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Biocatalytic protein membranes fabricated by electrospinning

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ABSTRACT

In this study, a protein-based catalytic membrane was produced by electrospinning. Membrane activity was characterised in terms of response current for various glucose concentrations. We focused on the preparation of a scaffold by converting a globular protein to other structural forms using catastrophic solvents. A scaffolding protein, bovine serum albumin, and an enzyme, glucose oxidase (GOD), were selected as a model natural carrier matrix and a biologically active agent, respectively. Beta-mercaptoethanol (β-ME) was used to convert the globular protein to an amyloid-like form. A structural stabilising agent, 2,2,2-triflouroethanol (TFE), was used to maintain the final α-helical structure of the amyloid-like protein. The TFE:PBS (phosphate-buffered saline) ratio and various electrospinning parameters were analysed to minimise activity loss. Using this approach, we applied electrospinning to an active enzyme to obtain biocatalytic nanofibrous membranes. After optimising the protein electrospinning process, the activities of the protein nanofibrous membranes were monitored. GOD remained active in the new membrane structure. The highest enzyme activity was observed for the membranes prepared with a 1.5:1 (v:v) TFE:PBS solvent ratio. In that particular case, the immobilized enzyme created a current of 0.7 μA and the apparent activity was 2547 ± 132 U/m^2.

1. Introduction

Electrospinning is a popular technique to produce nanofibrous structures using polymeric substances for a variety of applications [1–5]. The power of electrospinning mainly depends on the applicability of the technique to a number of different polymers with minor optimisation steps. Using the appropriate optimisation conditions, one can adjust the thickness, morphology, and length of a desired polymeric nanofibre [6]. Although electrospinning has been successfully applied to many synthetic polymeric materials, few studies have examined its application to natural polymers. One of the challenges in using natural polymers for electrospinning is their closed and densely packed structure. Combining proteins with polymers is one approach to overcome this limitation [7,8]. Additionally, some studies have recently examined direct electrospinning of proteins to form biological nanofibres [9]. Globular proteins, such as albumin [9–12], haemoglobin [5], and myoglobin [13], have been electrospun.

Our inspiration for protein electrospinning arose from the natural biological fibres secreted by diverse organisms. Biological protein fibres are generally secreted by specialised instruments, as in the case of the secretion of silk by *Bombyx mori* [14,15]. Silk is one of the oldest known protein fibres with functional properties. It has previously been reported that spider silk contains amyloid-like fibres when it is assembled in vitro [16]. Although biological fibres have many applications, the production of these natural fibres is not cost effective or efficient [17,18]. In this context, the utilisation of electrospinning to produce protein-based natural fibres is promising. Using this approach, different proteins with various functionalities can be electrospun for biotechnology applications. One of the best methods for fabricating a biofunctional scaffold is to load a bioactive agent to the scaffold. The performance of the bioactive agent is strongly dependent on supporting structures at the nanoscale. For example, nanofibres minimise diffusion limitations [9]. However, owing to their structural arrangement, the electrospinning of proteins requires an intensive optimisation strategy. As mentioned in the work of Dror and coworkers for the electrospinning of bovine serum albumin (BSA), disulphide bonds should be broken to obtain an electrospinnable solution using a catastrophic solvent. This process causes the conversion of a protein into an amyloid-like form. Among various alcohols, beta-mercaptoethanol (β-ME) is mainly used

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to open the tertiary structure of proteins and α-helical structures are stabilised by 2,2,2-triflouroethanol (TFE) [10].

In this article, a BSA-derived amyloid-like structure was used as a natural polymer for the electrospinning process to enhance the coating and supporting properties during the entrapment of a bioactive agent. In this scenario, the glucose oxidase (GOD) enzyme was chosen as a model bioactive agent. The negative effects of catastrophic solvents on enzymatic activity were studied. The ratio of the solvent to stabilising agent was carefully adjusted, as were the electrospinning parameters to minimise activity loss. The preliminary results for the performance of electrospun biocatalytic membranes are reported based on apparent enzyme activity.

2. Experimental

2.1. Materials

Glucose oxidase (EC 1.1.3.4.) from *Aspergillus niger* (specific activity: 306 U/mg), TFE, β-ME, D-(+) glucose monohydrate, and Congo red were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents (NaCl, Na_2HPO_4, and NaH_2PO_4) used as buffering agents and BSA were supplied by Acros Organics (Morris Plains, NJ, USA). Double-distilled water was used throughout the experiments.

2.2. Preparation of solutions and electrospinning

The negative effect of catastrophic solvents on enzymatic activity was studied. BSA was dissolved in a mixture of TFE and phosphate-buffered saline (PBS) at a concentration of 12% (w:v) at room temperature. The tertiary structure of the protein was decomposed with 10 equiv. bond β-ME and stabilised by the addition of TFE at the following ratios: 1.5:1.0, 3.0:1.0, 4.5:1.0, and 9.0:1.0. The BSA-containing solvent was stirred continuously at 25 °C for 4 h. After obtaining a clear solution of protein, the powdered enzyme corresponding to 200 U/mL final activity was added, stirred for 5 min, and the final solution was transferred to an injector to begin the electrospinning process immediately**.** Accordingly, the interaction period for the enzyme, GOD, and catastrophic solvents was limited to diminish activity prior to the electrospinning process. The preparation protocol for the solution is summarised in Fig. 1.

For the electrospinning setup, a direct current voltage supplier (MCH 303D2; Gamma High Voltage Research Inc., Ormond Beach, FL, USA) and a syringe pump (NE-1000; New Era Pump Systems Inc., Farmingdale, NY, USA) were used. The mixed solution was loaded into a 5-mL syringe with a vertically fixed metal needle (inner diameter, 0.80 mm). In the vertical setup, the collector was placed on the floor and the syringe pump was located above the collector. The flow rates (Q) were maintained between 0.20 and 0.60 mL/h (Fig. 1). Fibres were collected on glass slides and aluminium foil. The applied voltage and the distance between the tip and the collector varied between 9 and 20 kV and 10–20 cm, respectively. All experiments were performed at room temperature (about 25 °C). The samples were then stored for 24 h in a vacuum desiccator.

2.3. Characterisation

Fiber morphologies were investigated by optical microscopy (Nikon Eclipse LV100, Nikon Instruments Inc., Melville, NY, USA) and scanning electron microscopy (SEM, FEI-Quanta 200; Hillsboro, OR, USA). The chemical surfaces of membranes were characterised by FTIR-ATR (Fourier transform infrared spectroscopy attenuated total reflectance) (Perkin Elmer, Waltham, MA, USA). After production, electrospun membranes were stained with Congo red using the methods by Puchtler et al. [19] in order to verify the formation of an amyloid-like structure [19].

2.4. Measurement of enzyme activity

The reaction sequence to monitor glucose utilisation of flavin adenine dinucleotide (FAD) is given as follows:

$$\beta\text{-D-Glucose} + \mathrm{GOD(FAD)} \rightarrow \beta\text{-D-Gluconolactone} + \mathrm{GOD(FADH_2)} \quad (1)$$

$$\mathrm{GOD(FADH_2)} + \mathrm{O_2} \rightarrow \mathrm{GOD(FAD)} + \mathrm{H_2O_2} \quad (2)$$

$$\mathrm{H_2O_2} \rightarrow \mathrm{O_2} + 2\mathrm{H^+} + 2\mathrm{e^-} \quad (3)$$

Apparent enzyme activity was measured by amperometric detection. Amperometric measurements were performed using the DropSens μStat 200 (Llanera, Spain). The DropSens electrode was polarised at 650 mV to decompose hydrogen peroxide (H_2O_2), which is formed during the reaction of glucose with oxygen in the presence of GOD (1, 2). The electrons that result from the decomposition of hydrogen peroxide are proportional to the glucose concentration (3). They were tracked amperometrically and the response current (I) was recorded in units of μA. The details of the amperometric measurements of enzymatic activity were described previously [20].

In the experimental setup, the electrospun membranes were placed in a 50-mL PBS solution and stirred for 10 min until equilibrium was reached. A stock glucose solution (200 mM) was added to the PBS solution to achieve a final concentration of 0.05–50 mM glucose, and the solution was stirred continuously. Enzyme activities corresponding to different substrate concentrations were investigated based on response currents. This procedure was repeated several times until deviations from linearity were observed for all membranes. The optimum solvent

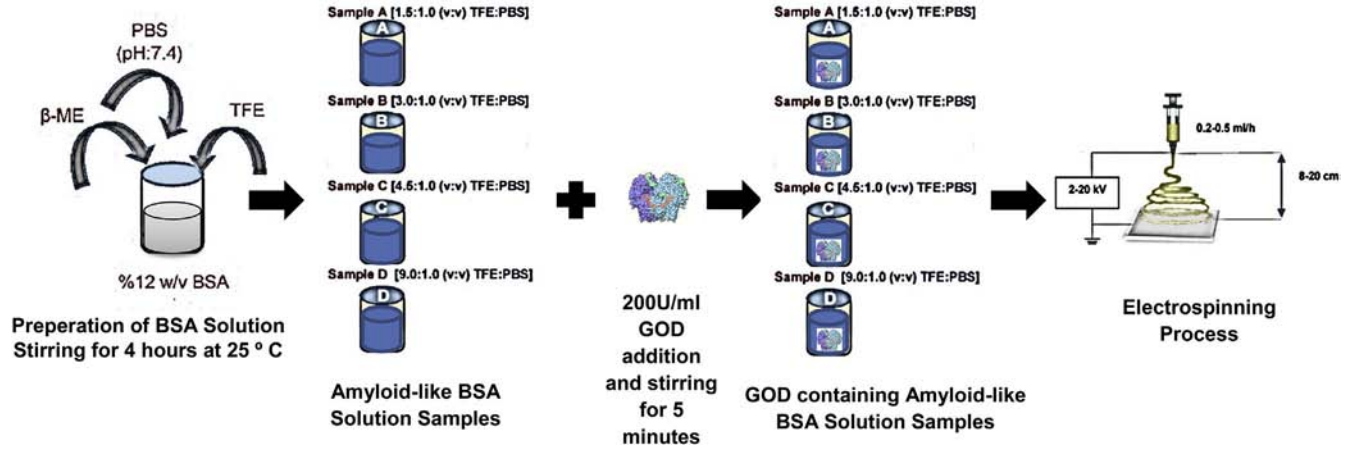

Fig. 1. Schematic representation of solution preparation and electrospinning setup.