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Full Length Article

Synthesis and characterization of bacterial cellulose and gelatin-based hydrogel composites for drug-delivery systems

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ABSTRACT

Bacterial cellulose and gelatin were successfully used to develop a hydrogel composite material. Hydrogel was synthesized by copolymerization between bacterial cellulose and gelatin. Scanning electron microscopy (SEM) images showed that the bacterial cellulose chain was uniform in size and shape. Glutaraldehyde was employed as a crosslinking agent. H-bonds were formed via the reaction between the amine and hydroxyl groups, which were the functional groups of the gelatin and bacterial cellulose, respectively. The hydrogel composite presented excellent properties in terms of its thermal stability, chemical resistance, and mechanical properties. Moreover, the swelling ratio of the hydrogel network, in water, was estimated to be 400–600%. Importantly, the hydrogel composite developed during this study is considered a good candidate for drug-delivery systems.

1. Introduction

In recent years, naturally derived polymers, including proteins and polysaccharides, have been widely utilized as biomaterials. Numerous strategic methods have been extensively developed for many medical-technology applications. For instance, one of the most attractive applications involved the use of hydrogels. Hydrogel materials have been commonly employed in many fields, such as wastewater treatment, chemical sensors, and medical technology [1,2]. Hydrogels have great potential because they can absorb a large amount of water or biological fluid, and offer high porosity as well as a soft consistency. Hydrogels are known as reversible gels if molecular entanglements, such as ionic, H-bonding, or hydrophobic forces, play a key role in forming the network [3,4]. These entanglements are often reversible and can be dissolved by changing the environmental conditions, such as the pH, ionic strength of the solution, or temperature. Therefore, hydrogels offer numerous advantages and can be used for the controlled release of pharmaceuticals [5].

To date, with the growth of the global population, hydrogels have been increasingly developed for use in pharmaceutical technology [6,7]. The design of hydrogels using bio-based materials is considered one of the most effective routes for sustainable development. With regard to the synthesis of bio-based materials for hydrogel applications, gelatin is considered the most effective bio-based polymer because it offers many advantages, such as non-toxicity, high water absorption, biodegradability, and biocompatibility. It is notable that these properties render gelatin as an excellent candidate with regard to drug-delivery vehicles. Hydrogels in drug-delivery systems can be used to deliver drugs over a specific time period via a controllable release mechanism. The use of hydrogels offers many advantages, such as reduced drug dosages, costs, and side effects. It is important to note that the use of hydrogels in drug-delivery systems relies on their swelling ability. The swelling mechanism occurs due to an increase in the distance between crosslinked polymer chains, which allows drug molecules to be released and absorbed into the bloodstream [8–13]. Researchers have focused on identifying materials that are non-toxic, environmentally friendly, and highly biocompatible. In previous studies, natural materials, such as polysaccharides, have been employed as reinforcement materials during the production of hydrogels [14–18]. Bacterial cellulose is considered to be one of the most effective reinforcement materials. Bacterial cellulose has a similar structure to that of cellulose, with ultrafine fibers. The most effective source of bacterial cellulose is considered to be *Acetobacter xylinum*: its cellulose has β-1,4-glycosidic bonds between two glucose molecules [19–22]. Previous studies have stated that bacterial cellulose could be used in hydrogel composites for healthcare research because of their excellent biocompatibility and biodegradability. Bacterial cellulose has many versatile advantages. The Young's modulus of a single fibril can be as high as 114 GPa [23]. Bacterial cellulose also exhibits other attractive features, such as a high degree of crystallinity (89%) [24], high degree of

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polymerization (14,400) [25], and a high specific surface area (37 m^2/g) [26]. Moreover, bacterial cellulose also offers a large surface area, high aspect ratio, and low bulk density, as well as hydrophilicity. It is important to note that the existence of a small amount of bacterial cellulose in gelatin-based hydrogels can offer significant enhancement with regard to their tensile strength and dimensional stability when employed under externally applied forces.

In this paper, we present the design of a bacterial cellulose and gelatin-based composite hydrogel. The effect of glutaraldehyde as a crosslinking agent was investigated. Preliminary experiments with regard to drug-delivery systems have been performed.

2. Experimental

2.1. Materials

Bacterial cellulose was successfully extracted from the nata de coco product (Chaokoh coconut gel in syrup, Ampol Food Processing Ltd., Nakornpathom, Thailand). This is an indigenous dessert, in which the main component is bacterial cellulose. Bacterial cellulose extracted from nata de coco was characterized and reported in previous work [27]. Its characteristics match those of bacterial cellulose extracted from *A. xylinum* cultures. Food-grade gelatin and glutaraldehyde were purchased from Sigma Aldrich, Co. Ltd. They were employed as a matrix material and crosslinking agent, respectively. All chemical reagents were used as received without further purification.

2.2. Methods

2.2.1. Bacterial cellulose extraction and purification

Bacterial cellulose was extracted from nata de coco, which was rinsed with distilled water to remove excess sugar and blended in a laboratory blender to obtain nata de coco pellicles. These pellicles were treated with 0.1 M NaOH at 80 °C for 1 h to remove any remaining microorganisms, medium components, and soluble polysaccharides. The purified bacterial cellulose was then thoroughly washed with distilled water until a neutral pH was achieved.

2.2.2. Bacterial cellulose and gelatin hydrogel composite preparation

A bacterial cellulose and gelatin-based hydrogel composite were successfully synthesized owing to the reaction between bacterial cellulose and gelatin. To achieve this, 10 wt% of gelatin was completely dissolved in water, and then a bacterial cellulose suspension was poured into the gelatin solution. Glutaraldehyde was employed as a crosslinking agent. The reaction was performed at 55 °C for 4 h. The chemical reaction that occurred between the bacterial cellulose and gelatin, with 1 wt% of glutaraldehyde, is exhibited in Fig. 1. Subsequently, the hydrogel composite was washed with deionized water to remove any unreacted chemicals and stored at 4 °C. The properties of the as-synthesized hydrogel were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM), and Brunauer–Emmett–Teller (BET) analysis.

In this experiment, neat gelatin was also studied for comparison. The ratios of gelatin and bacterial cellulose were determined as 25:1, 50:1, 100:1, 200:1, 300:1, 400:1, respectively.

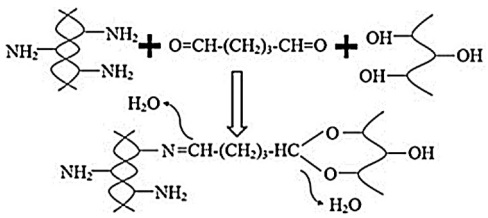

Fig. 1. Chemical reaction between bacterial cellulose and gelatin with glutaraldehyde.

The gelatin and bacterial cellulose-based hydrogel composites were evaluated based on their swelling capability. The water swelling ratio and equilibrium water content of the gelatin and bacterial cellulose hydrogel composite were determined using a gravimetric technique at ambient temperature. The hydrogel composite was immersed into deionized water. The investigation periods were determined as 24 h and 48 h. Prior to measurement, the hydrogel composite was dried in an oven at 45 °C until a constant weight was achieved. The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio} = (\text{W swollen} - \text{W dry})/\text{W dry} \times 100\%$$

2.2.3. Drug-loading and release experiments

Loading experiments, where methylene blue (MB, used as a model drug) was loaded into the hydrogel composites, were conducted using the swelling-diffusion method. The molecular structure of methylene blue has a positive change, similar to doxorubicin and hydroxyurea. They were commonly used as anticancer drug [28,29]. First, the hydrogel composites were dried in an oven at 45 °C until a constant weight was achieved, and then allowed to swell in a MB aqueous solution (5 mg/mL) at 37 °C for 48 h. The swollen hydrogels were rinsed with deionized water and dried again to obtain drug-loaded hydrogels. The concentration of the drug remaining in the loading solution was determined using a UV–vis spectrophotometer (UV-2600, Shimadzu, Japan) at 658 nm. The drug entrapment efficiency (EE) of the hydrogel composites was calculated using the following equation [30]:

$$EE\ (\%) = [(W_o - W_f)/W_o] \times 100,$$

where W_o is the total amount of MB in the solution prior to loading, and W_f is the total amount of MB in the solution following loading.

In the case of the drug-release experiments, the drug-loaded hydrogel composites were immersed into 50 mL of deionized water at 37 °C for 48 h under conditions of constant vibration (70 rpm). At predetermined time intervals, aliquots (0.5 mL) of the release medium were removed, and an identical volume of fresh medium was added. The concentration of the drug in the release medium was quantified via spectrophotometry (UV-2600, Shimadzu, Japan) at 658 nm. Each release experiment was performed in triplicate. The cumulative percentage release was calculated as follows [30]:

$$\text{Cumulative percentage release} = W_t/W_l \times 100,$$

where W_t is the amount of MB released from the hydrogel at time *t*, and W_l is the amount of MB loaded onto the hydrogel.

2.3. Characterization techniques

2.3.1. Fourier transform infrared spectroscopy

FTIR was performed using a Bruker Vector 22 mid-IR spectroscope (Bruker, Germany). All the FTIR absorption spectra were recorded over the wavenumber range of 4500 cm^{-1} to 500 cm^{-1} at a resolution of 8 cm^{-1}, with 1024 scans, using a deuterated triglycine sulfate (DTGS) detector. A straight line between the two lowest points in the respective spectra region was selected as a baseline. The bacterial cellulose and gelatin-based hydrogel composites were cast onto glass slides prior to investigation.

2.3.2. Field emission scanning electron microscopy

The morphological properties of the bacterial cellulose and gelatin-based hydrogel composites were investigated using a field-emission scanning electron microscope (FE-SEM, Hitachi, S-4800) at an acceleration voltage of 2 kV. Prior to investigation, the samples were stored in desiccators to avoid exposure to humidity. The hydrogel composites were prepared using a freeze-drying technique to remove any existing water. Each sample was placed on a carbon tape and sputtered with gold particles prior to analysis.