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Sericin-binded-deprotenized natural rubber film containing chitin whiskers as elasto-gel dressing

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ABSTRACT

Here, we aims to demonstrate a simple concept in biomaterials design by using natural resources solely as raw materials to fabricate elastic wound care dressing. Elasto-gel films comprise of silk sericin (SRC), natural rubber (NR), and chitin whisker (CTW) were developed. A glue-like protein SRC found in silk cocoons is beneficial for the treatment of wounds due to its superior skin moisturizing ability. However, the pure SRC film is generally difficult to be fabricated because of its weak structural feature. This limitation was overcome by using NR as a binder which consecutively rendered elasticity and strength of the films. CTW was chosen as another component to promote ability of the films for tissue restoration. Before the film formation, protein in the natural rubber latex (NRL) was removed to avoid allergic and cytotoxic problems. The enzyme-treated NR/SRC (ETNR/SRC) films having different blend compositions were fabricated by solution casting technique. The highest amount of the SRC to gain an easy to handle ETNR/SRC film was 30%. The ETNR/SRC/CTW films having 20% SRC were fabricated and studied in comparison. Essential properties of the films as elastic wound care dressings were investigated and effect of the materials chemistry on the observed properties were discussed.

1. Introduction

Depending on the type, severity and position of wounds, there are a variety of wound care dressings. For example: hydrocolloid dressings are useful for light to moderately exuding wounds such as pressure sores, minor burn wounds and traumatic wounds; hydrogel dressings are best for wounds with minimal or no exudate such as dry chronic wounds, necrotic wounds, pressure ulcers and burn wounds [1]. Beside the above mentioned, there are other wound dressings available, such as elastic or compression dressings which are proper for the wounds thought to occur due to improper functioning of venous valves (*e.g.* venous ulcers). These dressings then work not only to promote the formation of new skin tissue but also to slowly stretch out vein walls and improve overall circulation as well as restrict swelling at the place of injury [2]. Generally, the most frequently mentioned aims for wound healing are to accelerate the healing process and also lessen scarring and scabs. Medical research has shown that moist wound treatment is the best way to accomplish these aims. New cells for the formation of new skin tissue thrive upon moist conditions. Furthermore, the formation of a crusty scab is prevented under the moist wound environment since the moist promotes growth and migration of new cells and ensures that essential proteins for the closing of wounds stay where they belong in order to perform their repair task [3]. Skin moisturizing agent is therefore an important ingredient for the wound care products.

Silk, a natural macromolecular protein produced by Lepidopteron insects of the family Bombycidae and Saturniidae, consists mainly of two proteins; fibroin and sericin. While fibroin is a fibrous protein having glossy surface and desirable mechanical properties which thus render it to be widely used in textile manufacturing, sericin is a glue protein surrounding the fibroin fibers that most of it must be removed as a waste during the degumming process of silk production [4]. Sericin (SRC) is hydrophilic in nature. It contains 18 amino acids including essential amino acids and 30% is serine which is the main amino acid of natural moisture factor (NMF) in human skin [5]. The SRC is thus an excellent moisturizing agent. Its moisturizing effect is proved by the observed decrease in skin impedance, increase in hydroxyproline level, and hydration of epidermal cells. Furthermore, smoothness of the upper layer of the skin surface is enhanced as a result of moisturization [6]. *In vivo* experiments showed that the SRC peptides

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have no immunogenicity and can be used effectively for medical approach [7]. Some reports indicated that SRC enhances attachment and growth of human skin fibroblast cells [8,9], stimulates collagen production [10] and cell migration [11], as well as promotes the healing of second-degree burn wounds both *in vitro* and clinical test in human [12]. However, so far, SRC containing products are mostly available as cream or liquid, and principally used as cosmetics (*e.g.*, Dr. Temt®, Blossom®, Mayu Dzukushi®, and Neogen®). The SRC has always been neglected in the field of tissue engineering due to its weak structural properties and high water solubility. Fabrication of the SRC films and sponges is difficult because of the wide range of SRC molecular weights, ranging from 10 to over 300 kDa [13]. The obtained materials are also fragile. To overcome this problem, researchers have blended the SRC with other natural or synthetic polymers such as poly(vinyl alcohol), poly(oxyethylene)-poly(oxypropylene) block copolymer, and glycerine to fabricate 2D and 3D materials [14]. However, only small composition of the SRC could be included in all blend films ($\leq$3.0% w/v). Mandal et al. [15] reported the fabrication of 3D SRC/gelatin scaffolds and 2D films using non-mulberry *Antheraea mylitta* silk cocoon SRC protein. Both scaffold and film formation was feasible using glutaraldehyde and 12 N HCl as crosslinking and group-activating agents, respectively. Although the crosslinking agent was present, the researchers mentioned that, caution was exercised in handling pure SRC films as they could break easily. Work related to an attempt to fabricate the SRC film by avoiding the usage of toxic chemicals for both extraction and crosslinking was also done. Dash et al. [16] reported that silk gland SRC protein has a potential as a SRC source for the film formation without any crosslinking agent, but, specification of the constituent's part means limitation for the mass production. Even though Lim et al. showed that only small amount of the SRC ($\sim$5% and 10% w/w) blended with poly(vinyl alcohol) gave rise to a remarkable improvement in the materials' cellular function, chemical modification of the SRC to SRC-methacrylate before blending was required to improve its bonding ability [17].

Natural rubber (NR) is an elastic hydrocarbon polymer usually described as poly(*cis*-1,4-isoprene), and is found in the sap of certain plants. The most commonly known source of natural rubber is the Para rubber tree (*Hevea brasiliensis*). It is harvested mainly in the form of latex, which thereafter called, natural rubber latex (NRL). The NRL is a cloudy white liquid, similar in appearance to milk. It exhibits colloidal behavior having rubber particles suspended in water. The rubber particle diameter is between 5 and 3000 nm, and they are surrounded by a complex mixture of proteins, lipids, and long-chain fatty acids, which impart negatively charge. Most of the protein (75%) in the NRL is freely dissolved in the serum fraction while a minor fraction (25%) is bound to the surface of the rubber particles [18]. Fig. S1† shows schematic drawing of the NR suspension. This latex will turn into a rubbery mass within 12 h after it is exposed to the air and the obtained rubbery mass has excellent mechanical properties, outstanding elasticity, resilience, flexibility at low temperature, facile adhesion, ability to disperse heat, and relatively high water resistance [19]. Therefore, it is possible to mix the NRL with the SRC aqueous solution and produce water-insoluble SRC/NR films having desirable mechanical properties. Concisely, rubber particles can disperse in the SRC aqueous solution matrix and simultaneously act as a binder of the SRC film.

Nevertheless, the NRL can give rise to allergic problem which is of great concern here where the obtained NR containing films are willing to be used on human body. There are two types of allergy related to NRL; one caused by the natural proteins and another by chemicals used to alter the NRL to desirable materials. They are respectively called Type I and Type IV allergy [19,20]. The usage of NRL for biomedical applications with avoiding toxic chemicals have been proposed to depart from Type IV allergy while reduction of protein from the NRL to avoid Type I allergy could be done by centrifugation and proteolytic enzyme treatment [21–23]. After all, latex allergy normally attacks individuals who are habitually introduced to rubber materials such as rubber manufacture workers, and individuals who have had several operations or several medical procedures where latex materials were employed. Patients who get allergic reactions to foods, like kiwifruit, chestnuts, bananas, avocados, and tomatoes, have a greater chance of acquiring latex allergic reactions since the protein in these items is basically similar to that contained in the NR. So that, the majority of the population is not a clinical risk. With the exception of these allergic proteins, the NRL is an important inductor for the wound healing process due to the presence of a vascular growth factor which leads to its ability to stimulate natural angiogenesis and cellular adhesion [24–26].

Apart from appropriate moist wound environment, additional bioactive molecules such as drugs and growth factors are important for the tissue regeneration. Chitin—a long-chain polymer of an *N*-acetylglucosamine—is well-recognized for its ability to regenerate wound tissues and to be a substrate for lysozyme digestion. Once the injury occur, the wound healing process begins immediately by the release of different growth factors, cytokines, as well as enzymes, including lysozyme—an antibacterial enzyme that occurs naturally in all human mucous [27,28]. After contact with the lysozyme, chitin could be hydrolyzed to smaller molecules, *i.e.* glucosamine, which could enhanced the wound closure rate as a consequent of the greater fibroblast proliferation, collagen synthesis, and proliferation of hair follicles [29–31]. Chitin polymer is known to form microfibrillar arrangements embedded in a protein matrix, which is a major constituent of the exoskeleton of arthropods. These microfibers, hereafter called chitin whiskers (CTWs), usually have diameters range from 2.5 to 2.8 nm, with a length as large as 25 nm [32]. An enormous surface area is their main advantage over the original chitin flake and powder. This feature enables CTWs to interact effectively with compounds existing in living tissues. Thus, their ability to induce the formation of granulation tissues and recover wounds are favorable.

By integrating beneficial properties of the SRC, the NRL, and the CTWs through a simple solution-casting technique, the aim of this work is to fabricate the NR/SRC/CTW nanocomposite films as an alternative wound care product. Specifically, we aims to develop the novel elastic dressings applicable for the wounds in which the application of pressure to cushion the wound is required. The SRC functions as a moisturizing agent while inclusion of the NR is done as a means to assist the film forming ability and introduce elasticity as well as water insolubility. Besides, the CTWs functions as a supplement for the tissue regeneration. In order to stay clear of allergy, the NRL was centrifuged and treated with proteolytic enzyme before mixing. Substantial advantages of the present material over the other SRC-containing 2D and 3D materials are the exclusion of crosslinking and/or group-activating agents and the significantly greater amount of the SRC incorporation.

2. Experimental section

2.1. Materials

Bombyx mori silk cocoons and *Penaeus merguiensis* shrimp shells were supplied by Queen Sirikit Sericulture Center (Nakorn Ratchasima, Thailand) and Surapon Food Co., Ltd. (Samut Prakarn, Thailand), respectively. Natural rubber latex of *Hevea brasiliensis* tree cultivated from Phatthalung (Thailand) was used as an NR source and was preserved in ammonia solution. Hydrochloric acid (37%) was purchased from RCI labscan (Thailand). Tris-HCl (molecular biology grade) was purchased from Scharlau Chemie (Barcelona, Spain). All other chemicals were used as received.