Role of gallium and silver from phosphate-based glasses on in vitro dual species oral biofilm models of *Porphyromonas gingivalis* and *Streptococcus gordonii*

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**Abstract**

Phosphate-based glasses (PBGs) are excellent controlled delivery agents for antibacterial ions such as silver and gallium. The aim of this study was to assess the potential utility of novel PBGs combining both gallium and silver for use in periodontal therapy. To this end, an in vitro biofilm model with the putative periodontal pathogen, *Porphyromonas gingivalis*, and an initial colonizer, *Streptococcus gordonii*, was established. The effect of increasing calcium content in gallium–silver-doped PBG on the susceptibility of *P. gingivalis* was examined. A decrease in degradation rates (30.34, 25.19, 21.40 μg mm⁻² h⁻¹) with increasing PBG calcium content (10, 11, 12 mol% respectively) was observed, correlating well with gallium and silver ion release and antimicrobial activity against planktonic *P. gingivalis* (approximately 5.4 log$_{10}$ colony-forming units (CFU) reduction after 24 h by the C10 glass compared with controls) and *S. gordonii* (total growth inhibition after 32 h by C10, C11 and C12 glasses compared with controls). The most potent PBG (C10) was evaluated for its ability to inhibit the biofilm growth of *P. gingivalis* in a newly established constant-depth film fermentor model. The simultaneous release of silver and gallium from the glass reduced *P. gingivalis* biofilm growth with a maximum effect (1.92 log$_{10}$ CFU reduction) after 168 h. Given the emergence of antibiotic-resistant bacteria and dearth of new antibiotics in development, the glasses, especially C10, would offer effective alternatives to antibiotics or may complement current therapies through controlled, localized delivery of gallium and silver ions at infected sites in the oral cavity.

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**1. Introduction**

Periodontal diseases are a group of inflammatory diseases of the gingiva and the supporting structures of the periodontium. Plaque-related chronic periodontitis accounts for up to 60% of tooth loss in the UK and the annual cost of NHS periodontal therapy in 2001/2002 was £174 million [1]. This is of serious concern, as recent studies provide increasing evidence that periodontitis may be a risk factor for severe systemic conditions such as arteriosclerosis, myocardial infarction and stroke; increase the risk for preterm, low birth weight babies; and pose threats to those with chronic diseases, such as diabetes, respiratory diseases and osteoporosis [2,3]. Conventional periodontal therapy involves scaling or root planning, but in more severe cases antimicrobial agents such as doxycycline, metronidazole, minocycline or combinational antimicrobial chemotherapy are used as adjuncts. However, bacteria growing in a biofilm have been reported to be 1000 times more resistant to antimicrobial treatments than their planktonic counterparts [4] and are responsible for >80% of microbial infections in humans [5]. The putative periodontal pathogen *Porphyromonas gingivalis* is detected in dental plaque samples within 6 h following professional tooth cleaning [6,7], and numbers of *P. gingivalis* increase at sites of periodontal disease [8]. It has also been reported that a preformed streptococcal substratum is required for its incorporation into a biofilm [9]. The early appearance of *P. gingivalis* in the development of dental plaque biofilms was substantiated by the findings from Periasamy and Kolenbrander [10], who reported that *P. gingivalis* has the ability to interact with a variety of different stage colonizers and that it exhibits widespread mutualism with initial (*Streptococcus gordonii* and *Actinomyces oris*), early (*Veillonella sp.*), middle (*Fusobacterium nucleatum*) and late colonizers (*Aggregatibacter actinomycetemcomitans*). Further, *P. gingivalis* displayed specificity with initially colonizing streptococci as it forms biofilms with *S. gordonii* [10] but not with *Streptococcus mutans* [9] or *S. crista* [11]. We hypothesized that the ability of *P. gingivalis* to co-aggregate with *S. gordonii* [10] would allow us to establish in vitro dual-species biofilm models which could be used to evaluate the antimicrobial action of novel silver- and gallium-doped phosphate-based glasses (PBGs).