Photodiagnosis and Photodynamic Therapy (2014) 11, 290–299

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/pdpdt

Photodynamic effects on human periodontal-related cells *in vitro*

Jing Qiao, Shiying Wang, Yi Wen, Huimei Jia DDS*

Department of Periodontology, Peking University School and Hospital of Stomatology, The First Outpatient Center, Xishenku Avenue 37, Xicheng District, Beijing 100034, China

Available online 19 April 2014

KEYWORDS
Photodynamic therapy;
Human periodontal ligament cells;
Human gingival fibroblasts;
Periodontal therapy

Summary
Background: Photodynamic therapy (PDT) may be especially effective in combination with conventional periodontal therapy by its antimicrobial activities, but PDT may also exhibit other mechanisms that promote the healing of periodontal tissue. Therefore, the purpose of the present study was to evaluate the photodynamic effect of PDT on human periodontal ligament cells (hPDLCs) and human gingival fibroblasts (hFBs) *in vitro* and other possible mechanisms to promote periodontal healing.
Methods: The proliferation of hPDLCs and hFBs was assessed by MTT assay. Cell attachment on cementum slices of hPDLCs and hFBs was evaluated by MTT assay. Type I collagen synthesis of hPDLCs and hFBs was analyzed using enzyme linked immunosorbent assay. The alkaline phosphatase (ALP) activity in hPDLCs was measured by p-nitrophenyl phosphate substrate reactions.
Results: PDT treatment induced constant time-dependent growth of hPDLCs and hFBs at 24 h, 72 h and 6 days ($P < 0.05$). PDT treatment also promoted time-dependent hPDLCs and hFBs attachment on the cementum slices at 24 h, 72 h and 6 days compared to the controlled cells ($P < 0.05$). Type I collagen synthesis of hPDLCs and hFBs was markedly stimulated by PDT in a time-dependent manner ($P < 0.05$). Likewise, a significant increase in the specific ALP activity in hPDLCs was observed ($P < 0.05$).
Conclusions: The findings of this study indicate that PDT exhibited no cytotoxicity to hPDLCs or hFBs. Instead, it stimulated proliferation, attachment and collagen synthesis of hPDLCs and hFBs and ALP activity of hPDLCs. These effects might signal similar PDT activity on periodontal-related cells, and expanding the scope of its potential therapeutic utilization is very appealing.

* Corresponding author at: 100034 Xishenku Avenue Jia 37, Xicheng District, Beijing, China. Tel.: +86 10 58595054; fax: +86 10 62174226.
E-mail address: jhuimei2013@aliyun.com (H. Jia).

http://dx.doi.org/10.1016/j.pdpdt.2014.04.001
1572-1000/

Introduction

Periodontitis is a chronic inflammatory disease triggered by periodontopathogens that leads to the destruction of supporting structures of the teeth, including pocket formation in the gum tissue, attachment loss, bone destruction and, ultimately, possible tooth loss [1]. The main approach to treat periodontitis involves the removal of supragingival and subgingival plaque biofilm by mechanical therapies, such as scaling and root planing [2]. Mechanical debridement aims at improving clinical conditions by lowering the microbial load either by physical removal of plaque or by radical alteration of the subgingival habitat. However, the use of mechanical instrumentation has its limitations. Indeed, the efficacy of subgingival instrumentation is largely dependent on the experience of the operator. Complete removal of bacterial deposits therefore remains very difficult to accomplish [3,4]. To further facilitate bacterial reduction, complementary methods, such as the use of systemically and locally administered antibiotics, directly target subgingival species residing in the plaque biofilm or in the adjacent epithelial tissues lining the periodontal pocket [5,6]. However, the emergence of resistance to antibiotics is increasingly concerning. In addition, an organized biofilm protects periodontal pathogens and greatly limits the antibiotic action [7—10]. For these reasons, innovative and efficacious approaches for the efficient removal of periodontal bacteria are currently being sought.

Photodynamic therapy (PDT) was first introduced as a medical therapeutic strategy around 100 years ago [11]. PDT utilizes the activation of a photoactivatable agent, the photosensitizer, bound to its target, with a certain wavelength of light to produce singlet oxygen and other highly reactive agents that are extremely toxic to target cells and bacteria [12—14].

Its characteristic mode of action makes PDT particularly appealing as an alternative to conventional antibiotic therapy for conditions such as periodontitis. Although several *in vitro* studies have demonstrated the effectiveness of PDT in suppressing periodontal pathogens, the usefulness of the clinical application of PDT in the treatment of periodontal diseases is still questionable [15—17]. Furthermore, it is important to determine whether host tissues would be affected by light doses and sensitizer concentrations that are effective against bacteria. A few studies have reported the photodynamic effects on host periodontal tissues. Soukos et al. [18] investigated the photodynamic effects of toluidine blue on human oral keratinocytes and fibroblasts *in vitro*, and Luan et al. [19] evaluated the safety of toluidine blue-mediated photosensitization on periodontal tissues in mice.

However, more proof of the effects of PDT on human periodontal related cells and tissues is needed. Current research has proven that PDT treatment may be especially effective in combination with conventional periodontal therapy, helping to increase bacterial killing and inactivate bacterial virulence factors and host cytokines that may impair periodontal restoration, but PDT may also exhibit other mechanisms that promote the healing of periodontal tissue [20—22].

Therefore, the purpose of the present study was to evaluate the photodynamic effect of PDT on human periodontal ligament cells and human gingival fibroblasts *in vitro*. This was an attempt to identify and characterize the effects of PDT on adjacent normal tissue and any mechanisms to promote periodontal healing it may exhibit.

Materials and methods

Cell isolation and cell culture

Human periodontal ligament cells (hPDLCs) were obtained from extracted teeth removed for orthodontic reasons from young healthy volunteers. The periodontal ligament tissues attached to the middle one-third of the roots were removed by a surgical scalpel and then minced, placed in 35-mm culture dishes in Dulbecco's modified minimum essential medium (DMEM; Gibco BRL, Grant Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 U/ml penicillin G and 100 μg/ml streptomycin and were overlaid with sterile cover slips. Cultures were maintained at 37 °C in an incubator with an atmosphere consisting of 95% air, 5% CO_2 and 100% relative humidity. Cells were passaged until confluence was reached. Then cells were sub-cultured in fresh DMEM containing 10% FBS under the standard incubation conditions. Cells of the third passage were used for the experiment.

Human gingival fibroblasts (hFBs) were obtained from gingiva removed during crown lengthening surgeries from periodontal healthy patients. Gingival tissue samples were minced into small pieces using microdissection scissors and washed with serum-free DMEM. The gingival tissue was then digested in serum-free medium containing antibiotics and 1 mg/mL type IV collagenase (Worthington Biochemical, Freehold, NJ, USA) at 37 °C in a 5% CO2 humidified atmosphere for 3 h. The supernatant was then removed, and the remaining gingival tissue was placed in six-well culture dishes, allowing cells to migrate from the explants. After confluence was reached, cells were trypsinized and cultures expanded. Cells of the third passage were used for the experiment.

In all cases, patients were informed of the nature and extent of the study, and their informed consent was obtained according to the Helsinki Declaration. The study was approved by the Ethics Committee of the Peking University School and hospital of Stomatology, Beijing, China.

Preparation of cementum slices

Healthy cementum slices were obtained from teeth freshly extracted for orthodontic reasons. These teeth had no caries or fillings. The soft tissue on the root surfaces was gently removed with a scaler, and then the middle one-third of the tooth roots was taken and prepared with emery milestone. The final size of cementum slices was 4 mm × 4 mm. These slices were disinfected with penicillin and streptomycin, and then stored in DMEM medium for later use.