused on silver nanoparticles owing to their important scientific and technological applications in life sciences such as biosensors, peptide probes, anti-microbial agents, burn treatment, catheters, vascular grafts, human skin, prostheses, cancer therapeutics, and coatings of other medical devices [11–18]. Silver is particularly employed as nanoparticles in colloidal solution. For decades, many researches have been conducted on the presence of AgNPs at the implant site of the human body which successfully leads to the enhancement of antibacterial and antimicrobial activity [19]. Therefore, intense study on developing an ideal scaffold based on silver nanoparticles with free-periprosthetic infections goes on continuously, in the effort to resist a wide range of bacterial pathogens during the healing period.

It is well known that AgNPs is a zero-valent silver with a size ranging from 1 to 100 nm in at least one of its dimensions. Because of its strong antimicrobial activities, AgNPs became a potent metal in the tissue regenerative field [20]. In tissue-engineered skin substitutes, the moist

* Corresponding author.
E-mail address: farahhanani@ump.edu.my (F.H. Zulkifli).

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environment caused by fluid-impregnated dressings has been investigated to promote ulcer healing, thus reducing sufferings of patients [21,22]. Although silver is relatively inert, its interaction with moisture present on the surface of skin as well as fluid in the wound bed leads to the release of silver ions. Silver ions which are highly reactive, will bind to bacterial DNA and RNA, denaturing them, and inhibit bacterial growth [23]. However, the minute size of nanoparticles enables its penetrations into the stratum corneum of the skin and may interfere with a variety of cellular mechanisms [24]. Due to this reason, the size, morphology, and distribution of silver nanoparticles must be optimized by controlling the synthesis route, ensuring competency in preventing contamination and further physical damage to the wound area.

A variety of techniques have been reported to synthesize AgNPs, notably *via* physical and chemical approaches. Physical methods using laser ablation [25], radiolysis [26], and aerosol techniques [27] requires expensive instruments and consume high amount of energy [28]. Besides, these methods usually require agents that are both toxic and environment-polluting [29]. A convenient chemical method, for instance, photochemical reduction [30], $NaBH_4$ and UV irradiation photoreduction [31], electrochemical reduction [32], and heat evaporation including chemical vapor deposition [33] offer better options of being both low cost and ecofriendly. Green synthesis approaches require a simple method, are environmentally benign, and involve cost efficient processes. The synthesis depends on three important parameters which are (i) solvent medium, (ii) reducing agent, and (iii) stabilizing or capping agent [34,35]. Generally, biopolymer solutions such as poly (vinyl) alcohol (PVA), polyethylene oxide (PEO), chitosan or chitosan derivatives, and cellulose derivatives are chosen as reducing or capping agent due to its ability to act as an excellent host material for nanoparticles, besides other characteristics such as non-toxic, biodegradable, renewable, and able to utilize nature-friendly solvent.

In this work, we report the preparation and characterization of HEC incorporated with AgNPs nanocomposite scaffolds by the freeze-dry technique. HEC is one of the cellulose derivatives that possess hydroxyl groups in their chemical structure, thus acting as reducing agent and polymer matrix for HEC/AgNPs nanocomposite materials. HEC proves to be advantageous for having properties such as water soluble, biocompatible, and the capability to reduce silver ions, Ag^+, to silver nanoparticles in a few minutes, by heating treatment at room temperature. Furthermore, preliminary results of the application of HEC/AgNPs scaffolds in the cytotoxicity effects against human fibroblast (hFB) cells were also reported, without the use of any external reducing agent.

2. Materials and methods

2.1. Materials

Hydroxyethyl cellulose ($M_w = 250{,}000$) and silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich. Analytical reagent grade glutaraldehyde solution (25%) was purchased from Merck-Schuchardt, Germany. Phosphoric acid was purchased from Merck. KGaA-Darmstadt, Germany. Acetone was purchased from R&M Marketing, Essex, UK. Phosphate buffer saline (PBS) was purchased from Gibco Life Technologies, USA. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Life Technologies, USA. All the chemicals were of highest purity and used without further purification. All solutions were prepared using Millipore water.

2.2. Synthesis of HEC/AgNPs composites

The HEC solution with the concentration of 10 wt% was prepared by dissolving 10 g of HEC powder in 100 mL Millipore water for 2 h at room temperature. 200 to 800 μL of 0.05 M $AgNO_3$ in water was added to the HEC solution with constant stirring at 75 °C for 2 h, in a dark environment. The HEC solution acquired was light yellow in color indicating the reduction of Ag^+ to Ag^0. This interaction can be schematically represented as shown in Scheme 1. 2.5% GA was added to the solution for crosslinking purposes.

2.3. Preparation of HEC/AgNPs scaffolds

Scaffolds were prepared by the freeze-drying method. The solution was poured into the flasks and kept in a deep freezer at −80 °C for 24 h. These frozen samples were lyophilized in the freeze-dryer (FreeZone 6 Liter Benchtop Freeze Dry System, Labconco) at −50 °C for 72 h to obtain porous scaffolds. Subsequently, the scaffolds were then kept in a chamber saturated with GA vapor for 72 h for crosslinking formation, undergo heat treatment at 140 °C for 10 min, and dried in a vacuum oven for a day.

2.4. Porosity study

The porosity of the HEC/PVA scaffolds was measured using the water displacement method. The scaffolds were cut into 1 cm × 1 cm × 1 cm cubes and immersed in a known volume (V_1) of water in a Falcon tube for 30 min. The total volume of water and the water-impregnated scaffold was recorded as V_2. The water-impregnated scaffolds were then removed from the Falcon tube and the residual water volume was recorded as V_3. Experiments were carried out in six replicates for all types of scaffolds. The porosity of the scaffolds was obtained by Eq. (1) [36]:

$$\text{Porosity}\ (\%) = (V_1 - V_3)/(V_2 - V_3) \times 100\% \quad (1)$$

2.5. Swelling ratio study

The water uptake or swelling analysis was analyzed for 7 days. The lyophilized scaffolds were cut into 1 cm × 1 cm × 1 cm cubes and placed in a 24-well plate. The scaffolds were weighed (W_d) before being submerged in PBS (pH 7.4) and kept in the 37 °C incubator. The wet weight of the samples (W_t) was determined after 1, 3 and 7 days by gently blotting them on filter paper [37,38]. Experiments were carried out in triplicates for all types of scaffolds. The swelling ratio was calculated according to Eq. (2).

$$\text{Swelling ratio}\ (\%) = (W_t - W_d)/W_d \times 100\% \quad (2)$$

2.6. In vitro degradation study

The lyophilized scaffolds were cut into 1 cm × 1 cm × 1 cm sizes and sterilized under UV irradiation for 2 h. The scaffolds were placed in a 24-well plate containing 3 mL of PBS incubated at 37 °C. *In vitro* biodegradation test was studied by immersing a pre-determined weight (W_o) of scaffolds in PBS (pH 7.4) at 37 °C for 1, 3 and 7 days. Three paralleled scaffold of each degradation period were taken out at different period of time, washed with distilled water, and kept dry in the oven for 2 h. The final weight of samples was recorded as W_d. The percentage of degradation was calculated using Eq. (3) below:

$$\text{Weight loss}\ (\%) = (W_o - W_d)/W_o \text{ x } 100\% \quad (3)$$

$$AgNO_3 + \text{aq. HEC} \rightarrow Ag^+ + \text{HEC} \xrightarrow{\text{Reduction}} [Ag^o/\text{HEC}]$$

Scheme 1. Reduction of silver ion by HEC.