
Viral impermeability of hypoallergenic, low protein, guayule latex films

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Abstract: Guayule latex proteins do not cross-react with antibodies raised against latex proteins in commercially available products manufactured from *Hevea brasiliensis* latex. Thus guayule latex is a promising raw material for the manufacture of hypoallergenic latex products, safe for use by people suffering from IgE-mediated Type I "latex allergy." Also, guayule latex is a low protein material and therefore unlikely to cause widespread sensitization. Latex products commonly are used as essential barriers against the transmission of disease, and so guayule hypoallergenic latex medical products would be a viable alternative only if they possess effective viral barrier properties. To address this question, fingers of prototype hand-dipped guayule latex examination gloves were tested for their permeability to a

surrogate challenge virus, ϕ X174. This virus has a diameter of 27 nm and is similar in size to the smallest human pathogenic viruses. Prototype guayule latex condom films were tested using synthetic blood over a range of pressures and, after 4 years of storage, with synthetic blood and with the ϕ X174 virus. We concluded that guayule latex films taken from prototype hand-dipped gloves and condoms provide effective barriers to virus transmission and that they remain effective (at least in condoms) after long-term storage. © 1999 John Wiley & Sons, Inc. *J Biomed Mater Res*, 47, 434–437, 1999.

Key words: hypoallergenic latex; latex allergy; latex prototypes; *Parthenium argentatum*; viral barrier properties

INTRODUCTION

Parthenium argentatum (Gray), commonly known as guayule, is a perennial shrub native to the Chihuahuan desert of the United States and Mexico. Guayule produces high molecular weight natural rubber comparable in quality to that currently produced commercially from plantation-grown *Hevea brasiliensis* (the Brazilian rubber tree).^{1,2} The recent widespread occurrence of life-threatening, IgE-mediated "latex allergy" to the residual proteins in currently available latex products makes the development of a safe, alternative source of natural rubber highly desirable.^{3,4} It has been shown that latex allergy sufferers and the latex antibodies do not react to the proteins in guayule rubber latex, which suggests, therefore, that it may be able to supply the required source of safe, hypoallergenic,

natural rubber.^{5–7} Also, guayule latex itself contains very little protein and so is unlikely to cause widespread allergic reactions. Current commercialization efforts are based on the extraction of intact rubber particles from guayule shrub in aqueous suspension and the subsequent purification of these particles as a high quality, low protein, hypoallergenic latex.^{8,9} Although guayule latex has been characterized¹⁰ and prototype latex medical and consumer products have been made (including examination, surgical, industrial, and household gloves, surgical balloons and tubing, catheters, children's balloons, and condoms),¹¹ commercialization has not yet been realized. Guayule would provide a viable alternative only if dipped products made from its hypoallergenic latex, such as gloves and condoms, provide effective viral barrier properties. In this paper, we report on the effectiveness as viral barriers of guayule latex films taken from hand-dipped prototype gloves and condoms.

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MATERIALS AND METHODS

Latex prototypes

Guayule latex was purified from ca. 2,000 kg of fresh weight of branches cut from guayule plants field-grown at

the Maricopa Agricultural Center of the University of Arizona during a production run carried out in the spring of 1994 using a scale-up of a patented procedure.⁸ The shrub was homogenized using a hammer mill with a ¼-inch screen in the presence of aqueous 0.1% sodium sulfite and 1% polyvinyl polypyrrolidone. After filtration, ammonia (as NH₄OH) was added to a concentration of 0.2%. The homogenate was clarified, and the latex purified by three cycles of phase separation followed by four cycles of creaming with ammonium alginate, with the latex being rediluted between each cycle to remove the non-latex components. The purified latex contained 0.2% ammonia, 0.05% ammonium alginate, and 40% rubber on a dry weight basis. The purified latex was stored at 4°C until used.

One-year-old latex successfully was compounded to match the tensile properties of commercially available *H. brasiliensis* latex films, as demonstrated by ASTM test D412-92 (Standard Test Methods for Vulcanized Rubber and Thermoplastic Rubbers and Thermoplastic Elastomers - Tension) using 6-mm Die "C" test dumbbells (Harry F. Bader, Akron Rubber Development Laboratory, Akron, Ohio¹¹), and then it was used to manufacture hand-dipped latex examination gloves and condoms (Fig. 1). The film thickness averaged 132 µm for the examination gloves and 65 µm for the condoms. The products were stored in airtight plastic bags until testing.

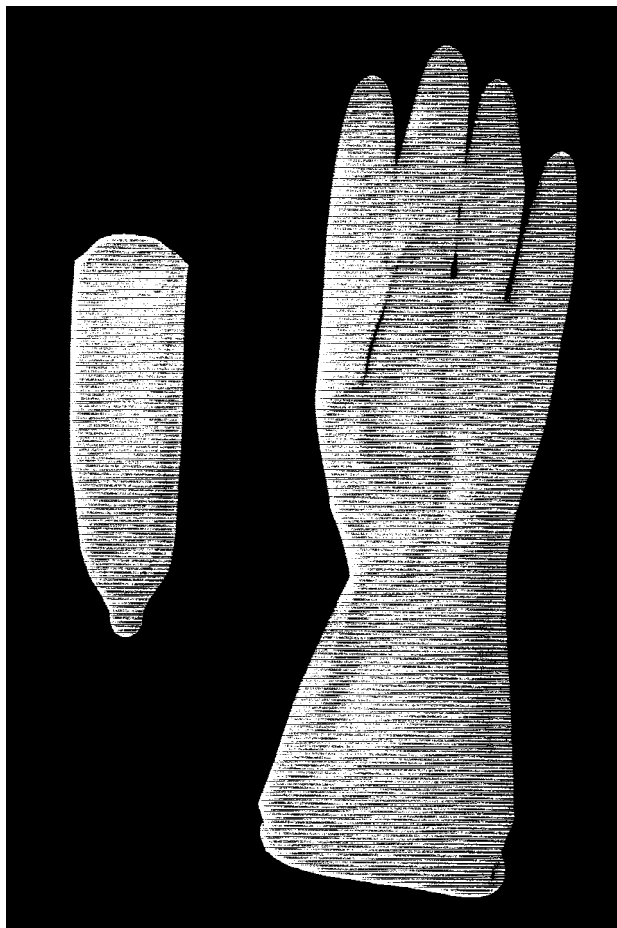


Figure 1. Guayule latex examination glove and condom.

The challenge virus

The bacteriophage ϕ X174 was chosen since at 27 nm in diameter it is as small as the smallest human pathogenic viruses, and it is non-infectious to humans, stable,¹² and has been used effectively as a challenge virus with other *H. brasiliensis* latex products.¹³⁻¹⁵ For comparison, common pathogenic human viruses include hepatitis B (42 nm), human papilloma (45–55 nm), herpes simplex (120–150 nm), cytomegalovirus (120–150 nm),¹⁶ and human immunodeficiency virus (90–130 nm).^{17,18} The virus was assayed biologically (by infectivity) using standard microbiological methods, that is, the standard double agar layer method for coliphage plaque formation with *E. coli* C as the host bacterium.¹⁹

Examination glove testing

Testing was performed on 4-month-old guayule latex examination gloves. Gloves were examined visually, and those with obvious flaws from the hand-dipping process were discarded. A sequential water leak and viral penetration test that had been used previously with *H. brasiliensis* latex and nitrile medical gloves was employed. For the standard 1000-mL water leak portion of the test,²⁰ the whole glove was suspended from the end of a long glass cylinder (7 cm od), filled through the cylinder with 1 L of Dulbecco's phosphate-buffered saline (DPBS, pH 7.0) containing 1.2 to 1.8 $\times 10^6$ pfu/mL of the ϕ X174 challenge virus, and visually inspected for 2 min for obvious leaks, which are visible down to about 300 nL. Gloves (or fingers) with visible leaks, which resulted in virus-containing buffer flowing down onto one or more of the test fingers, were eliminated from the barrier test. Seventeen fingers that were not visibly contaminated were tested for virus penetration. The index, middle, and ring fingers of intact gloves were placed individually into 50-mL plastic centrifuge tubes containing 40 mL of DPBS as collection buffer and left in place for 60 min to collect any virus penetrating each glove finger. The collection buffer was assayed for the presence of ϕ X174. This virus penetration test can detect leaks as small as 33 nL and a single hole down to 2 µm in diameter.

Condom testing

The permeability of 7-month-old guayule latex condoms was determined by a slight modification of ASTM F16 70-95 (Test Method for Resistance of Protective Clothing Materials to Synthetic Blood), using sections cut from the open and closed ends of the condoms. In this procedure, a piece cut from a condom was placed in a test cell to form a 25.6 cm² barrier to pressurized synthetic blood (2.4% Acrysol G110, 1% Direct Red 081 Dye, W.L. Gore and Associates, Elkton, Maryland). The films were restrained from stretching under pressure by organza (polyester) 200–235 µm mesh fabric. Sixty mL of synthetic blood were poured into the chamber of the test cell and pressure was applied to the liquid, using an

attached air line, in 0.5-psi increments to a maximum of 3.5 psi. The condom films were inspected for 1 min at each pressure. This test can detect single holes down to 3–4 μm in diameter and leaks as small as 100 nL.²¹

In a follow-up study, to examine the condom film stability after long-term storage, four untested condoms were examined 4 years after the first tests were performed. The films were tested with both the dye test (ASTM F16 70-95) used in the initial condom tests and with the ϕX174 virus penetration test, a slight modification of the ASTM F16 71-95 (Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using ϕX174 Bacteriophage Penetration as a Test System). This virus penetration test determined whether the ϕX174 virus in the test cell (same set-up as in the dye test) passed through the latex barrier after 1 min at 2 psi by rinsing the view side of the latex film for subsequent collection and assay of any virus that penetrated the barrier. The single hole detection limit of this test is 0.9–1.0 μm and leaks less than 0.1 nL.²¹

RESULTS AND DISCUSSION

Several gloves and condoms were eliminated from the barrier tests because they had visible defects, which was not unexpected because they were all hand-dipped prototypes. It was assumed that additional smaller defects also were present in some samples. Mechanized dipping undoubtedly would improve the uniformity of the finished products. Nonetheless, defect-free sections of the hand-dipped prototypes still would yield pertinent information on the basic barrier impermeability of guayule latex films.

None of the 17 glove fingers from intact examination gloves, as tested with the 1000-mL leak test, allowed virus penetration over 60 min, indicating that the basic guayule latex glove material, when defect-free, was an effective barrier to viral transmission.

The condom films were half the thickness of the examination glove films (65.4 μm and 132.1 μm , respectively). Of the original condoms, six film samples taken from the open ends of the condoms did not leak under any pressure used with the synthetic blood test (Table I). Small localized leaks were observed in three of six films cut from the closed ends of the condoms, one leak being detectable before any pressure was applied. No new leaks occurred at pressures above 2.0 psi. In all, only four small holes were detected in 12 samples totaling 307 cm^2 of hand-dipped guayule latex condom films, showing that most of the basic condom film was impermeable to synthetic blood passage.

Of considerable interest, when four condom films were tested after 4 years of storage, all passed the dye test, and three of the four passed the challenge virus transmission test (which had not been performed on

TABLE I
Number of Leaks Observed in Tests with Pressurized 2% Synthetic Blood of Films Cut From the Open or Closed End of Hand-Dipped Prototype Guayule Latex Condoms

Film Type	Condom Number	Pressure (psi)							
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Open end	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0
Closed end	1	0	0	1	1	2	2	2	2
	2	0	0	0	0	0	0	0	0
	3	0	0	0	1	1	1	1	1
	4	0	0	0	0	0	0	0	0
	5	1	1	1	1	1	1	1	1
	6	0	0	0	0	0	0	0	0

Pressure was increased in 0.5-psi increments and left 1 min at each level for visual detection of leaks.

the condoms during the initial round of testing). The sample that failed the virus test (from the closed end of a condom) allowed 0.4 nL to penetrate, indicative of a hole < 1.3 μm in diameter. Thus the permeability of the condoms did not degrade significantly in 4 years.

Although we have only anecdotal evidence on the physical feel of the prototype products, the more than twenty people who have tried the gloves found their tactile properties attractive. Also, we observed that glove films could not be cut with scissors (DuraSharp, 1000 stainless, Viking Office Products, Los Angeles, California); the films bent around the scissor blades and had to be well stretched before cutting could be achieved. This property was observed both in the fresh gloves and after 4 years of storage, suggesting improved break resistance over currently available products. These properties also may lead to enhanced performance if guayule latex is used to produce surgical gloves.

CONCLUSIONS

Our data show that most tested samples (three of four condom samples and 17 of 17 glove samples) showed no virus penetration. Thus guayule latex film prototype examination gloves and condoms effectively can prevent virus passage if no hand-dipping defects are present, and they retain this effectiveness even during long-term storage. With refinement of the manufacturing process, the quality level of the finished products undoubtedly will rise, which would make guayule latex an effective new natural rubber material for use as a barrier in condoms and gloves.

Hypoallergenic guayule latex, then, could supply an important natural rubber market in which *H. brasiliensis* latex cannot safely be used, that is, for hypoallergenic latex products.

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