



Amnion-Chorion Membrane Products: Processing Impacts on Antimicrobial Properties

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Introduction

Methods developed within the past few decades have made it possible to create shelf-stable amnion-chorion membrane products, allowing these products to be generally available at reasonable costs. Processing methods vary, however, in how these tissues are washed/rinsed and how they are dehydrated, leading to products with different physical and biological properties. This study aimed to compare three commercially available amnion-chorion membrane products available in the dental/maxillofacial market to elucidate how distinct tissue processing methods affect the final composition of the product.

Washing/Rinsing Methods

Saline-based Solutions: Salt-based solutions and biologically compatible buffers (e.g., PBS) are employed to rinse tissues contaminated with blood. The ionic strength and pH of these solutions can be tuned to optimize the removal of clots, blood, and undesired contaminants in preparation for the dehydration process.

Detergent-based Solutions: Mild detergents, specifically non-ionic detergents, can be used to increase “cleaning” strength to remove clots, blood, and undesired contaminants. Detergent washes are also be used to disrupt cells within tissues, making it possible effectively decellularize the tissues in preparation for the dehydration process.

Dehydration Methods

Lyophilization (freeze-drying): Cleaned tissues are first frozen and then put into a vacuum chamber. The vacuum has effect of pulling the water out of the tissue through a process called sublimation in which water goes directly from a solid form to a gas. Because the tissue is frozen solid, it doesn’t shrink down as the water gets pulled out, leaving behind voids (air pockets) in the tissue where the ice crystals had been.

Heat + Low Pressure (vacuum oven): Cleaned tissues are placed in a chamber in which the temperature is increased to promote drying. By applying a slight vacuum to the chamber, complete drying can occur without elevating the temperature to a level that will damage (cook) the tissue. As water leaves the tissue, the extracellular matrix collapses, and the membrane becomes thinner because there is no water to add volume to the tissue.

Three commercially available membrane products were obtained, each created with distinct processing methods, and evaluated for their antimicrobial effect on bacteria associated with periodontal disease using a Bacterial Inhibition Assay (described in the box to the right).

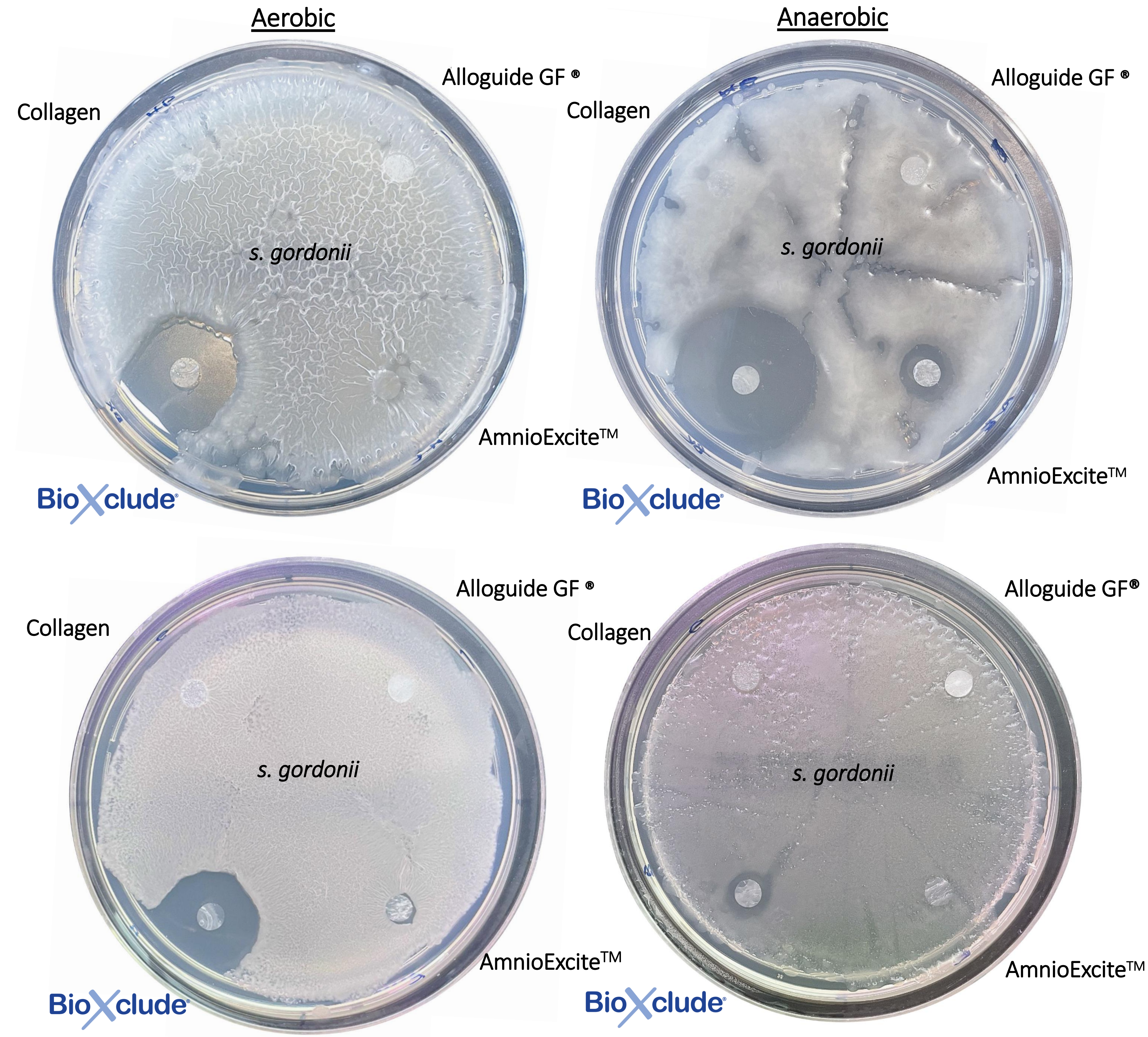
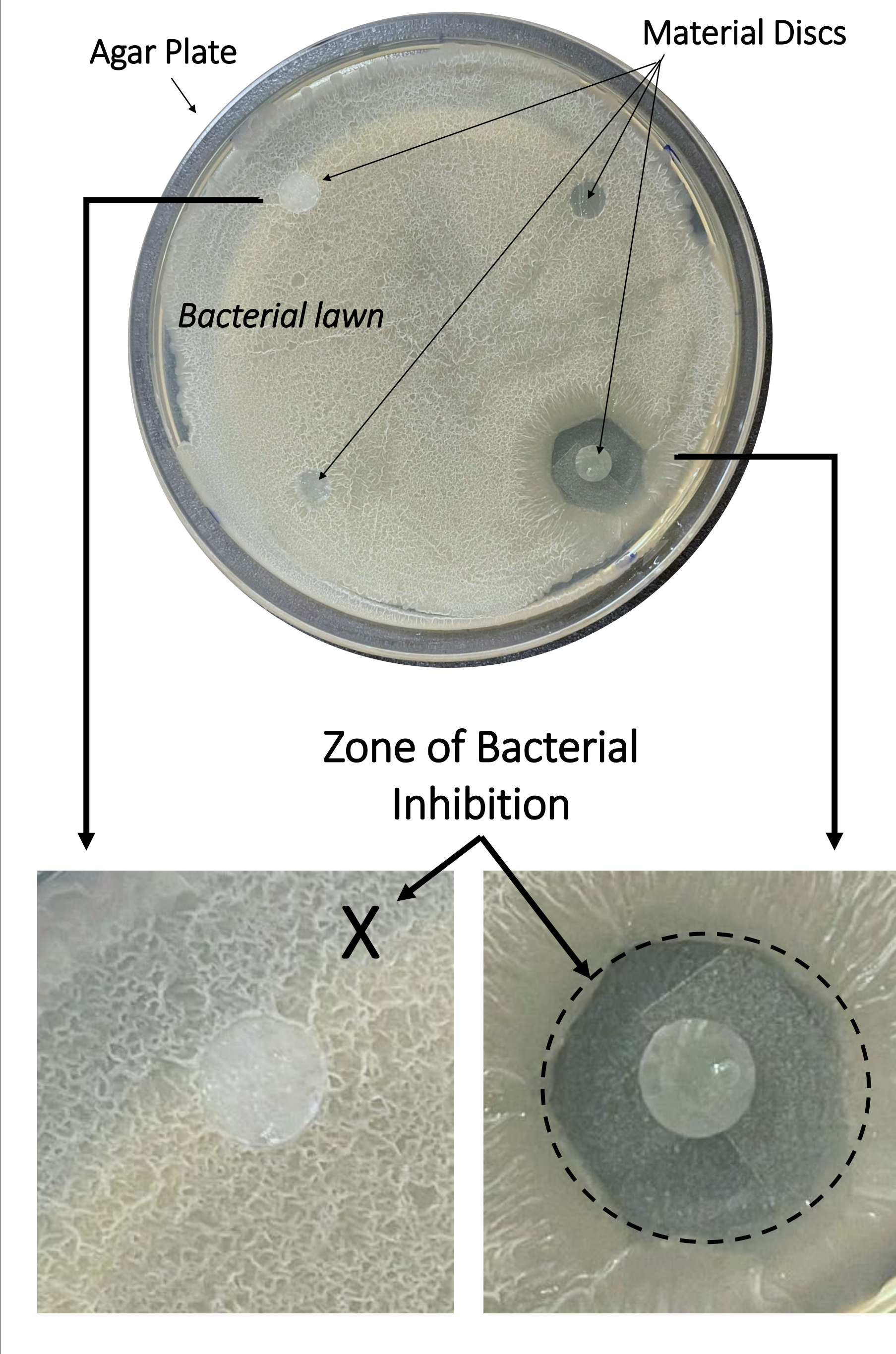
BioXclude® (Snoasis Medical): Tissues are processed using a patented process in which the amnion and chorion layers are separated to facilitate saline-based wash steps and de-epithelialization of the amnion layer. The amnion and chorion layers are reassembled before dehydration using a heat + low pressure method.

AmnioExcite™ (Salvin Dental): Amnion and chorion layers kept together through washing steps that employ a patented process in which detergents are used to completely decellularize the tissues. Cleaned membranes are then dehydrated by lyophilization.

Alloguide® GF (Ace Surgical): Amnion and chorion layers kept together through washing steps that include proprietary freeze-drying solution. Cleaned membranes are then dehydrated by lyophilization.

Bacterial Inhibition Assay

Bacterial Inhibition Assays are performed to evaluate the ability of materials to prevent the growth of bacteria. Briefly, bacteria are spread evenly across the surface of an agar growth plate followed by the placement of material discs over the top. The plates are then incubated to allow growth (propagation) of the seeded bacteria, which ultimately results in the bacteria forming a visible “lawn” on the surface. Where growth of the bacteria is inhibited, a clear “Zone of Inhibition” can be observed where the “lawn” is absent (see below).



Methods

Streptococcus gordonii, (ATCC 35105), a pathogenic strain of bacteria associated with periodontal disease, was grown in BHI broth (Fisher, cat#EBT0061) and then spread evenly on BHI agar plates (Fisher, cat#B21569X). 5mm diameter discs of each membrane product were created with tissue biopsy punches and placed, chorion side down, on the surface of the seeded agar plates. Discs created from Neodent Membrane Flex (NMF) (Collagen Matrix, Inc.), a collagen membrane with no known antimicrobial properties, was used as a negative control. Plates were then incubated for 48 hours at room temperature (top plates) or overnight at 37°C (bottom plates) in either normal atmospheric conditions (aerobic, plates on right) or in a 2.5L AnaeroPack chamber (Fisher, cat#23246385) containing an Anaerobic Gas Generator pack (Fisher, cat# 23246376) to create an anaerobic environment (right).

Results

A clear “Zone of Inhibition” can be observed around the BioXclude discs (and AmnioExcite discs, but to a lesser extent), indicating that the membrane contains soluble factors that prevent bacterial growth, even at a distance from the membrane. Alloguide and NMF Collagen discs did not shown any evidence of limiting the growth of *S. gordonii* in this assay. No underlying processing step was specifically identified to account for these observed differences.

Conclusion

BioXclude®’s ability to inhibit *S. gordonii* far exceeds that of AmnioExcite™ and Alloguide GF®, indicating a beneficial effect unique to the patented process used to make BioXclude®.