



## Developing on Implant Biorubber Materials without Using Acid for Coagulation

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### Abstract:

**Background:** Natural rubber latex (NRL) from *Hevea brasiliensis* is a colloidal anionic system formed by rubber particles (1,4-cis-polyisoprene) stabilized by phospholipids and protein molecules. Rubber biomaterials using as a novel technology could develop to apply as biomaterial based on a new manufacturing process, several new biomedical applications have been proposed since NRL is very biocompatible, stimulating cellular adhesion, the formation of the extracellular matrix, and promoting the replacement and regeneration of tissue.

**Objective:** This study aimed to deproteinization from fresh natural rubber latex (NRL) and to coagulate the deproteinized natural rubber latex (DNRL) for using as implant biomaterials with novel technology without using acid for coagulation.

**Methods:** Coagulated DNRL films is often used to prepare the blended films by solution-casting technique. Its films presents interesting physical properties in elasticity.

**Results:** The deproteinized NRL containing various CaO gave lower modulus values comparing with the control films.

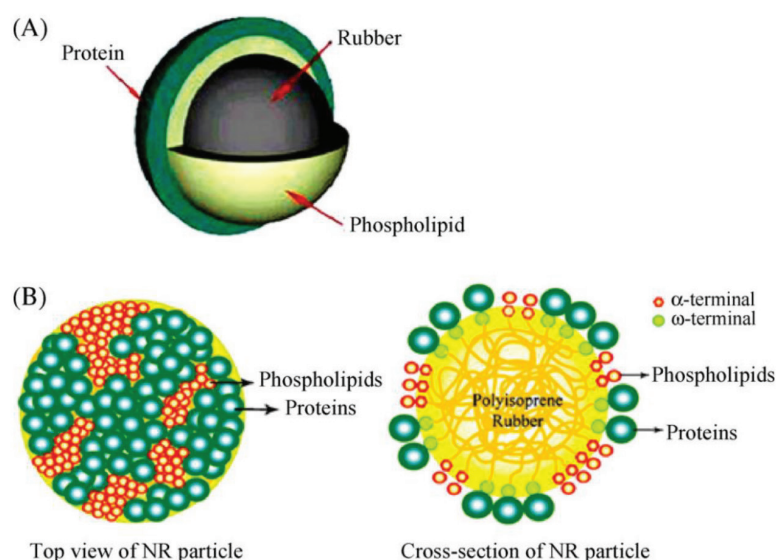
**Conclusion:** In this experiment, the blended films of DNRL and various CaO could form appropriate films. The physical and mechanical properties of the blended films depended on type and content of CaO addition. From the good elasticity of blended films, they could develop to apply as the production of a biomaterial of NRL that has been used to replace vessels, esophagus, pericardium, and abdominal wall.

**Keywords:** Deproteinization, Fresh natural rubber latex, Rubber, Biomaterials, CaO

## Introduction

*Hevea brasiliensis* (H.brasiliensis), popularly known as the Para rubber tree, is a plant species that belongs to the Euphorbiaceae family. It is the most economically important member of the genus *Hevea* because the milky latex extracted from the tree is the primary source of natural rubber<sup>1</sup>. Most of the harvested latex is coagulated for the manufacture of dry rubber products, including automotive tires. The latex of *H. brasiliensis* can be stabilized in an uncoagulated form with the use of ammonia, which allows the latex to be used for the manufacture of other products, such as surgical gloves<sup>2</sup>. Natural

rubber latex (NRL) from *H. brasiliensis* is a colloidal anionic system formed by rubber particles (1,4-cis-polyisoprene) stabilized by phospholipids and protein molecules (Figure 1)<sup>3,4</sup>. One-third of the weight of *H. brasiliensis* latex is made of natural rubber, but 1–2% of its weight consists of hundreds of proteins<sup>5</sup>. Other constituents such as lipids, Quebrachitol, ribonucleic acids, and organic salts are also present<sup>6,7</sup>. Based on a new manufacturing process, several new biomedical applications have been proposed since NRL is very biocompatible, stimulating cellular adhesion, the formation of the extracellular matrix, and promoting the replacement and regeneration of tissue<sup>8</sup>.



**Figure 1** The structure model of the natural rubber latex particle surface.

(A) A current model of an NRL particle, and (B) the new model (adapted from Ref. 3).

Cockle shells (aragonite) is one of the more abundant crystalline polymorphs of calcium carbonate ( $\text{CaCO}_3$ ) with more than 95% purity. The trace elements were measured as mercury (Hg) < 0.25 ppm, arsenic (As) 0.75 ppm, lead (Pb) 0.31 ppm and cadmium (Cd) 0.31 ppm<sup>9</sup>. Moreover, it contains fractions of heavy metals from natural sources, but all elements are within the range permitted by ASTM's implant materials standard.

This work aims to deproteinization from fresh NRL and then to coagulate natural rubber biomaterials using active CaO extract from mollusk shells with novel technology without using acid for coagulation.

## Objective

In the present work, the purpose focused on the deproteinization from fresh NRL and to coagulate the deproteinized natural rubber latex for using as implant

biomaterials with novel technology without using acid for coagulation.

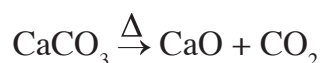
## Method

### Deproteinization Processes

The solutions were chosen for protein extraction: 2% sodium lauryl sulfate (SLS) and 0.1%w/w, 2.0 %w/w KOH. Natural rubber latex 250 g stirred in a 500 mL extract solution. After shaking at room temperature for 3 hours, the mixture was mixed with 2%w/w alum ( $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ ) and heat up to 60°C and then immediately subjected to protein precipitation. In the protein precipitation step, the low proteins rubber was collected by centrifugation at 10,000 rpm and 4°C for 10 minutes, and the collected cream fraction was diluted with distilled water. Finally, the deproteinized natural rubber latex (DNRL) was redissolved in deionized water and stored at 4°C.

### Active CaO preparation

Five hundred gram of fresh cockle shells were immersed with 15%v/v  $\text{H}_2\text{O}_2$  for 24 hours and then calcined at 900°C for 1 hour under an oxidation atmosphere. Thermal treatment in this condition has changed CaO into high purity and active to reaction<sup>9</sup>. Then milled calcium oxide into powders by using a high-speed ball mill and stored in a dedicator. The transform into calcium oxide by freeing carbon dioxide ( $\text{CO}_2$ ) according to the following equation:

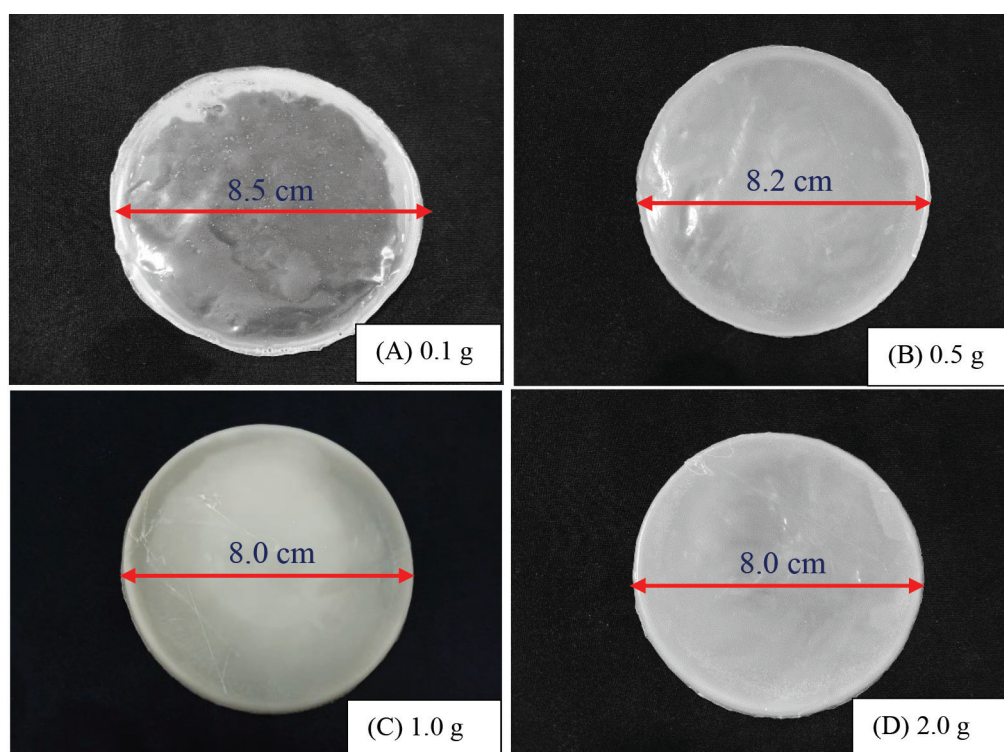


### Coagulating of Natural Rubber Biomaterials

Coagulation of DNRL studies was carried out in 1,000 mL beakers by 100 g of DNRL and various CaO addition for 0.1, 0.5, 1.0, and 2.0 g. The mixtures were stirred for 30 min at room temperature (27-30°C). Then each mixture was cast in the glass plates. After that, let dried at 60°C for 24 h using an air-circulating oven. The physical testing of dried rubber film was analyzed by a universal testing machine based on ASTM D 412. Five dumbbell test pieces were cut from each film and the average thickness was calculated and then attached between the grips of a tensile testing machine and pulled at a rate of 500 mm/min.

### Results

From the deproteinization process, the total protein content in NRL, determined by the methods as described in ASTM D3533<sup>10</sup>, it was reduced for more than 72.35% comparing with that in the fresh NRL<sup>5</sup>. Coagulated DNRL films is often used to prepare the blended films by solution-casting technique. Its films presents interesting physical properties in elasticity. The thickness of films was measured at five different areas using a micrometer. Figure 2 show the coagulated DNRL films could form the yellowish transparent films. The physical and mechanical properties of the DNRL blended films are shown in Table 1.



**Figure 2** Coagulated DNRL films by different amount of CaO addition; 0.1, 0.5, 1.0 and 2.0 g of CaO are present in (A), (B), (C) and (D), respectively.

**Table 1** Physical and Mechanical Properties of Coagulated DNRL films

Sample	Component		Properties	
	Deproteinized NRL (g)	CaO (g)	Thickness (mm)	Young Modulus (MPa)
A00	100	-	0.92±0.01	4.55±0.08
A01	100	0.1	1.70±0.02	3.86±0.06
A02	100	0.5	2.52±0.01	3.24±0.02
A03	100	1.0	3.47±0.01	2.39±0.05
A04	100	2.0	3.95±0.02	1.41±0.02

## Discussion

The deproteinized NRL films containing various CaO gave lower modulus values comparing with the control films (A00 as films without CaO). This result indicated that additive CaO provided thickness and softness films. The UTM of the samples with various CaO were lower than that of the control films. UTS of A01

and A02 which contained 0.1 and 0.2 g of CaO, respectively, which suggesting the immiscibility of the components. The physical and mechanical properties of the blended films depended on type and concentration of CaO. From the good elasticity of blended films, they could develop to apply as biomaterial films.



## Conclusion

In this experiment, the blended films of DNRL and various CaO could form appropriate films. The physical and mechanical properties of the blended films depended on type and concentration of CaO. From the good elasticity of blended films, they could develop to apply as the production of a biomaterial of NRL that has been used to replace vessels, esophagus, pericardium, and abdominal wall. Moreover, NRL was also coated with calcium phosphate (Ca/P) and tested for biomedical application. Biomaterials added with Ca/P present biological, chemical, and mechanical properties very similar to the mineral phase of the bone besides the ability to bond to the host tissue. A hemolytic test was performed, and this material did not affect the blood cells, being so ready for animal tests.

## Acknowledgement

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