

# All Natural Anti-microbials for Oral Care Products

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## Summary

**Problem:** Nearly half of American adults have a mild, moderate, or severe form of gum disease which can lead to tooth loss and is strongly associated with other chronic diseases such as diabetes and heart disease (Figure 1).<sup>1</sup> Current oral care hygiene results in over *one-fourth of Americans losing all their teeth*.<sup>2</sup> Annually, \$54 billion in lost productivity can be attributed to gum disease.<sup>3</sup>

Robert Genco, DDS, PhD, researcher and past president of the American Association for Dental Research stated that, “periodontal disease is one of the most prevalent non-communicable chronic diseases in our population, similar to cardiovascular disease and diabetes.”

Moreover, consumers demand for natural oral care products is increasing.<sup>4</sup> Each year, they purchase tens of millions of natural or cosmetic brands like Toms of Maine and Therabreath.<sup>5</sup> However, these leading brands do little to reduce the bacteria that causes periodontal disease.<sup>6</sup> **The combination of 1) ineffective natural products and 2) a demand for natural products has little effect on reducing the stunningly high prevalence of periodontal disease.**

**Current Solutions:** Many over-the-counter mouthwashes carry an antigingivitis/antiplaque indication and help control gum disease.<sup>7</sup> These contain 22% *alcohol* (Listerine) and *cetylpyridinium chloride* (Scope). However, none are completely natural, and many people don't like the burning sensation of alcohol. Some parents fear oral rinses due to confirmed reports of children being harmed or even dying from mouthwash intoxication.<sup>8</sup> Because of this, consumers feel all-natural products are safer and gentler resulting in increased demand.<sup>9</sup>

Currently, no all-natural mouthwashes carry an antigingivitis indication or an ADA Accepted label.<sup>10</sup> Testing in our laboratory shows that commercially available **natural oral care products have about as much anti-bacterial efficacy as water**.<sup>11</sup>

## Innovation

Our company, Lokena Technologies, has developed a patent pending<sup>12</sup> mouthwash that is **orders of magnitude more effective at reducing oral pathogens than currently commercially available natural**

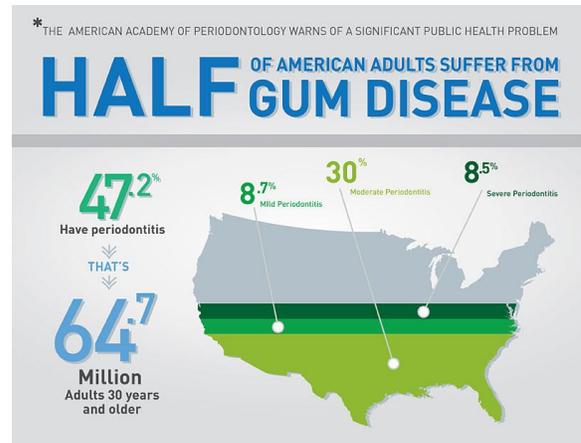


Figure 1: Prevalence of Periodontitis in Adults in the United States<sup>1</sup>

Water, Pomegranate extract, Sorbitan monolaurate, Benzoic Acid, Sodium benzoate, Sorbitol, Xylitol, Grapefruit Seed Extract, Zemea, Glycerin, Plantasol (caprilylcapryl glucoside), Thymol, Menthol, Peppermint oil, Methyl Salicylate, Spearmint, Eucalyptol



**Figure 2:** (left) List of ingredients. (right) Lokena all-natural oral rinse. Some natural color has been added to this batch. With no color added the mouthwash is completely clear.

**and cosmetic mouthwashes** as can be seen in the studies section below. Moreover, it is equally or more effective than over-the-counter mouthwashes. In short, this groundbreaking formulation has the potential to be the first all-natural mouthwash that meets FDA requirements for antimicrobial efficacy.

Lokena Mouthwash has the strength of synthetic chemical mouthwashes without the real or perceived safety concerns. It doesn't burn or dry the mouth because it doesn't contain alcohol. It is refreshing, tastes great, and freshens breath. Initial user feedback shows that it lasts overnight and keeps breath fresh overnight.

This oral rinse contains a synergistic combination of antimicrobial and anti-inflammatory essential oils and plant extracts (Figure 2). It looks clean and is clear (Figure 3). The formulation has been developed and used by its developers for over a year.

**Background:** The antibacterial mouthwash originated in Dr. Shanta Modak's lab at Columbia University. Dr. Modak has been elected as a Fellow of the National Academy of Inventors. The Modak lab has produced over 40 patents related to infection control over the last 35 years and some of these innovations are now FDA cleared and are in use in hospitals across the country.<sup>13</sup>

After successfully creating formulas to kill bacteria in skin wounds, the Modak lab began to develop botanical alternatives for oral care. The oral care technology has progressed over the past 4 years through multiple iterations. Nearly \$400,000 in research funding has been applied toward perfecting the formula. Dr. Chaturanga De Silva, was instrumental in the development of the formulation during his doctoral and post-doctoral work at Columbia University.

The patent pending formulation has been licensed from Columbia University to Lokena Technologies which is led by Joseph May and supported by Ben Rollins, both serial entrepreneurs.



Figure 3: Compared to other essential oil-based mouthwashes, Lokena is effective, completely clear and solubilized.

**Reducing Periodontal Disease:** The potential for a natural and effective oral rinse may play a significant role in reducing periodontal disease because it addresses the needs of a consumer that will not use the current products. Furthermore, its development will lead to other natural oral care products such as toothpaste, mints, gum, and even periodontal mouth bandages for serious gum disease.

**Mouthwash Market:** The mouthwash market size in the U.S. is **\$1.8B** with a projected annual growth of 3.3%. Alcohol based antiseptic rinses make up 42%, followed by non-alcohol based antiseptics with 19% and Fluoride-based with 24% (Figure 4).<sup>6</sup>

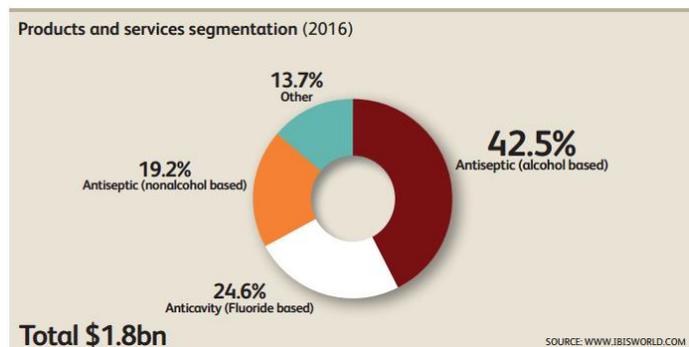


Figure 4: Comparison of mouthwash types

Studies have been carried out and earlier versions of the mouthwash were published in the Journal of Applied Science and Technology.<sup>14</sup> The Federal Register for Oral Health Care Products (21 CFR Part 356) recommends that over-the-counter antigingivitis products demonstrate their effectiveness with a 20-second Kill-Time study (ASTM E2783) and a Plaque Glycolysis and Regrowth Model study (PGRM).

Lokena has demonstrated impressive efficacy of its mouthwash by performing the Kill-Time study against many of the challenge microbials recommended in the Federal Register and the American Dental Association program for gingivitis product testing.<sup>15</sup>

## Challenge Microbials

Microbials	Internally Tested Microbials	Representative Microbials per Federal Register	Representative Microbials for ADA Acceptance Program	Gram positive / negative	Type
Actinomyces viscosus		x	x	Gram-positive	facultative aerobe
Candida albicans	x	x		Gram-positive	fungus
Fusobacterium nucleatum		x	x	Gram-negative	anaerobe
Porphyromonas gingivalis*	x	x	x	Gram-negative	anaerobe
Prevotella intermedium		x	x	Gram-negative	anaerobe
Streptococcus mutans	x	x		Gram-positive	facultative Catalase +ve
Tannerella forsythia		x	x	Gram-negative	anaerobe
Actinomyces naeslundii			x	Gram-positive	facultative aerobe
A. actinomycetemcomitans*	x		x	Gram-negative	facultative anaerobe
Bacteroides loescheii			x	Gram-negative	anaerobes
Campylobacter rectus			x	Gram-negative	facultative anaerobe
Eikenella corrodens			x	Gram-negative	facultative anaerobe
Pseudomonas aeruginosa	x		x	Gram-negative	facultative anaerobe
Staphylococcus aureus	x		x	Gram-positive	facultative Catalase +ve
Treponema denticola			x	Gram-negative	anaerobes
Wolinella recta			x	Gram-negative	facultative anaerobe
Enterobacter aerogenes	x			Gram-negative	facultative Catalase +ve
Acinetobacter baumannii	x			Gram-negative	aerobe
MRSA	x			Gram-positive	facultative Catalase +ve

Figure 5: Challenge microbials tested internally (second column), microbials recommended for testing per the Federal Register (3rd column), microbials recommended for testing in the ADA Acceptance program testing (fourth column).

**Kill-Time Study:** The Kill-Time study measures the antimicrobial activity of the mouthwash at a specific point in time. In general, the 3 Log<sub>10</sub> reduction is considered the minimum level of performance that would indicate a product has substantive killing activity versus a particular microorganism.<sup>16</sup> Anything less indicates that relatively huge numbers of the microorganisms remain viable after treatment with the product – for example, a 1 Log<sub>10</sub> reduction in a population of one million bacteria (a small number, where bacterial contamination is concerned) means that 100,000 bacteria remain.

As can be seen in Figure 6, the Lokena mouthwash demonstrated *more than a 3 Log<sub>10</sub> reduction* for the challenge microbials. As a comparison, TOMS, TheraBreath, and PeriActive had little to no effect on the challenge microbials. ***Lokena was 1,000 to 10,000 times more effective than the leading natural mouthwashes.***

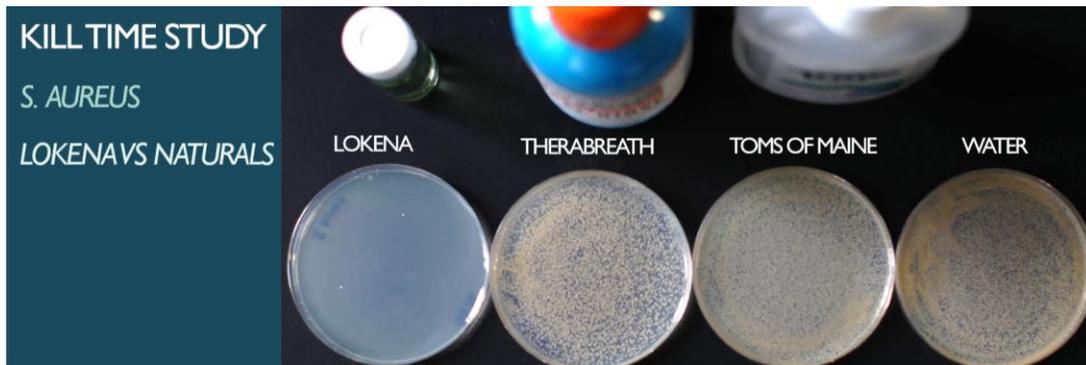
Companies like TheraBreath publicly market “that anaerobic bacteria are the primary cause of bad breath due to their ability to consume vast amounts of proteins and excrete foul-smelling volatile sulfur compounds as a result of rapidly digesting the proteins in the mouth.” However, their products do relatively nothing to effectively reduce the referenced bacteria.



**Figure 6:** Kill-Time study demonstrating the reduction of bacteria by Lokena Mouthwash. TheraBreath is a cosmetic oral rinse. TOMS and PeriActive are all-natural oral rinses that show little effectiveness.

These tests are carried out in triplicates and repeated for at least 6 tests for each mouthwash and microbial. Figure 6 shows the average and standard deviation of the 6 samples.

While the data results in the graph are impressive, the following images (Figure 7) really show how little reduction in bacteria occurs with other all-natural products. The rapid kill studies (ASTM-2783) were conducted and photographed for oral pathogens such as *S. aureus* and *P. aeruginosa*. Each visible dot on the plate represents a colony of bacteria.

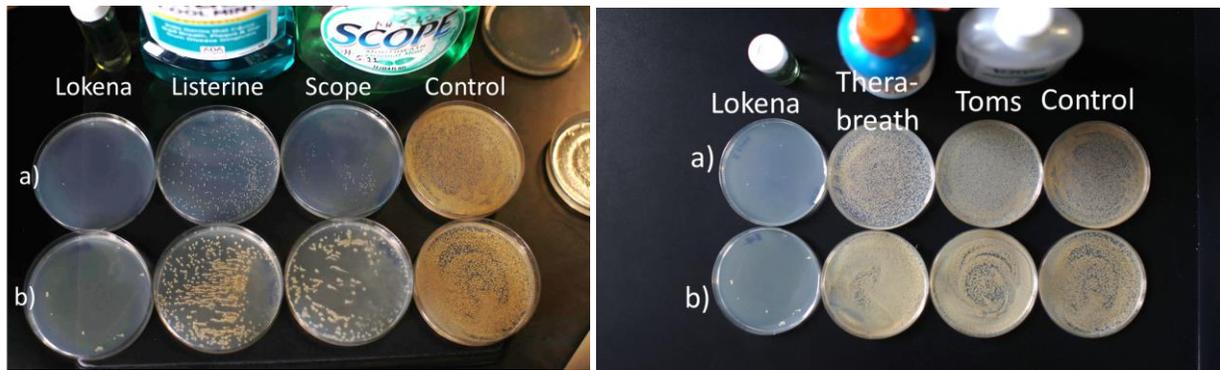




**Figure 7:** The images above show the results of the kill time study (ASTM-2783). The top two compare the leading natural mouthwashes and the bottom two images compare synthetic mouthwashes. For this study, 108 CFU per ml microbial cultures were prepared in appropriate media. 0.9 ml oral rinse is kept in contact with 0.1 ml microbial cultures for 20 seconds in culture tubes and subsequently neutralized by adding 9 ml of inactivating media. The samples are subsequently plated and incubated overnight for bacterial growth.

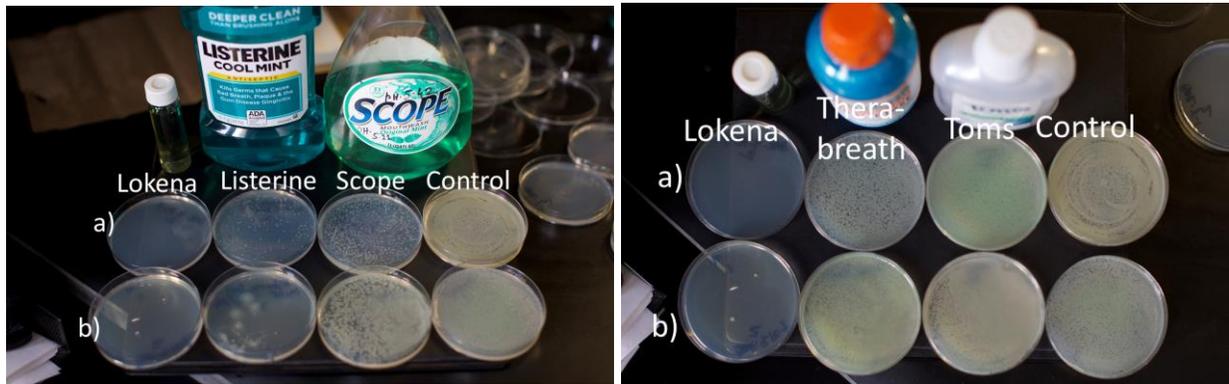
**Study of the Efficacy of Residual Oral Rinse on Colonized bacteria:** We also simulated the effect of oral rinse remaining on the gum and teeth on colonized bacteria. Bacteria colonized on the agar plate for 4 hours in an incubator and were then exposed to 1 ml of oral rinse by spreading it on the plate for 1 minute. The oral rinse on the agar plate was then inactivated with drug neutralizing fluid and then these fluid samples were plated and incubated at 37°C. The top agar plates in the Figures 8 and 9 represent the bacteria that is tightly held to the teeth after 1 minute of rinsing the mouth and the bottom plates represent the bacteria that is loosely held to the teeth after rinsing. As can be seen from the images, natural options are not effective.

### Challenge Microbial: *S. aureus*



**Figure 8:** Visual representation of Time-Kill of a) strongly bound bacteria and b) loosely bound bacteria on the surface of the agar plate. In all cases tested, Lokena shows significantly higher efficacy than competing brands. Test organism *S. aureus*.

### Challenge Microbial: *P. aeruginosa*



**Figure 9:** Visual representation of Time-Kill of a) strongly bound bacteria and b) loosely bound bacteria on the surface of the agar plate. In all cases tested, Lokena shows significantly higher efficacy than competing brands. Test organism *P. aeruginosa*.

**Figure 10:** Lokena has many advantages over the leading natural mouthwash products.

### Product Extensions

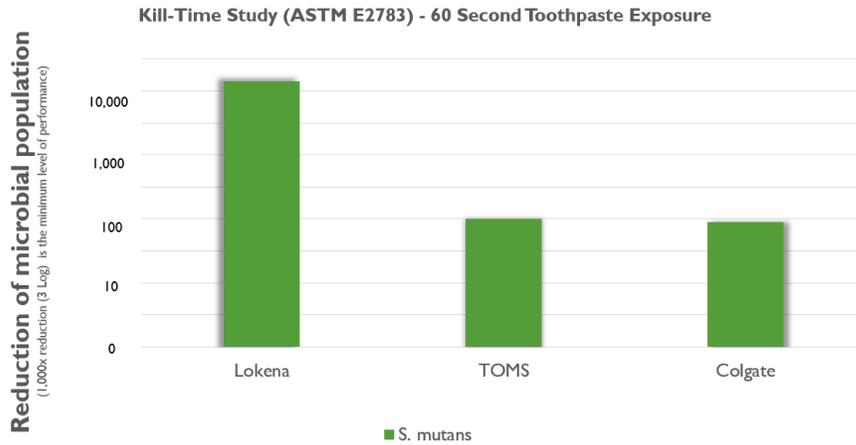
The anti-bacterial formula has many applications beyond mouthwash.

- **Toothpaste:** The formula can be applied to toothpaste. This would be the first toothpaste of any kind that effectively reduces *S. Mutans*, the leading cause of cavities.
- **Mints/Gum:** The formula can be used in mints that actually kill bad breath germs, rather than just cover them up.
- **Hand Sanitizer / Soaps / Wound Care:** The Modak lab has show efficacy of other natural personal care products. Data is available upon request.

### Toothpaste

Decades of research have conclusively demonstrated that *Streptococcus mutans* (also known as "*S. mutans*") is the major contributor to cavities.<sup>17</sup>

If this is the case, then why don't today's toothpastes eliminate *S mutans*? Even Colgate which contains the chemical Triclosan only has a 2 Log10 (100x) reduction of the bacteria. Lokena, with its all natural formula has a 10,000 times reduction, which is above FDA standards and significantly higher than other pastes.



## Summary

Nearly half of American adults have Gum disease. Currently, over a quarter of Americans ages 65 and older *have lost all their teeth*. Oral care must improve.

Consumer demand for natural oral care products is increasing; however, these natural and cosmetic mouthwash products have little effect at reducing bacteria that cause gum disease.

Lokena is a novel, non-alcohol, 100% natural oral rinse that reduces gum disease. It performs as good as, or better than, over-the-counter drug and alcohol-based oral rinses and doesn't burn. Lokena toothpaste kills 100x more cavity-causing bacteria than the leading brands and it contains all-natural ingredients.

Nothing like this exists on the market today.

## Bibliography

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- <sup>6</sup> See preliminary study section of this proposal
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- <sup>10</sup> Based on an internal review of major retailers of mouthwash including Walgreens.com, Amazon.com, Walmart.com. Completed 1/2/2018
- <sup>11</sup> See preliminary studies section in this proposal.
- <sup>12</sup> Modak, Shanta M, et al. *Botanical Antimicrobial Compositions*.
- <sup>13</sup> Links to products currently available on the market that were developed at the Modak laboratory: <http://www.goremedical.com/products/dualmesh> , [www.bd.com/documents/.../IP\\_Surgicept-waterless-surgical-hand-scrub\\_BR.pdf](http://www.bd.com/documents/.../IP_Surgicept-waterless-surgical-hand-scrub_BR.pdf), <https://www.teleflex.com/usa/product-areas/vascular-access/vascular-access-catheters/arrow-ergopack-system/infection-control/arrowgard-antimicrobial-technology/>
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