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Comparative effectiveness of hand and ultrasonic instrumentations in root surface planing in vitro

Mahmood Khosravi[1,2], Zahra Samadi Bahrami[1], Mahdi Saheb Jam Atabaki[2], Mohammad Ali Shokrgozar[1] and Fazel Shokri[1,3]

[1]National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran; [2]Department of periodontics, Isfahan School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran; [3]Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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Abstract

Background/aims: A variety of techniques are employed for planing and scaling of the superficial root surfaces, of which hand and ultrasonic instrumentations have been preferentially used in routine periodontics clinics. This study was undertaken to compare the effectiveness of ultrasonic scalers and hand curettes in facilitating fibroblast attachment to the scaled root surfaces.

Materials and Methods: Sixteen patients with periodontally involved teeth and nine subjects without periodontal diseases (control subjects) were selected. Two single-rooted teeth were extracted from each subject. Mesial and distal surfaces of teeth were selected in treated and untreated groups, respectively. The mesial surface of each tooth was randomly chosen to be treated either by hand curettes or ultrasonic instrumentation. The degree of cell attachment on the root surfaces of treated and untreated groups from control subjects and patients was then determined by the use of a gingival fibroblast line established and employed at early passages. The attachment and proliferation of gingival fibroblasts on the root surfaces were evaluated using neutral red assay and scanning electron microscopy (SEM).

Results: Fibroblast survival and proliferation on the surfaces of untreated periodontally involved roots were found to be significantly lower compared with control untreated surfaces ($p < 0.0001$) or treated surfaces from patients ($p < 0.0001$). No significant difference, however, was observed between root surfaces treated either by hand curettes or ultrasonic scalers.

Conclusion: These results indicate the beneficial effectiveness of both techniques in root treatment and planing.

Key words: cell cytotoxicity; fibroblast attachment; hand curettes; root planing; ultrasonic instrumentation

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The cementum of roots exposed to plaque-infected periodontal pockets undergoes several changes that decrease biocompatibility, including hypermineralization and adsorption of endotoxin, antigen–antibody complexes and products of microbial metabolism (Morris 1975, Adelson et al. 1980). The reattachment of gingival tissues to previously diseased root surfaces is a major goal of periodontal therapy. However, this can only be achieved if the root surface is rendered biologically compatible to the adjacent tissues (Lowenberg et al. 1986, Frantz & Polson 1988, Babay 2000). The classical methods of treating root surfaces to increase biocompatibility have been scaling and root planing with chemical reagents such as citric acid (Lowenberg et al. 1986), EDTA (Babay 2000) and tetracycline HCl (Frantz & Polson 1988, Babay 2000) or mechanical intervention by hand (Jones & O'Leary 1978, Fukazawa & Nishimurak 1994) or ultrasonic instrumentation (Van Gogswaardt & Behrens 1990, Smart et al. 1992, O'Hehir 1993, Chan et al. 2000, Drisko et al. 2000, Busslinger et al. 2001, Kocher et al. 2001). Recently, laser therapy has been proposed as a potentially effective method for root preparation (Aoki et al. 2000, Kreisler et al. 2002, Yilmaz et al. 2002). Comparison between hand and ultrasonic instruments in removing plaque using both stereomicroscopic and scanning electron microscopy (SEM) evaluation showed that neither methods

removed all stained accretions (Breininger et al. 1987, Garnick & Dent 1989, Copulos et al. 1993, Drisko 1993). Nevertheless, the accretions consisted not only of plaque but also of fibrin, debris and cuticle. Previous studies have demonstrated that ultrasonic scalers were more effective than hand root planners in removing endotoxins from periodontally involved root surfaces (Breininger et al. 1987, Smart et al. 1992, Copulos et al. 1993). Controversial results; however, have been reported on the comparative effectiveness of these two procedures in root planing (Garnick & Dent 1989).

In the present study, neutral red cytotoxicity assay and SEM were used to examine the growth of gingival fibroblast cells and their attachment to normal and diseased root surfaces in order to evaluate and compare the effectiveness of hand and ultrasonic methods in root planing.

Materials and Methods

Preparation of specimens

This study was conducted in vitro on tooth samples already scheduled for extraction due to severe periodontal complications, based on prior diagnosis by expert periodontists. The control teeth were extracted from subjects with no indication of periodontal involvement, mostly affected by orthodontal complications requiring tooth extraction. Therefore, all tooth extractions were a part of treatment protocols. A written consent was signed by all the patients allowing us to use the pulled out teeth in our study.

Twenty-five patients (16+9) attending the Dentistry Clinic of Isfahan School of Dentistry were included in this study. Sixteen patients, nine females and seven males, 42–70 years of age, were diagnosed with moderate to advanced periodontitis. Teeth scheduled for extraction were evaluated both clinically and radiographically for periodontal disease involvement. The inclusion criteria have been loss of attachment more than 5 mm on the whole proximal surfaces or bone loss more than half of the root length, together with involvement of at least two single-rooted teeth with hopeless prognosis due to severe bone loss and mobility. The selected patients had not received any kind of professional periodontal therapy in the preceding 6 months. Teeth with carious lesion or filling material on the root surfaces were excluded from the study. Two single-rooted teeth were extracted from each patient. Mesial and distal surfaces of the teeth were selected in experimental (treated) and control (untreated) groups, respectively. The mesial surface of each tooth was randomly chosen to receive one of the following treatments: (a) hand instrumentation with rigid Gracy curettes, numbers 1 and 2 (HU-Friedy, Chicago, IL, USA), 50 vertical pull stroke (Cogen et al. 1983), (b) ultrasonic instrumentation using the highest power setting, copious water flow and a P10 (Cavitron Dentsply, New York, NY, USA) set at 1 min (Breininger et al. 1987). The distal surface of the tooth was not manipulated and served as an untreated control. The teeth were then sectioned into mesial and distal halves using a Gillings–Hamco thin sectioning machine (Hamco Machines, Rochester, NY, USA) equipped with a Buehler diamond wayfaring blade (Buehler Ltd, Lake Bluff, IL, USA) and copious water spray. Each half was generated into a section with $5 \times 1 \times 1$ mm dimensions. Nine control subjects (six females and three males, age ranging from 12 to 60) scheduled for teeth extraction due to orthodontal complications ($n = 7$) or prosthetic problems ($n = 2$) with no indication of periodontal disease were also included in the study for comparative analytical purposes. The exclusion criteria for selection of the control subjects are the same as those of the periodontal patients. Specimens from healthy subjects were prepared as already described for patients. Specimens were dipped in cell culture medium (RPMI-1640, Sigma, St. Louis, MI, USA) containing penicillin (300 U/ml), streptomycin (300 μg/ml), gentamycin (150 μg/ml) and fungizone (1 μg/ml) for 24 h (Rosenberg & Ash 1974, Checchi & Pelliccioni 1988) to achieve sterilization. All the specimens were subsequently assessed for fibroblast attachment and proliferation.

Establishment of human gingival fibroblast (HGF) cell line

A small biopsy (1 cm^3) of normal attached gingival tissue in the lower molar region was obtained from a healthy subject under aseptic conditions and local anesthetic. No lesion or sign of inflammation was observed prior to biopsy. The tissue was stored for 2 h in 10 ml complete culture medium (RPMI-1640, Sigma) containing 2 mM L-glutamine, 10% fetal calf serum (FCS) (GIBCO, Paisley, UK) supplemented with penicillin (300 U/ml), streptomycin (300 μg/ml), gentamycin (150 μg/ml) and fungizone (1 μg/ml). The tissue section was then washed several times with the same medium. The last wash step was carried out in a complete culture medium supplemented with standard concentrations of penicillin, streptomycin, gentamycin and fungizone (100 U/ml, 100, 50, and 0.3 μg/ml, respectively). The section was subsequently minced into small pieces of $\sim$ 1 mm diameter in a glass dish and transferred into a sterile 25 cm^2 plastic flask (Greiner, Frickenhausen, Germany), incubated at 5% CO_2 atmosphere, 37°C, and saturated humidity. The outgrowth of fibroblasts from most of the attached tissue sections was microscopically visible after 2–3 weeks incubation. Upon reaching 40–70% confluency, the fibroblasts were detached by treatment with trypsin (GIBCO) [(0.25% w/v in isotonic phosphate-buffered saline) (PBS)] and sub-cultured to another flask. The cell line (HGF_2, NCBI C166, National Cell Bank of Iran, Tehran, Iran) was finally propagated and expanded in 75 cm^2 tissue culture flasks and a batch of the cells at a given passage number (passages 3–7) was cryopreserved in liquid nitrogen as a stock seed for evaluation of in vitro interaction between the HGFs) and the root slice, using neutral red assay and SEM.

In vitro interaction between the HGFs and the root slices

Root slabs obtained from patients and healthy controls sterilized for 72 h in culture medium containing a high concentration of antibiotics were subsequently rinsed with culture medium containing a standard concentration of antibiotics and placed in wells of a sterile culture plate (Greiner). HGFs (3×10^4 cells/well) were then suspended in complete culture medium supplemented with a normal concentration of antibiotics, dispersed on root surfaces and allowed to interact with each other for 5 days in 37°C and 5% CO_2 atmosphere. The total number of viable HGFs attached to root surfaces at the end of this period was then determined using neutral red assay. In addition, the attachment and distribution of HGFs on root surfaces were analyzed morpholo-

gically using stereomicroscope and SEM.

Neutral red assay

Neutral red assay was used to determine the total number of attached HGFs to treated and untreated root surfaces. The assay was performed basically as reported by Babich & Borenfreund (1990) with some modifications (Azar et al. 2000). Briefly, suspended HGFs were incubated with root surfaces as described above. Culture supernatant was subsequently removed and each well was filled with 200 μl of neutral red dye (80 μg/ml) (Merck, Damstadt, Germany), freshly prepared in RPMI-1640 from a stock solution of 5 mg/ml in distilled water. After 3 h incubation at 37°C, viable cells were evaluated microscopically with stereomicroscope. The attached cells were next washed and fixed with $CaCl_2$ (1%) in CH_2O (5%) and finally lysed with CH_3COOH (1%) in C_2H_5OH (50%) in order to extract the dye from viable cells. The optical density (OD) of extracted dye was then measured with a visible spectrophotometer at 540 nm. Cells treated with distilled water representing 100% cytotoxicity and cells incubated with culture medium alone representing 100% viability were included as positive and negative controls, respectively. The OD corresponding to positive control was subtracted from all test OD values. The total number of viable HGFs attached to root surfaces was estimated from a standard cell titration curve (OD/cells) using different dilutions of HGFs. The percentage of cytotoxicity for each test sample was estimated according to the following calculation:

$$\%\ \text{Cytotoxicity} = \left(1 - \frac{\text{Mean OD of test sample}}{\text{Mean OD of negative control}}\right) \times 100$$

Scanning electron microscopy (SEM)

For this study, 15 root slabs from three patients and two control subjects (treated with hand curettes and ultrasonic instrumentation or untreated) were randomly selected. Only five subjects were included for SEM study due to the limited availability of extracted teeth slabs. After incubation with suspended HGFs, the sections were washed two times in 0.1 M PBS and fixed in 2.5% gluteraldehyde. Following 1 h incubation at room temperature, the slabs were rinsed two times with 0.1 M PBS and fixed in 1% osmium oxide (OsO_4) in 0.1 M PBS for 1 h. They were subsequently rinsed two times in PBS, dehydrated in a graded series of ethanol treatment (Merck) dipped in tert butanol (Merck) and stored in -20°C until use. The specimens were later dried in a freeze dryer (Ey ELA, Rikakikai, Japan) for 6 h, affixed to aluminum stubs and coated with 10 nm of gold–platinum using a sputter coater (gold coater) (BIO-RAD, SC-500, Hemel Hempstead, UK). A scanning electron microscope (Stereoscan 360, Cambridge, England) operated at 25 kV was used to visualize the cells and take photographs at different magnifications.

Statistical analysis

Comparison of results obtained from treated and untreated samples within each patient or control subject group was analyzed by one-way analysis of variance and comparisons between the two groups were analyzed by *t*-test procedure, as appropriate. Differences were considered significant at *p*-values of less than 0.05.

Results

Cell attachment to treated and untreated root slices

The mean number of attached fibroblasts determined by neutral red assay to treated (hand curettes and ultrasonic instrumentations) and untreated root slices in control and patient groups is presented in Table 1. One-way analysis of variance showed a significant difference ($p < 0.0001$) in the mean number of attached fibroblasts to treated (hand curettes and ultrasonic instrumentations) and untreated root slices in the patient group. No significant difference, however, was observed between root surfaces treated either by hand curettes or ultrasonic scalers. According to one-way analysis variance procedure, no significant difference was observed between the mean number of attached fibroblasts to treated and untreated root slices in control groups. When treated and untreated samples were compared between the two groups (control and patients) by *t*-test, a significant difference ($p < 0.0001$) was only apparent between untreated root sections in these groups (Table 2).

Table 1. Outcome of fibroblast attachment to periodontally involved and control root surfaces

Groups	Type of treatment	Mean number of HGFs attached to 1 cm^2 of root surfaces
controls ($n = 9$)	hand curette (A1)	19898 ± 4267
	ultrasonic (A2)	20281 ± 5860
	untreated (B)	22788 ± 4345
patients ($n = 16$)	hand curette (A1*)	25859 ± 11278
	ultrasonic (A2*)	27189 ± 14507
	untreated (B*)	4875 ± 26551

*Indicates patients as apposed to control individuals.
HGFs, human gingival fibroblasts.

Table 2. Statistical comparison of number of attached fibroblasts to root surfaces between control and patient groups

Groups*	*p*-Value
A1 and A1†	NS
A2 and A2†	NS
B and B†	<0.0001

*Refer to Table 1 for definition of groups. NS, not significant.
†Indicates patients as apposed to control individuals.

Morphology of HGF cells attached to treated and untreated root surfaces

Following staining with neutral red, HGFs were visualized on root slices with stereomicroscope. No substantial difference in the confluency of HGF cells on treated (hand curettes or ultrasonic scalers) and untreated root slices was observed in the control group. The attached cells in highly confluent areas were flattened, extended and spindle-shaped (Fig. 1a). However, there were a few viable HGFs on untreated root surfaces in the patient group. These cells were scattered and morphologically round-shaped (Fig. 1b-I). The morphology of HGFs attached to instrumented root slices, either by hand or ultrasonic device, was found to be very

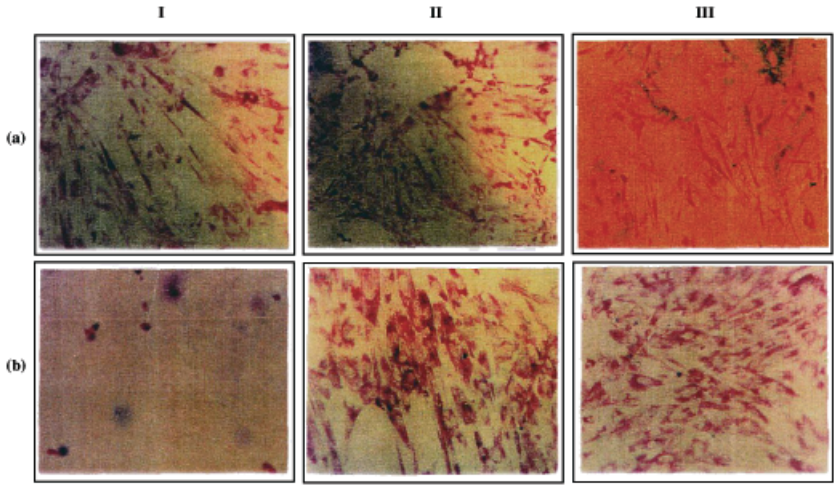

Fig. 1. Representative stereomicroscopic photomicrographs (× 200) of attached human gingival fibroblasts on the control and patient root slices. (a) Control samples, (b) patient samples, I: untreated root slices; II: hand curette-treated root slices; III: ultrasonic-treated root slices.

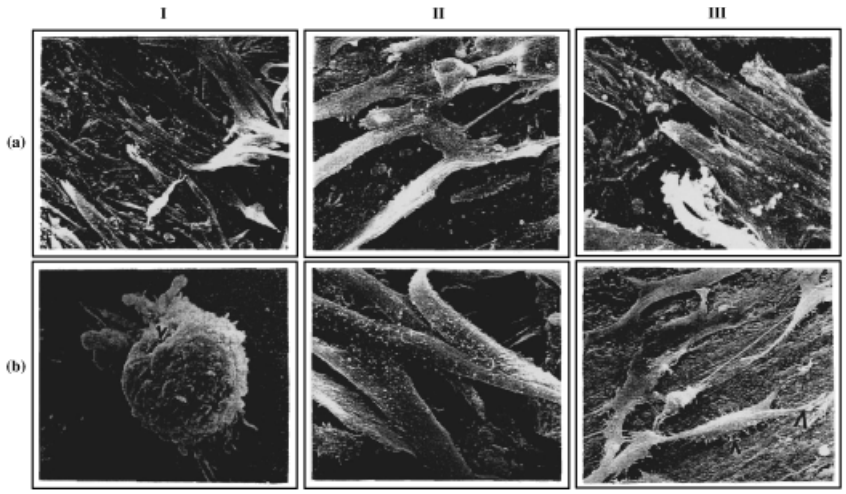

Fig. 2. Representative scanning electron microscopy photomicrographs of human gingival fibroblasts attached on the control and patients root slices. (a) Control samples, (b) patient samples, I: untreated root slices (× 500, × 4000); II: hand curette-treated root slices (× 1000, × 2000); III: ultrasonic-treated root slices (× 2000). Marker (v) illustrated in bI represents the distribution of cell membrane and markers (Λ) illustrated in bIII indicate filipodias and adult lamellapodias of the cell surface.

similar to that of the healthy group (Fig. 1b-I and III). The SEM photomicrographs of untreated and treated slices from the healthy group revealed highly confluent, regular, elongated and spindle-shaped cells with abundant adult lamellapodia. No readily apparent difference was observed between the morphology of cells attached to untreated and treated root surfaces (Fig. 2a). However, only a few perforated globular cells with low confluency could be observed on untreated root slices in the patient group (Fig. 2b-I). The HGFs attached to the treated root surfaces were morphologically similar to those of the control group, with no significant difference between the two instrumentation methods (Fig. 2b-II and III).

Discussion

The major cemental changes associated with periodontal diseases are: (a) penetration and adsorption of endotoxin to cementum; (b) hypermineralization of cemental surface; and (c) degeneration of collagen matrix (Fukazawa & Nishimurak 1994). These changes contribute to a large extent to the detachment of gingival tissues from diseased root surfaces. Our results of significantly decreased attachment and growth of HGF to uninstrumented root slices extracted from the patients, as compared with those of the healthy subjects (Table 1), support this proposition. The pathogenic changes of cementum in periodontally involved teeth are found mainly within superficial layers and thus curettage of deeper cementum should be avoided (Fukazawa & Nishimurak 1994). The cementum is covered with various proteins, which facilitate cell attachment. In periodontal diseases, however, these proteins are largely degraded by proteolytic enzymes and covered with endotoxins produced by the invading microorganisms. Ultrastructural studies have revealed that deeper cemental tissue becomes a suitable base for both cell and gingival tissue attachment following superficial curettage of periodontally diseased cementum (Noguchi et al. 1985). Planing and scaling of the superficial root surfaces have been successfully performed by a variety of techniques, the most important ones being hand scalers (Jones & O'Leary 1978, Fukazawa & Nishimurak 1994), ultrasonic instrumentation (Van Gogswaardt & Behrens 1990, Smart et al. 1992, O'Hehir 1993, Chan et al. 2000, Drisko et al. 2000, Busslinger et al. 2001, Kocher et al. 2001), laser scaling (Aoki et al. 2000, Kreisler et al. 2002, Yilmaz et al. 2002) and demineralization or chemical scaling (Lowenberg et al. 1986, Frantz & Polson 1988, Alleyn et al. 1991, Biagini et al. 1992, Babay 2000). Hand and ultrasonic instrumentations have been more widely used in periodontics, due to simplicity, cost-effectiveness and more objective operatory control.

In the present study, hand and ultrasonic instrumentations were compared by evaluation of in vitro attachment and growth of gingival fibroblasts to instrumented root surfaces, using a combination of ultrastructural microscopic observation and the neutral red assay. To our knowledge, the neutral red assay employed in this study has not already been used in periodontal investigations. This methodology, which was originally introduced for assessment of cell viability and cytotoxicity (Babich & Borenfreund 1990, Shokri et al. 2000), has recently been found to be comparable to other commonly used cytotoxicity as-

says, giving objective and sensitive results (Heidari et al. 2002). Owing to its simplicity and objectivity, we employed this method for the first time in endodontics investigations to evaluate in vitro cytotoxicity of a new epoxy resin-based sealer on HGFs (Azar et al. 2000). This assay is based on active adsorption and accumulation of the neutral red dye by viable cells attached to root surfaces. The OD of extracted dye is proportional to the number of viable cells that can be readily extrapolated from a standard curve (Azar et al. 2000). This technique gives more objective and precise results in comparison with microscopic enumeration of stained cells attached to root slices, either by stereomicroscope or SEM, taking into account the non-homogeneous confluency of the fibroblasts attached to different portions of root surfaces as well as subjective variations and fluctuations in microscopic counting. One of the important methodological considerations taken in our study is high-dose antibiotic sterilization of root slices used for HGF attachment. In contrast to autoclave sterilization employed in some previous studies (Adelson et al. 1980, Noguchi et al. 1985), or demineralization with acidic or basic solutions (Lowenberg et al. 1986, Frantz & Polson 1988, Alleyn et al. 1991, Biagini et al. 1992, Babay 2000), this technique avoids unnecessary harsh treatment of root surfaces that could lead to loss of some of the effective biological factors or degeneration of collagen matrix surrounding the root, which could eventually lead to a negative influence on HGF attachment (Pitaru et al. 1995).

Our results indicate that the two methods of instrumentation were equally effective. These findings were substantiated by both neutral red assay and morphological observations achieved by both stereomicroscope and SEM (Table 1 and Figs. 1 and 2). Our findings are in agreement with those reported by other investigators demonstrating similar effectiveness of these two procedures in calculus removal (Van Gogswaardt & Behrens 1990, Alleyn et al. 1991, Biagini et al. 1992, Drisko et al. 2000, Busslinger et al. 2001, Drisko 1998). Controversial results have also been reported, indicating the superiority of the ultrasonic scalers in providing more biocompatible surfaces and more effective removal of endotoxins from periodontally involved root surfaces (Breininger et al. 1987, Drisko 1993, Copulos et al. 1993, Leon & Vogel 1987). These differences could be attributed to the methodological variations employed in these studies, as mentioned above.

In conclusion, no significant difference in the attachment and growth of gingival fibroblasts on periodontally diseased root surfaces treated with either hand or ultrasonic instrumentation was observed. Therefore, both methods can equally provide biologically compatible surfaces for the attachment of gingival tissues. The decision as to which of these debridment techniques is best for a given situation is to be made by the periodontist. It is noteworthy that this is an in vitro investigation and, as a consequence, the results observed here could be different in vivo.

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Address:
Fazel Shokri
Department of Immunology
School of Public Health
Tehran University of Medical Sciences
Tehran, PO Box 6446-14155
Iran
Fax: +98 21 6462267
E-mail: fazshok@ yahoo.com