Periodontal Pathogens and Reactivation of Latent HIV Infection: A Review of the Literature
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Introduction
According to the World Health Organization, approximately 34 million people are infected with the human immunodeficiency virus (HIV), and although there have been many advances in HIV research in recent years, including therapies such as highly active antiretroviral therapy, this disease still constitutes one of the most significant public health problems in the world.1 In the U.S. alone, the Center for Disease Control (CDC) estimates the number to reach 1.2 million in 2012.2 Infection with HIV interferes with the immune system as a result of the virus’s ability to infect cells of the immune system, such as helper T-cells (specifically CD4+ T-cells), macrophages and dendritic cells.3-5 Once past this acute phase of the infection, HIV replicates at very low levels for the next 8 to 10 years. One of the difficulties in treating HIV infection stems from its ability to remain latent within these CD4+ T-cells, which function as memory cells and remain in the body for years.4 People who have a compromised immune system caused by HIV are more highly susceptible to other infections, including periodontal disease.6

According to the CDC, 75 to 90% of American adults have some form of periodontal disease (gingivitis or periodontitis).2,6 Periodontal disease, whose primary etiology is bacterial biofilm, including Porphyromonas gingivalis, causes a chronic inflammatory response by the release of bacterial and host cell products.3 P. gingivalis has previously been shown to be a significant risk factor for many systemic diseases, including heart disease, diabetes and low birth-weight, among others.6 Recent studies suggest that bacteria found in the oral cavity, including P. gingivalis, have the ability to reactivate the latent HIV virus within infected cells.3-5 A 2009 study suggests that P. gingivalis produces a fatty acid called butyric acid, which may induce reactivation of the latent HIV-1 virus.3

Abstract
Purpose: Infection by the human immunodeficiency virus (HIV) causes the host to have a compromised immune system due to the virus’s ability to infect cells of the immune system, such as helper T-cells (specifically CD4+ T-cells), macrophages and dendritic cells. HIV remains latent within these cells, which function as memory cells and remains in the body for years. People who have a compromised immune system caused by HIV are more highly susceptible to other infections, including periodontal disease. Until recently, very little attention has been given to the potential interactions between chronic oral infections, such as periodontal disease and latent HIV reactivation/upregulation. This review focuses on the literature available between 2009 and 2011, evaluating the potential link between bacterial infections, including oral infections caused by periodontal pathogens, the reactivation of latent HIV leading to the potential failure of highly active antiretroviral therapy and acquired immunodeficiency syndrome (AIDS) progression. It has been hypothesized that infections by periodontal pathogens can stimulate reactivation of HIV-latently infected cells. Studies showed that soluble factors produced in response to periodontal pathogens by gingival cells could be indirect contributors to HIV-1 promoter activation. It was also found that the oral bacteria stimulated the HIV promoter activation in a dose-dependent and time-dependent manner. While these preliminary studies present a potential link between oral periodontal pathogens and HIV reactivation, additional clinical and epidemiological studies are needed to clarify the causal link and mechanisms of HIV latency reactivation associated with oral pathogens.

Keywords: Periodontal disease, HIV latency, HIV reactivation, AIDS, oral bacteria
This study supports the NDHRA priority area, Clinical Dental Hygiene Care: Assess how dental hygienists are using emerging science throughout the dental hygiene process of care.
Since HIV infection is considered pandemic with over 34 million people worldwide living with the disease,\(^1\) it is highly presumable that dental hygienists are encountering patients in their practices at all stages of infection. A large component of patient education provided by a dental hygienist consists of explaining the link between systemic disease and periodontal disease. Thus, for patients who have contracted HIV, discussing the possible link between HIV reactivation and periodontal pathogens could result in earlier risk identification and better control and prevention of disease progression.\(^7,8\) Therefore, the purpose of this review is to look at the current literature available on the link between infection by oral pathogens and the reactivation of latent HIV virus leading to possible risk identification for prevention and control of disease progression.

**Target Cells for HIV Infection and Latency**

HIV can infect and integrate into dendritic cells, monocytes/macrophages and CD4+ T-cells, thus causing them to be long-lived reservoirs of the latent virus.\(^3,5,9\) This pool of infected host cells is established during the early stages of acute HIV infection, and once integrated becomes virtually invisible to the immune system, and is one of the main reasons why complete eradication of the HIV infection cannot be currently achieved.\(^5,9\) Due to these obstacles, there has been an increase in research using these cell types in the hopes of finding new treatments and better methods of prevention.\(^10\)

**Indirect Mechanisms of Periopathogens on HIV-1 Promoter Regulation**

González et al looked specifically at the ability of supernatants (cytokines/chemokines) produced by human gingival fibroblasts (Gin-4) and oral epithelial cells (OKF4) to modulate HIV promoter activation in macrophages when challenged with periodontal pathogens.\(^5\) BF24 monocytic cell lines were used which mimic a latent HIV-1 infection. These cell lines were transactivated with a HIV-1 promoter, and then stimulated with the periopathogens P. gingivalis, Fusobacterium nucleatum or Treponema denticola. Both Gin4 and OKF4 supernatants enhanced HIV-1 promoter activation, with a notable enhancement when supernatants from OKF4 were challenged with extract from F. nucleatum and P. gingivalis. The cells that were pulsed with F. nucleatum showed a significant cytokine/chemokine increase in GM-CSF, Interleukin-6 and Interleukin-8, which have the capacity to modulate HIV-1 promoter activation. In addition, González et al also evaluated the ability of supernatants from resident gingival cells and bacterial extracts from periodontopathogens to promote synergistic HIV-1 promoter activation.\(^5\) There was a significant increase in activation of the HIV-1 promoter in BF24 macrophages incubated in media with increasing concentrations of T. denticola, P. gingivalis and F. nucleatum. The results indicated an increase in HIV-1 promoter activation as compared to the response when challenged by bacteria alone without supernatants. This effect was shown to be additive, not synergistic in nature.

Iami et al focused on the possible effects of infection with P. gingivalis on HIV-replication, thus leading to a progression towards AIDS.\(^3\) The effects of P. gingivalis on HIV-1 latency were examined using human cell lines (ACH-2 and U1) derived from CD4+ T-cells and macrophage cells that were infected with the HIV-1 provirus. Their results showed that P. gingivalis facilitates the reactivation of latent HIV-1 cells through chromatin remodeling, which may indicate a pathophysiological link between HIV progression to AIDS and infection with P. gingivalis. Specifically, P. gingivalis produces butyric acid in concentrations in the significant range of 4.7 to 13.8 mM in dental plaque, which promotes expression of the HIV-1 latent virus, and implies that infection with P. gingivalis bacteria could be a risk factor in HIV-AIDS progression. This was one of the first studies to show a molecular link between AIDS progression and a bacterial metabolite.

**Primary reactivation of HIV latently infected cells**

In 2009, Huang et al evaluated the capacity of bacteria found in the oral cavity to stimulate HIV promoter activation.\(^4\) A T-cell line (1G5), the macrophage line (BF24) and the THP-1 line were transfected with the HIV long terminal repeat promoter. These were then stimulated with bacterial sonicates from the following bacteria: P. gingivalis, Prevotella intermedia, F. nucleatum, Actinomyces viscosus, Aggregatibacter actinomycetemcomitans, Streptococcus mutans, Campylobacter rectus and Tannerella forsythia. When the 1G5 line was stimulated with sonicates, both the gram-negative and gram-positive species were able to elicit positive stimulatory activity. The BG24 macrophage lines were also tested on the same bacterial sonicates, and F. nucleatum showed significant increases at lower doses, while P. gingivalis showed a greater HIV promoter activation than T. denticola and F. nucleatum. S. mutans had a minimal effect on the macrophages. Different bacteria had different responses when
tested on dendritic cells, and although all of the bacteria did cause an increase in promoter activity, P. intermedia and C. rectus were the most effective.

Two years later, looking specifically at dendritic cells as reservoirs for the latent HIV virus that could be stimulated by oral bacteria leading to HIV reactivation, Huang et al obtained dendritic cells from the THP89GFP cells, which were then transfected with the HIV-1 genome. The dendritic cells were then subjected to different bacterial challenges, including the following oral pathogens: P. gingivalis, S. mutans, F. nucleatum, Candida albicans and P. intermedia, using TNF as a positive control. They compared the reactions of both mature and immature dendritic cells to produce HIV promoter activation. They found that the oral bacteria activated the HIV promoter in the dendritic cells. There were significant differences between the reactions of individual bacteria, with P. gingivalis, P. intermedia and F. nucleatum having a significant effect on promoter activation, peaking at about 8 hours. An optimal dose of P. gingivalis, F. nucleatum and S. mutans was around 1x107/culture, with higher levels showing a decrease in HIV promoter activity. P. intermedia had a much larger range of stimulation, ranging from 1x106 to 2x107/culture. Consistent with what has been seen in previous studies, S. mutans and C. albicans had a significantly lower effect on HIV promoter stimulation than their Gram-negative counterparts. Huang et al also found that the oral bacteria stimulated the HIV promoter activation in a dose-dependent and time-dependent manner.

Although there have been several studies that have investigated HIV reactivation by a monospecies challenge, there has been some recent interest in examining the effects of polymicrobial bacterial challenges, such as the synergistic colonies found in the subgingival biofilm of the oral cavity, which would better reflect in vivo conditions. Therefore, building on their previous work, Huang et al evaluated the theory that HIV infected patients who have a polymicrobial oral co-infection, such as periodontitis, have a risk factor for HIV reactivation. The cell lines used in this study were a monocytic leukemia subclone (BF24) and (THP-89GFP), infected with the HIV-1 strain. Huang et al chose the following pathogenic bacteria to mimic an oral infection: A. actinomycetemcomitans, P. gingivalis, P. intermedia, F. nucleatum, T. denticola, S. mutans, Streptococcus gordonii, and Streptococcus sanguinis. The different polybacterial and monobacterial treatments were exposed to the cell lines, and their responses measured using a Mann-Whitney U-test or Kruskal-Wallis analysis. The study grouped several types of bacteria together, such as P. gingivalis and P. intermedia, S. mutans, S. gordonii and S. sanguinis, as well as measured responses of the cell lines to bacteria such as A. actinomycetemcomitans, individually. The results revealed that there was a significant difference in HIV reactivation of the BF24 and THP89GFP cell lines in the presence of Gram-positive versus Gram-negative bacteria and that many of the Gram-negative bacteria could act synergistically with each other to produce HIV promoter activation and viral replication. Gram-positive bacteria did not show any synergistic effects on the cell lines.

**Discussion**

The literature reviewed in this paper primarily encompasses research done since 2009 on the possible link between infection by oral pathogens and the reactivation of latent HIV virus leading to possible disease progression. To date, all the studies included in this review were performed on specific cell lines (macrophages/monocytes, CD4+T-cells, dendritic cells) infected with the HIV-1 provirus.

The 2009 Iami et al study was one of the first studies to show a molecular link between AIDS progression and a bacterial metabolite, specifically butyric acid produced by P. gingivalis. This study focused specifically on the ability of butyric acid to promote HIV reactivation in individuals infected with HIV-1. Although this study focused primarily on P. gingivalis as the primary producer of butyric acid, it should be noted that there are many other periodontopathogens that produce this short chain fatty acid. Further studies should include the use of Clostridium, Fusobacterium and Eubacterium, as well as several other microorganisms that produce butyric, which may also be implicated in the replication and reactivation of HIV-1.

Following the work of Imai et al, González et al conducted the first study to show soluble factors produced in response to periodontal pathogens by gingival cells could be an indirect contributor to HIV-1 promoter activation. Although their research did provide a definitive link, the exact mechanisms used by oral bacteria to induce reactivation of HIV-1 in latently infected cells remains unclear.

Huang et al selected the following bacteria: P. gingivalis, P. intermedia, F. nucleatum, A. viscosus, A. actinomycetemcomitans, S. mutans, C. rectus and T. forsythia to test HIV reactivation in macrophages, dendritic cells and T-cells latently infected with HIV-1. Different bacteria had different responses...
when tested on dendritic cells, and although all of the bacteria did cause an increase in promoter activation, P. intermedia and C. rectus were the most effective. Consistent with what has been seen in previous studies, S. mutans and C. albicans had a significantly lower effect on HIV promoter stimulation than their Gram-negative counterparts. Huang et al also found that the oral bacteria stimulated the HIV promoter activation in a dose-dependent and time-dependent manner. These findings all suggest that oral infections are a potential risk factor for patients being treated for HIV infection. Therefore, reactivation of latent HIV in dendritic cells, monocytes/macrophages and CD4+ T-cells may result in failure of highly active antiretroviral therapies and ultimately lead to AIDS progression. Future studies are needed to address the in situ occurrence of these processes, as well as the mechanisms responsible for the interactions between periodontopathic genic bacteria and HIV reactivation.

**Conclusion**

Following the many advances in HIV research in recent years, and building on previous studies that have established oral pathogens as risk factors for many systemic diseases including heart disease and diabetes, interest has been generated in investigating the possible link between infection by oral pathogens and their ability to reactivate latent HIV virus in infected cells. Despite the fact that this research is in the very early stages, several studies have shown a positive correlation between infection by bacteria found in the oral cavity and HIV promoter activation. Due to the sensitive nature of working with individuals infected with HIV and the possibility of latency reactivation, the research currently being done will continue to be studied using cells lines cultured in laboratories. Although HIV is a highly complicated disease for which we have yet to find a cure, these studies provide useful preliminary information that can be shared with patients regarding the status of their oral health and its possible relationship to their current HIV status. Future laboratory and epidemiological studies are needed to clarify the causal link and mechanisms of HIV latency reactivation associated with oral pathogens that could lead to AIDS progression and possible failure of highly active antiretroviral therapies.

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References


