

## Original Article

# Effects of minocycline-HCl paste root conditioning on periodontal surgery: *in vitro* and *in vivo* studies

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**Abstract:** Objectives: This study was aimed to investigate effects of 2% minocycline-HCl paste root conditioning on periodontal surgery. Materials and methods: *In vitro*, cementum slices affected by periodontitis were randomly conditioned with 2% minocycline-HCl paste, 2% minocycline-HCl liquid, and 0.9% saline. NIH3T3 cells were cultured and attached to the each slide, and the viability and proliferation of cells were observed; *In vivo*, 21 deep periodontal pockets were treated by periodontal surgery, and the exposed root surfaces were randomly conditioned with 2% minocycline-HCl paste or 0.9% saline. The periodontal parameters were measured at baseline, after 3 and 6 months of periodontal surgery. Results: *In vitro*, NIH3T3 cell showed better viability and proliferation at 3, 5, and 7 day in groups conditioned with minocycline-HCl than the group conditioned with 0.9% saline (at 3 day ( $P < 0.05$ ); at 7 day ( $P < 0.01$ )). Minimal differences were found between minocycline-HCl paste and liquid groups; *In vivo*, 3 months after periodontal surgery, the greater CAL reduction was found in the minocycline-HCl treated group than in the control group ( $P < 0.05$ ). The similar results were found for both CAL and PD ( $P < 0.05$ ;  $P < 0.05$ ) between two groups at 6 months after surgery. PI and SBI variations showed no statistical differences between two groups after periodontal surgery. Conclusion: Our results suggested that root conditioning with minocycline-HCl paste during periodontal surgery improve the periodontal healing, which may be associated with the promotion of the periodontal cell attachment and growth onto the root surfaces.

**Keywords:** Root conditioning, minocycline-HCl, periodontal surgery

## Background

Root conditioning is an important part of treatment in periodontal surgery, and clinicians have started to approach different pharmacologic agents for root conditioning. The root surface affected by periodontitis is harmful to attachment because the structure is changed by the effects of toxic and other substances from periodontal pathogens, which prevent the attachment and growth of gingival and periodontal ligament cells to the root surface [1-3]. Although mechanical root planning can effectively remove the endotoxin from the affected root surface [4, 5], the smear layer created during the root planning further affects attachment [6].

Chemical root conditioning shall be carried out after mechanical periodontal debridement, and

can neutralize microorganisms and toxins by a low pH condition or antibacterial property [7-9]. At the same time, demineralization occurs and effectively removes the smear layer of root surfaces, and thereby exposes the dentinal tubules and the part of the matrix proteins to improve the wound-healing environment [6, 10-12]. In addition, exposed proteins may promote calcium deposition, which is a key factor affecting new cementum formation (Egelberg J. Regeneration and repair of periodontal tissues. *J Periodontal Res* 1987; 22: 233-42).

Minocycline-HCl is a semi-synthesized tetracycline widely used for periodontal treatment because of its antibacterial and anti-collagenase properties [13, 14]. Some researchers have reported the demineralization feature of minocycline-HCl *in vitro*. Atilla and Baylas [15] evaluated effects of citric acid, tetracycline-HCl,

## Therapy for periodontitis

and minocycline-HCl on the demineralization, and found that all of these agents can demineralize Ca and P contents and expose the collagen matrix on the root surface. Further, Minabe [16] found that minocycline-HCl can remove the dentin smear layer and neutralize the part of endotoxins by immersing the affected roots in minocycline solution. Another study has shown that root conditioning with minocycline-HCl can stimulate the attachment of human gingival keratinocytes and lead to rapid periodontal healing by promoting junctional epithelium reformation [17]. However, in clinic, the effect of minocycline-HCl on cementum surface is still unclear, as most of the previous studies have focused on dentin surface.

The present study investigated the effect of 2% minocycline-HCl root conditioning on periodontal surgery, *In vitro*, the cell experiment was performed to observe the attachment and growth of fibroblast cells onto root surfaces conditioned with different minocycline-HCl formations or 0.9% saline; *In vivo*, a clinical assessment was carried out to observe the effect of minocycline-HCl paste root conditioning on periodontal surgery.

### Materials and methods

#### *Preparations of cementum slice and root conditioning*

Written informed consents were given to those who agreed to participate in this study. Twenty-two freshly extracted premolars affected by severe chronic periodontitis were collected. The teeth were scaled and root-planned, and fabricated into a rectangular plate (4 mm × 2 mm × 0.5 mm) with one cementum surface. A total of 27 cementum slices were prepared and randomly divided into three groups: minocycline-HCl group, minocycline-HCl liquid group and control group. Night slices of each group were sponged for 1 min with 2% minocycline-HCl paste (Perioline, Sunstar, Osaka, Japan), 2% minocycline-HCl liquid and 0.9% saline, respectively, and then washed with 0.9% saline for 30 s. The conditioning procedure was repeated for 3 times. Slices were sterilized in 75% ethanol solution for 2 h and washed in distilled water for 5 min, and then kept in phosphate buffer solution (PBS) at 4°C for use.

#### *Cell culture*

All slices for control and 3 for each treated group were individually transferred into single

wells of a 24-well plastic tissue culture plate, and 0.5 ml cell culture medium containing 90% DMEM (Hyclone, Logan, USA) and 10% FBS (Hyclone) was added into wells to immerse slices. 1 ml NIH3T3 cell suspension (approximate  $4 \times 10^4$  cells) was added into each well, and the cells were cultured at 37°C incubator with an atmosphere of 5% CO<sub>2</sub>.

#### *Scanning electron microscope (SEM)*

After incubated for 24 h, 3 slices from each group were taken out and washed with PBS for 3 times. Slices were fixed with 2.5% glutaraldehyde for 2 h, and prepared for SEM (Quanta 200; FEI, USA) observation with an accelerated voltage of 20 kv and a magnification of 300 times. Five areas from each slices were randomly selected and photo were taken. Then the number of cells were counted and mean number of cells were calculated.

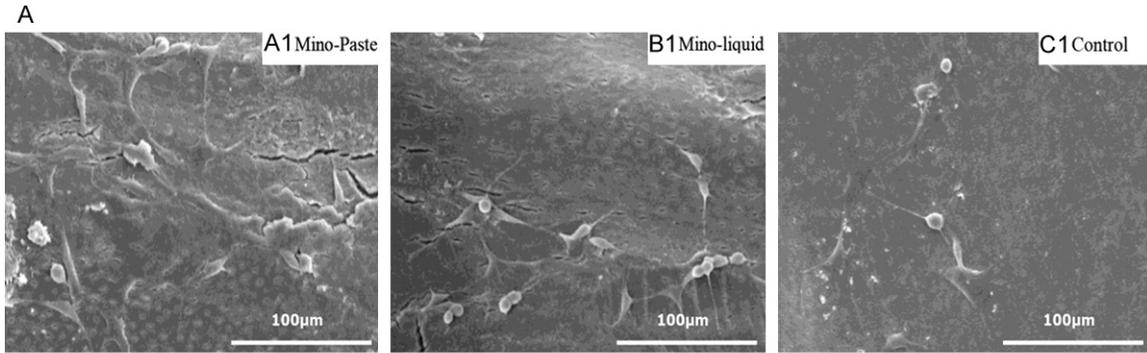
#### *Cell viability and proliferation*

After incubated for 24 h, 6 slices from each group were individually transferred into single wells of a 96-well plate, and 100 µL cell culture medium with 10 µL Alamar Blue reagent was added into each well. After incubated for 4 h, 100 µL medium in each well was transferred to single wells of a new 96-well plate, and absorbance values of medium were measured at 570 nm and normalized at 600 nm (PowerWave XS2, BioTek, USA). Then, slices in the 96-well plate were washed with PBS, and 100 µL fresh cell culture medium was added to each well with slice. After incubated for 20 h, the procedures were repeated to obtain the absorbance values at 1, 3, 5 and 7 day.

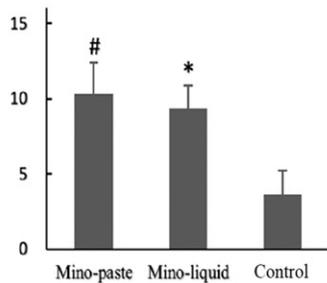
#### *Patient recruitment and group*

The ethical clearance was approved by Ethics Committee and Review Board of Hospital of Stomatology, Wuhan University. Sixty-one patients with chronic periodontitis, who were treated by non-surgical treatment at least 3 months ago, were recruited from the Department of Periodontology. Thirteen patients aged from 35 to 60 were selected after periodontal examination. Patients were selected on the basis of assessment of their clinical characteristics, which included: (1) probe pocket depth ≥ 6 mm with bleeding on probing; (2) clinical attachment loss ≥ 5 mm; (3) plaque index ≤ 2; (4) sulcus bleeding index ≤ 4; (5) tooth mobility less

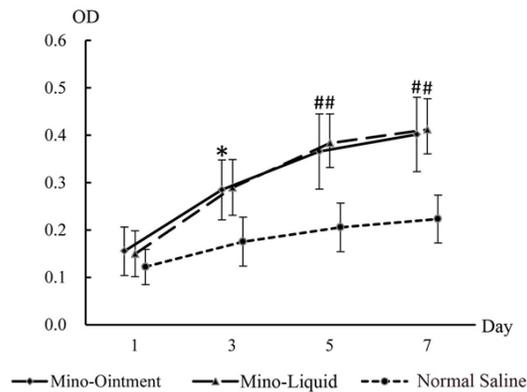
## Threapy for periodontitis



**B cell count**



**Figure 1.** SEM micrographs of NIH3T3 cells attached to the conditioned surface after 24h incubation with cementum slices ( $\times 300$  magnification, A). (B) showed the count of cells attached to the conditioned surface (\* $P < 0.05$ ; # $P < 0.01$ . One Way ANOVA with Post hoc Tukey test).



**Figure 2.** Viability and proliferation of NIH3T3 cells attached to the conditioned surface. Each date set is presented as the mean and 95% confidence interval. (OD: the absorbance value of cell culture medium; Day: cell incubation time; \* $P < 0.05$ , # $P < 0.01$ , One Way ANOVA Post hoc Tukey test).

than degree III. Patients who met the following criteria were excluded from the study: (1) systemic disease that can alter the course of periodontal disease; (2) systemic administration of antibiotics; (3) allergic to tetracycline; (4) a smoking habit; (5) pregnancy or lactation.

The recruited patients were randomly divided into two groups: the treatment group and the control group, in which, teeth would be condi-

tioned with 2% minocycline-HCl paste or 0.9% saline respectively.

### Surgical procedure

The modified Widman flap surgery was performed on teeth of selected patients as described by Ramfjord and Nissle [18]. Degranulation and debridement were completed using ultrasonic and hand instruments. Root surface corresponding to the deep periodontal pocket was sponged with minocycline-HCl paste or saline for 1 min, and then washed with 0.9% saline. The conditioning procedure was repeated for three times. Flaps were repositioned to their original location and sutured using a single interrupted suture technique.

### Clinical assessments

Clinical attachment loss (CAL) and probing depth (PD) were examined with Florida probe (Florida Probe Corporation, Gainesville, Florida, USA), and plaque index (PI) and sulcus bleeding index (SBI) were examined with William probe. Clinical assessments were measured by a single examiner on three chronic periodontitis patients, and repeated 24 h later. The intra-examiner reproducibility for CAL was measured with K coefficient ( $\pm 1 \text{ mm}$ ) = 0.85. All measurements were assessed at baseline, and after 3 and 6 months of periodontal surgery.

## Threapy for periodontitis

**Table 1.** Characteristics of 21 teeth sites of two groups at baseline

| Characteristic                  | minocycline-HCl paste (n = 0) | 0.9% saline (n = 11) | P     |
|---------------------------------|-------------------------------|----------------------|-------|
| Gender (Male/Female)            | 5/5                           | 6/5                  | 0.835 |
| Age (years)                     | 43 (36.75, 45.25)             | 40 (37, 46)          | 0.940 |
| Tooth (Anterior/Premolar/Molar) | 1/5/4                         | 1/4/6                | 0.793 |
| Sites (Mesial/Distal)           | 3/7                           | 6/5                  | 0.256 |

Data set was presented as the number of patients or the median (Q1, Q3). P-value was calculated with Chi-Square test or Mann-Whitney U test.

### Statistical analysis

*In vitro*, descriptive data were presented as mean and standard deviations. The inter-group differences were analyzed using One-Way ANOVA with Post hoc Tukey test; *In vivo*, the baseline balancing of two groups was tested with Pearson Chi-square test or Nonparametric Mann-Whitney U test. The difference before and after surgery was analyzed with Wilcoxon signed ranks test, and the difference between treatment group and control group was analyzed with Mann-Whitney U test. The statistical significance was determined as a P-value less than 0.05. All the data were analyzed using SPSS 17.0 statistical software (New York, USA).

### Results

#### SEM

The density and morphology of NIH3T3 cells attached to the surface of cementum slices was presented as in **Figure 1**. The surfaces conditioned with minocycline-HCl paste showed higher cell density than that conditioned with 0.9% saline, and minimal differences were found between minocycline-HCl paste and liquid groups. Cells presented a flat and spindle shape showing a characteristic of extension at the slice surfaces conditioned with minocycline-HCl, while cells were almost spherical showing less extension at the surface conditioned with 0.9% saline.

#### Cell viability and proliferation

The absorbance values of culture medium of NIH3T3 cells attached to slice surfaces were depicted as in **Figure 2**. The results showed that the absorbance values were higher in minocycline-HCl groups than in 0.9% saline

group, and the absorbance value was significantly higher in minocycline-HCl liquid group than 0.9% saline group at 3 day ( $P < 0.05$ ). The absorbance value was significantly higher in minocycline-HCl groups than 0.9% saline group at 5 and 7 day ( $P < 0.01$ ). No difference of absorbance values was found between two minocycline-HCl groups.

#### Clinical data collection

Among the 13 selected patients, 2 patients failed in the clinical assessments due to personal reasons. Data were collected from 21 teeth sites (10 sites in the treatment group and 11 sites in the control group) of 11 patients at baseline, after 3 and 6 months of periodontal surgery. The missing data were ignored in the analysis after checking their relevance to the results. There was no difference for the age and gender between the two groups. No significant difference was found for the 21 teeth sites between the two groups at baseline (**Table 1**).

#### Analysis of clinical assessments

CAL reduced after periodontal surgery in both treated and control groups, and the reduction from Baseline to Evaluation2 and Evaluation3 was significantly different between two groups ( $P < 0.05$ . **Table 2**, **Figure 3B** illustrated the distribution of teeth sites of the CAL reduction in two groups, which showed that the distribution in treated group was more right shift than that in control group.

No difference was found for PI before and after periodontal surgery between two groups. SBI was decreased after periodontal surgery, and the reduction of SBI in two groups was similar. PD level was also decreased in two groups after periodontal surgery, with greater reduction in the treatment group than in the control group (3 months,  $P < 0.05$ ; 6 months,  $P > 0.05$ . **Table 1**). The distribution of teeth sites of the PD reduction was similar to that of CAL as illustrated in **Figure 3A**.

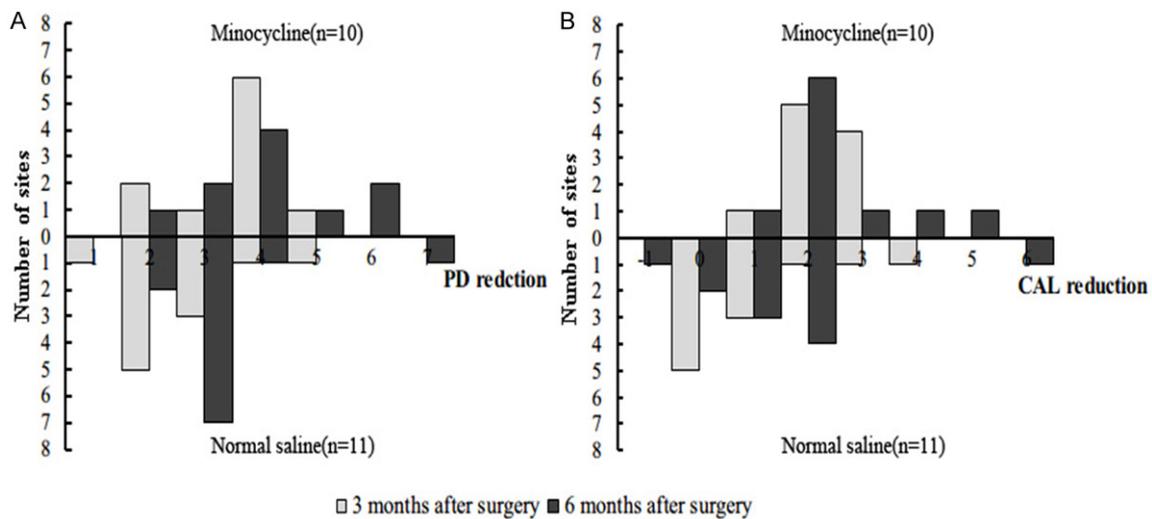
All data set was presented as median (Q1, Q3).  $\Delta$ : Variation values between different evaluation times ( $\Delta_{3,0}$  refers to variations from 3 month to baseline;  $\Delta_{6,0}$  refers to variations from 6 month to baseline). \*#Statistical significance

## Therapy for periodontitis

**Table 2.** Comparisons of periodontal measurements of clinical assessments at baseline, 3 and 6 month between two groups

| Periodontal measurement | Group     | Evaluation time |                |                | Variations     |                |
|-------------------------|-----------|-----------------|----------------|----------------|----------------|----------------|
|                         |           | Baseline        | 3 month        | 6 month        | $\Delta_{3-0}$ | $\Delta_{6-0}$ |
| PI                      | treatment | 1 (0.75, 1.25)  | 0.5 (0, 1)     | 1 (0, 1)       | 1 (-0.25, 1)   | 0 (-0.251, 1)  |
|                         | control   | 1 (0, 1)        | 1 (0, 1)       | 1 (0, 1)       | 0 (0, 1)       | 0 (-1, 1)      |
| SBI                     | treatment | 2 (0.72, 3)     | 0.5 (0, 2)*    | 0 (0, 1)*      | 1 (0, 2)       | 2 (0.75, 2)    |
|                         | control   | 2 (2, 3)        | 1 (0, 2)*      | 1 (0, 1)#      | 1 (1, 2)       | 2 (1, 2)       |
| PD (mm)                 | treatment | 7 (7, 7.25)     | 3.5 (3, 4.25)# | 3 (3, 4)#      | 4 (2.75, 4)*   | 4 (3, 5.25)    |
|                         | control   | 7 (6, 8)        | 5 (3, 5)#      | 4 (3, 4)#      | 2 (2, 3)       | 3 (3, 3)       |
| CAL (mm)                | treatment | 9 (8, 9)        | 6 (6, 7)#      | 6.5 (5.75, 7)# | 2 (2, 3)*      | 2 (2, 3.25)*   |
|                         | control   | 8 (7, 9)        | 7 (6, 8)*      | 7 (6, 8)*      | 1 (0, 2)       | 1 (0, 2)       |

Data set was represented as the number of samples or the Median (Q1, Q3). \* $P < 0.05$ . # $P < 0.01$ .



**Figure 3.** The relationship between the number of sites and variations of clinical assessments (PD (A) and CAL (B)) at 3 and 6 months after periodontal surgery. The upper and lower parts represented minocycline-HCl paste group and 0.9% saline group, respectively. Each data set was presented as the mean  $\pm$  SD (minocycline-HCl paste group:  $n = 10$ ; 0.9% saline group:  $n = 11$ ).

marks (\* $P < 0.05$ ; # $P < 0.01$ ). In Evaluation time row, it refers to intra-group statistical differences for comparisons between different evaluation time and baseline; In Variations row, it refers to inter-group statistical differences for comparisons of variation values between treatment and control groups.

### Discussion

The removal of bacterial deposits and the arrest of periodontal disease progression are the primary goals in the treatment of periodontal diseases [19]. Root conditioning with chemical agents is an effective way of removing the smear layer and exposing collagen after mechanical debridement, therefore, which pro-

motes the attachment and growth of periodontal cells at root surfaces. *In vitro*, our results showed that the cementum surface conditioned with minocycline-HCl promoted the cell viability and proliferation of fibroblast cells attached to root surfaces, which was similar to previous studies based on dentin surfaces [20, 17]. The results suggested that root conditioning with minocycline-HCl promoted the attachment and growth of the periodontal cells onto root surfaces after periodontal surgery.

*In vivo*, the results of clinical assessments suggested that root conditioning with minocycline-HCl paste improved the effect of periodontal surgery on the clinical attachment and accelerated periodontal healing. However, Erdinc *et al*

## Therapy for periodontitis

conducted a 4-week clinical trial with 10 aggressive periodontitis patients and found that root conditioning with tetracycline in periodontal surgery has no detectable clinical effect on the regeneration of clinical attachment [21]. The different results may be due largely to the type of periodontal pockets. Deep and narrow intrabony defects are the most predictable response to regenerative procedures [22]. All periodontal pockets of teeth sites included in present study were intrabony pockets for periodontal flap surgery. The fluidity of root conditioning agents may be another possible reason that have an effect on the periodontal healing. *In vitro*, little difference was observed between paste and liquid formations of minocycline-HCl, suggesting that root conditioning may not be affected by the fluidity of etching agents. However, in clinical practice, it is a safe and convenient way to apply the paste to the root surface, which can minimize the damage to gingiva, periodontal ligament and alveolar bone around the conditioned roots.

There are two main kinds of periodontal healing at the tooth: new periodontal attachment and long junctional epithelium formation, which are developed by periodontal ligament cells and gingival keratinocytes, respectively. A series of studies has shown that exposure of a collagenous matrix onto the dentin surface can facilitate the attachment of a fibrin network that mechanically inhibits the downgrowth of gingival epithelium, which may lead to the formation of new attachments [23-26]. *In vitro*, our results showed the increased NIH3T3 cell density, viability and proliferation in response to the surface conditioned with minocycline-HCl, which suggested that root conditioning with minocycline-HCl might cause the similar exposure of a collagenous matrix at root surfaces. However, the type of periodontal healing was still unclear and needed to be investigated further. Regardless of the type of periodontal healing, *in vivo*, the clinical attachment was improved after periodontal surgery with minocycline-HCl paste root conditioning, suggesting that a positive clinical prognosis could be maintained for 3 to 6 months.

In summary, our results suggested that root conditioning with minocycline-HCl promote the attachment and growth of the periodontal cells onto the root surfaces after periodontal surgery. Our results showed that root conditioning

with minocycline-HCl paste during periodontal surgery increase clinical attachment levels and improve periodontal regeneration, which may be associated with the promotion of the periodontal cell attachment and growth onto the root surfaces.

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### Disclosure of conflict of interest

None.

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## Threapy for periodontitis

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