PILOT EVALUATION OF A SIMPLE ADJUNCTIVE METHOD FOR IMPROVED REMOVAL OF ORAL BIOFILM DURING CONVENTIONAL SCALING AND ROOT PLANING THERAPY

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Various studies have evaluated the adjunctive use of chemical and antimicrobial treatments to assist in the mechanical removal of oral microbial biofilm from tissue surfaces during scaling and root planning therapy (SRP). The current study demonstrates the elimination of two classes of surrogate molecular markers from periodontal disease sites. This suggests the current agent may be a more effective adjunctive cleansing agent for complete biofilm removal. A patient with advanced chronic periodontitis was subjected to standard SRP therapy, supplemented by irrigation with HYBENX® (HBX). Samples of gingival crevicular fluid were collected with triplicate absorbent paper points from each of three quadrants at three time points: 1) at baseline prior to treatment; 2) after irrigation with the topical agent for 20 seconds and rinsing; and 3) after SRP followed by a second irrigation/rinsing treatment with the agent. Paper points were extracted to assay the presence of 13 bacterial species known to be primarily associated with periodontal disease using DNA pyrosequencing. In addition, the presence of Matrix Metalloproteinase-8 (MMP8), as well as IL-1β, IL-6, and TNF-alpha were also assessed by immunoassay of the paper point sample extracts. The combined adjunctive treatment indicated a complete absence of detectable bacterial DNA and the four inflammatory mediators from samples taken from the gingival sulci treated with HBX. The advantage of the current adjunctive topical treatment technique is that it is simple and easy to administer in conjunction with standard SRP techniques. It appears to provide a level of cleanliness not currently achieved with other SRP adjunctive procedures.

The importance of the role of the pathogenic oral microbial biofilm in the etiology of acute and chronic periodontal disease has been well established (1, 2). Molecular biofilm components have been determined to be a critical factor in the etiology of the pathological immune disease response ranging from mild gingivitis and mucositis to severe periodontal disease and peri-implantitis (2-4). Modern global dental standards of care mandate therapy to debride biofilm via mechanical scaling and root planing (SRP) in order to manage oral disease. Despite the use of meticulous procedures and more recent addition of various adjunctive components, such as antibiotics and rinsing agents, significant resolution
of long-term disease remains elusive. SRP provides transient diminishment of clinical pathology and temporary symptomatic relief. However, critical molecular biofilm residue remains after repeated SRP debridement causing virulent biofilm to redevelop and trigger chronic disease, often with the development of new antibiotic-resistant persister cells. A more effective debridement approach is needed that will enable complete removal of the residual molecular biofilm during SRP. The purpose of the current preliminary study was to assess the potential of a new liquid topical debridement agent, HYBENX® Oral Decontaminant (HBX), to achieve more fastidious cleaning by assessing the diminishment of molecular markers in the gingival sulcus. Based on the results of these assays, we describe a simple, non-invasive, inexpensive, fast, and safe adjunctive treatment method with the potential to remove residual biofilm from oral tissue surfaces more effectively.

MATERIALS AND METHODS

Patient treatment
A single quadrant of a 60-year-old male patient with chronic severe periodontitis was subjected to a 20-second irrigation with HBX followed by rinsing and subsequent standard SRP therapy combined with a second adjunctive 20-second HBX irrigation/rinsing procedure. Samples of gingival crevicular fluid were collected in triplicate with absorbent paper points in each of the three deepest pocket locations within one quadrant at three time points: 1) baseline prior to treatment; 2) after the first HBX irrigation; and 3) after SRP and a second HBX irrigation.

Molecular marker analysis
Paper points were pooled and an extract of the paper points performed and subjected to both 16s bacterial DNA analysis, using PCR amplification, and Luminex® Screening Immunoassay by two separate independent laboratories. The bacterial DNA assay assessed the presence of the primary complex of 13 bacteria species known to be associated with periodontal disease. Testing was performed by Oral DNA Labs (Eden Prairie, MN, USA) using methods modified from ‘Microbiological Goals of Periodontal Therapy’ (5). The immunoassay assessed the presence of a primary known periodontal disease immune modulator marker protein, Matrix Metalloproteinase-8 (MMP8), as well as the additional inflammatory markers IL-1ß, IL-6, and TNF-alpha. Immunoassays were performed by R&D Systems (Minneapolis, MN, USA) using the Human Luminex Screening Assay as described by Reis et al. (6).

RESULTS

The baseline sample yielded a significant presence of multi-species periodontal pathogen bacterial DNA (Fig. 1A). In addition, significant levels of the four immune modulators were also found in the pooled baseline sample (Fig. 1B). The second time point sample yielded a marked reduction in the levels of key periodontal pathogen bacterial DNA after the first irrigation with HBX solution Fig. 1C). Similarly, the level of inflammatory markers in that second sample was reduced to the limits of statistical non-detectability after this first HBX irrigation (Fig. 1B). The final time point yielded a further reduction of bacterial DNA to levels below the DNA detection limit after treatment with SRP and a second HBX irrigation (Fig. 1D). The immune modulators remained below detectable limits after the first HBX irrigation. The combined adjunctive HBX/SRP/HBX treatment indicated a complete absence of detectable bacterial DNA and the four inflammatory mediators from samples taken from the gingival sulci.

DISCUSSION
The correlation between the etiological contribution of microorganisms released from the Oral Microbial Biofilm and dozens of acute and chronic disease conditions throughout the body is becoming overwhelming (1). Better removal of oral biofilm holds the promise to expand dental benefits well beyond infection control in oral disease. Mechanical debridement of the periodontal environment, along with the use of various adjunctive antimicrobial agents, is a key concept in currently accepted methods to control periodontal disease. However, the limitation of these approaches in certain cases is becoming apparent with the advent of genetic and other molecular analysis methods to better assess the persistence of microbial biofilms with respect to non-cultivatable microbes and protein inflammatory mediators. Without complete eradication of the biofilm, chronic periodontitis persists. Repeated antibiotic therapy leads to worsening community health scenarios caused by persistent antimicrobial resistance. No current combination oral treatment
disease conditions such as chronic periodontitis (3, 4, 8).

We report here the potential for HYBENX® Oral Tissue Decontaminant solution to be used as an effective molecular cleansing adjunct in combination with conventional SRP techniques. Application of the simple solution quickly and completely denatures organic molecular biofilm components. This disrupts the molecular attachment mechanisms of the biofilm and easily enables mechanical removal of the matrix components by simple conventional rinsing action. This agent appears to be an effective in the destruction and removal of residual components from tissue surfaces at a molecular level. It facilitates physical removal and local cleansing of the tissue surface method has proven safe and effective in the complete removal of the molecular biofilm matrix components. Even microbial resistance to chlorhexidine is becoming evident (7).

In order to develop new more fastidious methods of removing biofilm at the molecular level, more precise measures must be used to assess biofilm removal during cleaning. A proven candidate has become the assessment of 16s bacterial DNA as an improved surrogate over older inadequate culture-based methods for assessing surviving biofilm microorganisms. In addition, recent studies suggest that immunoassay of molecular inflammatory markers such as matrix metalloproteinases, especially MMP-8, are a more relevant indicator of the extent of oral

**Fig. 1.** A) Baseline 16s DNA Detected Before HYBENX Treatment. Aa = Aggregatibacter actinomycetemcomitans; Pg = Porphyromonas gingivalis; Tf = Tannerella forsythia; Td = Treponema denticola; Cr = Campylobacter rectus; En = Eubacterium nodatum; Fn = Fusobacterium nucleatum/periodonticum; Pm = Peptostreptococcus (Micromonas) micros; Pl = Prevotella intermedia; Cs = Capnocytophaga sp. (gingivalis, ochracea, sputigena); Ec = Eikenella corrodens

B) Evaluation of biomarkers in paper point samples collected pre and post HYBENX irrigation treatment.
- Collection Set 1 (C1): Pooled paper points eluted with 150 μL of PBS, assayed at a 1:2 dilution.
- Collection Set 2 (C2): Pooled paper points eluted with 50 μL of PBS, assayed at a 1:6 dilution (Due to the 1:6 dilution, IL-6 and TNF-α were not detectable).

**C**) 16s DNA Detected After HYBENX Treatment.

**D**) 16s DNA Detected After HBX/SRP/HBX Treatment.

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of biofilm microorganisms. This could prevent the opportunity for microbial resistance to emerge. In addition, the primary inflammatory mediators in periodontal disease are destroyed.

This initial pilot study correlates well with other emerging clinical papers, which indicate improved clinical debridement procedures and greater patient comfort when HYBENX solution is used in an adjunctive procedure (9-11).

Conflict of interest: compensated by EPIEN Medical, Inc., Saint Paul, MN, USA.

REFERENCES