Original Article

Clinical and Radiographic Evaluation of Locally Delivered Plant Stem Cells for Treatment of Periodontitis: Randomized Clinical Trial

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Abstract

Background: Periodontitis causes the destruction of soft and hard tissues. Stem cells have immense potential in regenerative cellular therapy. This clinical trial aimed to evaluate clinically and radiographically the effectiveness of the local application of Edelweiss stem cells as a nonsurgical treatment for stage III periodontitis. **Materials and Methods:** The trial included 40 periodontal pockets in participants who have stage III periodontitis with probing pocket depth (PPD) ≥5 mm and clinical attachment loss (CAL) ≥5 mm. Pockets were randomly divided into two groups Group 1: was given oral hygiene instruction, scaling, root planing, and subgingival application of plant stem cells on gel foam carrier after that a periodontal dressing was applied. The procedures were repeated after 2 weeks. Group 2: was treated only by scaling and root planing. Gingival index, CAL, and PPD were measured at baseline and 3 months' posttherapy. The radiographical evaluation was done by digital long-cone parallel periapical radiographs at baseline and 6 months posttherapy. **Results:** Clinical parameters for both groups showed a statistically significant improvement. Regarding radiographic evaluation, there was a significant increase in bone density in favor of the study group. **Conclusions:** Locally applied Edelweiss stem cells can be considered a promising nonsurgical treatment modality for periodontal regeneration.

Keywords: *Edelweiss, periodontal regeneration, stem cells*

Introduction

Plant regeneration is a distinctive process at the cellular and tissue level as plant stem cells possess capabilities that aid in the excitation and regeneration of plants following injury. Plant extracts have a considerable effect on expansion and variation into multilineage cells when used as stimulants. Plant-derived bioactive chemicals exert precise control over mesenchymal stem cells (MSCs).[1] MSCs, in combination with medicinal plant extracts, may hold promise for stem cell and therapeutic regeneration.[2]

Periodontitis is an inflammatory process affecting the teeth-supporting structures. Stage III periodontitis reflects severe clinical attachment loss (CAL) equal to or more than 5 mm, deep periodontal pocket, severe bone loss reaching and beyond the middle of the roots, furcation involvement, and teeth loss due to periodontal destruction.[3]

Optimal periodontal regeneration cannot be achieved by conventional periodontal treatment such as scaling and root planning.[4] To achieve periodontal tissue regeneration, several regenerative techniques have been developed including guided tissue regeneration and bone grafts. However, the clinical effects of these strategies are uncertain and diverse. Furthermore, there are various risks and contraindications for surgical procedures.[5-7]

It is necessary to discover alternative regenerative techniques for restoring periodontal structure and functions. The antioxidant and regenerative ability of plant stem cells and/or stem cell extracts, make them useful for medical uses.[8] Medicinal plants/herbs have less toxic effects and are also inexpensive making them a promising treatment modality for periodontal regeneration.[1]

Edelweiss (*Leontopodium alpinum*) is a member of the *Asteraceae* family, a species of wildflower found in rocky limestone areas at high elevations, like the Swiss Alps. For a long period, Edelweiss was used as traditional therapy against pain,

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inflammation of the bronchi, diarrhea, dysentery, and fever. Numerous types of research have demonstrated the anti-inflammatory activity of Edelweiss extracts in mice, rats, human keratinocytes, and endothelial cells.[9]

Edelweiss root extracts contain components that increase cholinergic neurotransmission, indicating that they may be used as antidementia and antioxidant agents, such as leontopodic acid A and 3,5-dicaffeoylquinic acid. In addition, Edelwiss extracts proved to have antimicrobial action.[10] All these characteristics qualify them to be used for periodontal regeneration.

Materials and Methods

Forty interproximal periodontal pockets in participants diagnosed with stage III periodontitis were selected. Their ages ranged between 39 and 65 years old. To include the patients in this study, their consent was taken, and all procedures were explained before treatment. The trial was ethically accepted by the research ethics committee.

Inclusion criteria

Probing pocket (PPD) ≥5 mm, CAL ≥5 mm, absence of relevant medical conditions that interfere with periodontal tissue response to treatment, and ideal compliance, which is demonstrated by no missed sessions for treatment and a favorable tendency toward oral hygiene.

Exclusion criteria

The use of antibiotics agents 6 months before the study, smokers, diabetics, pregnant and lactating patients.

Patients with periodontal pockets who met all inclusion criteria were randomized using the sealed envelopes method into two equal groups as follows:

Group 1: participants got oral hygiene instructions, a single scaling, and root planing session, with the subgingival application of Edelweiss stem cells and plant extract.[10] The material was placed on a small piece of sponge and then inserted into the pocket [Figure 1], then a periodontal dressing was applied. The gel was repeatedly applied 2 weeks later.

Group 2: participants received a conventional scaling and root planing.

Study design

Clinical assessment

PPD and CAL were measured according to Ramfjord.[11]

Both clinical parameters were determined from a fixed position using a periodontal probe to the deepest probing depth. Customized acrylic stents were used to prevent angulation and positioning errors. Gingival index (GI): the severity of gingival inflammation was given a score from 0 to 3 according to the criteria proposed by Silness-Löe.[12]

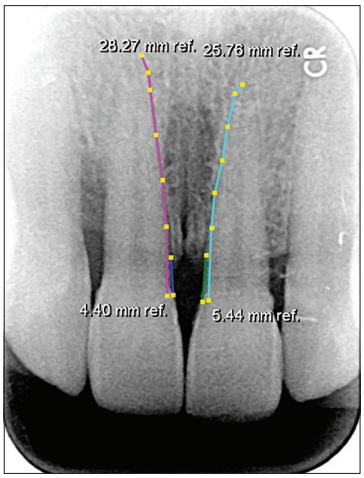

Figure 1: Application of gel foam loaded with plant stem cell and stem cell extract

The above parameters were measured at baseline and 3 months following therapy.

Radiographical assessment

The percentage of interproximal alveolar bone loss

Digital long-cone parallel periapical radiographs were done at baseline and 6 months postoperative. The mean of interproximal bone loss percentage was calculated from the cementoenamel junction (CEJ) to the crest of the bone and divided by the root length as the method described by Hausmann *et al*.[13] using SIDEXIS 4 software [Figure 2].

The alveolar bone mineral density

Interproximal alveolar bone mineral density was measured using Digora 2.7 software (Dental Imaging Technologies Corporation Company, Alpharetta, Georgia, United States). The mean of bone mineral density at a fixed area for the interproximal defect was measured starting from CEJ to 5 mm apical to the crest of the bone defect.

Postoperative care

All participants had postoperative oral hygiene instructions, involving rinsing with 0.1% chlorhexidine (twice daily for 2 weeks), and avoiding dental flossing for 10 days and hard or sticky foods, to avoid material dislodgement. Periodontal dressing removal was performed after 14 days. Supportive periodontal therapy was performed monthly till the end of the study period which includes, periodontal evaluation, and plaque control reinforcement. Furthermore, scaling and root planing were performed as necessary.

Statistical analysis

Paired *t*-test was assessed for comparison between two periods for normally distributed quantitative variables. Mann–Whitney test was used to compare two groups for not normally distributed quantitative variables. The Wilcoxon signed-ranks test was assessed for comparison between two periods for not normally distributed quantitative variables.

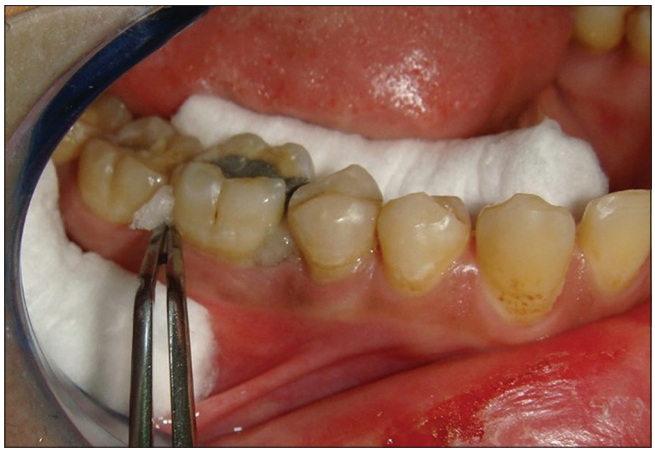

Figure 2: Measurement of mean of interproximal bone loss using SIDEXIS 4 software

Results

The patient's data were uploaded, processed, tabulated, and statistically analyzed using the Statistical Package for the Social Science (SPSS version 23, (IBM Company, New York, United States).

Clinical results

No subjects were lost or excluded over the following 6 months. The performed treatment was well-tolerated by all patients without any side effects. Throughout the study, the level of oral hygiene remained stable.

The two studied groups showed significant improvement in PPD, CAL, and GI at 3 months posttherapy than the mean baseline value ($P \leq 0.05$).

The intergroup comparison demonstrated a significant difference between the two studied groups for PPD and CAL after 3 months ($P > 0.05$) in favor of the study group. Regarding the GI, there was an improvement in favor of the study group, but it was not statistically significant [Table 1].

Radiographic results

The interproximal bone loss percentage results presented a statistically significant decrease in the mean values at 6 months as compared to their baseline values for the study group ($P < 0.05$), whereas the reduction in the control group, was insignificant ($P > 0.05$). At baseline and during the 6 month study period, there was an insignificance difference between the two treated groups ($P > 0.05$) [Table 2].

Regarding the interproximal alveolar bone mineral density, there were statistically significantly higher mean values at 6 months than their baseline values for the study group ($P < 0.05$), whereas the increase in the control group was insignificant ($P > 0.05$). The intergroup comparison showed no statistically significant difference between the two groups at baseline, whereas after 6 months, there was a statistically significant difference in favor of the study group ($P < 0.05$) [Table 2].

Discussion

In developing countries, it is important to always search for alternative forms of therapeutic modalities that are effective yet, cheaper, and less toxic. Stem cell study is generating public attention due to its therapeutic potentials.[14]

Recent technological advancements in herbal medicine, and stem cell therapy, have catch interest to be employed for periodontal regeneration. Hence, this study was carried out to assess clinically and radiographically the possible influence of the direct and local application of Edelweiss stem cells on stage III periodontitis as a nonsurgical treatment.

In a review article conducted by Xue *et al*.,[10] they stated that; a maximum of 80% of the population in developing nations use traditional medicine and medicinal plants for initial healthcare according to the World Health Organization.

Throughout the whole study period, there were no side effects detected as the performed treatment was well-tolerated by all the patients. This was in accordance with Tauchen and Kokoska,[15] who stated that there have been no major side effects reported in any of the published research on the pharmacological activity of Edelweiss. Furthermore, Cho *et al*.,[9] did not report any allergic reactions in volunteers throughout their study. Recently, Naser[16] proved that LACCE did not lead to any dangerous side effects on the skin. However, only Duwensee *et al*.,[17] reported the lipoprotein metabolism altering properties of leoligin as a health risk of Edelweiss.

A concentration of 1% Edelweiss stem cells and stem cells extract was selected in the current study according to Cho *et al*., 2020[9] who recommended the use of 1% Leontopodium Alpinum callus culture extract (LACCE) rather than a smaller concentration because they determined that the probable influence with a smaller concentration of LACCE on keratinocyte cells might be small. Furthermore, we used 1% LACCE in the current study as the material will be used topically and the antibacterial and anti-inflammatory effects may be decreased with the washing effect of saliva.

In the present study, results of the study group showed favorable clinical outcomes after 3 months posttreatment and there were statistically significant higher results regarding the interproximal alveolar bone mineral density after 6 months. This can be explained by the favorable properties of Edelweiss stem cells and stem cell extract.

Anticollagenase properties of Edelweiss were proved by the Institute of Biotechnological Research, reflecting the antiaging component derived from Edelweiss stem cell extracts for its protective and powerful anticollagenase and hyaluronidase activities. It is abundant in leontopodic acids

Table 1: Comparison between the two studied groups according to clinical data

	Study (*n*=20)	Control (*n*=20)	Test of significance	*P*
GI				
Baseline				
Mean±SD	2.6±0.5	2.65±0.49	*U*=190.0	0.799
Median (minimum-maximum)	3 (2-3)	3 (2-3)		
3 months				
Mean±SD	0.30±0.47	0.75±0.79	*U*=138.0	0.096
Median (minimum-maximum)	0 (0-1)	1 (0-2)		
Z (P_0)	4.008* (<0.001*)	4.030* (<0.001*)		
PPD				
Baseline				
Mean±SD	6.1±0.79	6±0.86	*t*=0.384	0.703
Median (minimum-maximum)	6 (5-7)	6 (5-7)		
3 months				
Mean±SD	3.25 0.55	4±1.03	*t*=2.881*	0.006*
Median (minimum-maximum)	3 (2-4)	4 (3-7)		
t_0 (P_0)	21.708* (<0.001*)	9.747* (<0.001*)		
CAL				
Baseline				
Mean±SD	5.65±0.67	5.7±0.92	*t*=0.196	0.846
Median (minimum-maximum)	6 (5-7)	6 (4-7)		
3 months				
Mean±SD	3±0.65	4±1.17	*t*=3.343*	0.002*
Median (minimum-maximum)	3 (2-4)	4 (2-7)		
t_0 (P_0)	24.218* (<0.001*)	7.033* (<0.001*)		

*Statistically significant at $P \leq 0.05$. *t*: Student *t*-test; *U*: Mann-Whitney test; t_0: Paired *t*-test; Z: Wilcoxon signed ranks test; *P*: *P* value for comparing between study and control; P_0: *P* value for comparing between baseline and 3 months. GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment loss; SD: Standard deviation

Table 2: Comparison between the two studied groups according to X ray data

	Study (*n*=9)	Control (*n*=7)	*t*	*P*
Bone loss percentage				
Baseline				
Mean±SD	34.89±10.44	35.09±11.52	0.036	0.972
Median (minimum-maximum)	33.1 (21.2-55)	30.6 (23.8-55)		
6 months				
Mean±SD	25.26±7.7	34.43±10.75	1.993	0.066
Median (minimum-maximum)	28.7 (15-35)	29.9 (24-53)		
t_0 (P_0)	4.587* (0.002*)	2.152 (0.075)		
Bone density				
Baseline				
Mean±SD	91.96±11.02	105.9±14.46	1.890	0.056
Median (minimum-maximum)	93.4 (79-106)	100 (89-132)		
6 months				
Mean±SD	143.9±29.96	108±13.23	3.213*	0.008*
Median (minimum-maximum)	143 (106-186)	102 (93-132)		
t_0 (P_0)	4.937* (0.001*)	2.228 (0.067)		

*Statistically significant at $P \leq 0.05$. *t*: Student *t*-test, t_0: Paired *t*-test, *P*: *P* value for comparing between study and control, P_0: *P* value for comparing between baseline and 3 months, SD: Standard deviation

A and B, which provide a potent antioxidant influence on the skin.[18]

Concerning the anti-inflammatory action of Edelweiss, Tauchen, and Kokoska[15] conducted a review and stated that several research data indicated significant anti-inflammatory benefits of Edelweiss-derived extracts and chemicals in both *in vitro* and animal models. These findings corroborate with previously reported ethnomedicinal information about Edelweiss as it is traditionally used in the treatment of various inflammatory disorders.

One of the first *in vitro* studies of the anti-inflammatory activity of Edelweiss was conducted by Schwaiger *et al*.,[19] In a more recent study, ethanol extract of callus cultures of Edelweiss has significantly decreased inflammatory responses (induced by cytokines, lipopolysaccharide, oxidized low-density lipoprotein, and ultraviolet light) in primary human keratinocytes and endotheliocytes *in vitro* as proved by Daniela *et al*.[20] Likewise, Dobner *et al*.[21] observed anti-edema activity.

These properties can be attributed to the fact that Edelweiss has a unique capacity for survival at severe altitudes and exposure to solar radiation, which provide significant antioxidant and anti-inflammatory properties. Edelweiss stem cells prevent the destruction of hyaluronic acid and collagen by enzymes.[22]

Dobner *et al*.,[23] investigated the *L. alpinum* and observed that, *L. alpinum's* had a significant antibacterial activity justifying its traditional usage for the management of abdominal pains, diarrhea, and dysentery. This was in line with literature data performed by Kankaanpää *et al*.[24]

On the other hand, Schmid *et al*.[25] detected the antisenescence action of an extract of Uttwiler spatlauber stem cells. Senescence is a natural process in which, after around 50 divisions, a cell loses its ability to divide further. This property can be of great value concerning periodontal regeneration.

The strategic use of herbal remedies in stem cell therapy has the potential to provide cost-effective, widely accessible, and nontoxic therapeutic effects. It will undoubtedly result in a new understanding of periodontal tissue regeneration based on stem cell research.[10]

Limitations and future study prospects

There were 40 defects included in this study. A larger sample size and a comparative experimental design are necessary for future studies. The methods we used to evaluate the regenerative effect included clinical examination and digital long-cone parallel periapical radiographs, although accurate results were obtained, the use of cone-beam computed tomography can be used in future studies for its higher sensitivity. Furthermore, long-term results of 1 year or more are needed to build up stronger evidence to prove the role of locally applied edelweiss stem cells on periodontal regeneration. In the future, it is essential to create novel biomaterials and new delivery systems for the growing field of regenerative periodontal therapy.

Conclusion

Several approaches using plant stem cells and/or stem cell extracts for regenerating damaged periodontium are under study with varying degrees of clinical applications. Our results indicate that Locally applied Edelweiss stem cells

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Nil.

Conflicts of interest

There are no conflicts of interest.

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