Clinical factors and cellular responses of in situ human alveolar bone-derived mesenchymal stromal cells associated with early peri-implant marginal bone loss: a prospective cohort pilot study

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Clinical factors and cellular responses of in situ human alveolar bone-derived mesenchymal stromal cells associated with early peri-implant marginal bone loss: a prospective cohort pilot study

Directed by Professor Chang-Sung Kim

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감사의 글

박사 과정을 마치기까지 많은 분들이 부족한 저에게 도움을 주셨습니다. 가장 먼저 이 논문이 완성되기까지 끊임없는 지도와 따뜻한 조언으로 이끌어 주신 김창성 지도교수님께 진심으로 감사드립니다. 또한 학문에 대한 열정을 일깨워 주신 김종관 교수님, 항상 아낌없는 관심과 격려를 보내주신 채중규 교수님, 치주과 의사로서 큰 본보기가 되어주신 조규성 교수님, 연구자로서 새로운 시각의 중요성을 깨닫게 해 주신 최성호 교수님, 날카롭고 창의적인 안목을 키울 수 있게 해 주신 정의원 교수님, 끝까지 세심하고 아낌없는 조언을 주신 이중석 교수님, 수련 기간 동안 많은 격려와 도움 주신 차재국 교수님께 깊이 감사드립니다. 또한 바쁘신 와중에도 심사를 맡아주시고 조언을 아끼지 않으신 이동원 교수님, 김영택 교수님, 임현창 교수님께도 감사드립니다. 아울러 대학원 과정 동안 곁에서 큰 힘이 되어 준 선후배 의국원 및 연구원들에게도 감사의 마음을 전합니다.

마지막으로, 언제나 한결 같은 마음으로 제게 믿음과 지지를 보내주시는 부모님과 듬직한 동생에게 사랑과 감사를 말하고 싶습니다.

2020 년 6 월

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Abstract

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Purpose: The aim of this study was to investigate clinical factors and cellular responses of in situ human alveolar bone-derived mesenchymal stromal cells involved in early peri-implant marginal bone loss.

Materials and methods: 37 completely or partially edentulous patients were enrolled in this study. Periapical radiographs were taken at the time of implant surgery, at 3-month follow up, and at 1-year follow-up. Univariate analysis and multiple logistic regression were performed to investigate the associations between marginal bone loss (>1 mm) and study variables. The mRNA expression levels of 21 bone-remodeling- and tissue-healing-

associated genes were analyzed by subgroup.

Results: 31 patients with 98 implants were followed. The incidence and mean amount of bone loss were higher for overdenture group than for other prosthesis group, and higher for the maxilla group than for the mandible group. In addition, the bone loss subgroup showed lower mRNA expression levels of runt-related transcription factor-2 (Runx-2), bone morphogenetic protein-2 (BMP-2) and peroxisome proliferator-activated receptor gamma-2 (PPARγ-2) and higher receptor activator of NKκB ligand/osteoprotegerin (RANKL/OPG) ratio than the subgroup without bone loss.

Conclusions: Within the limitations of the study, certain genes involved in bone remodeling (Runx-2, BMP-2 and PPARγ-2) and RANKL/OPG ratio are correlated with early peri-implant bone loss, with the type of suprastructure and the involved jaw being significant clinical factors.

Keywords: Initial bone loss, Clinical research, logistic regression, mRNA expression

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I. Introduction

High survival rates of dental implants have been found in many long-term clinical trials [1,2], which has led to the current focus of clinical research being on identifying more-specific outcomes, such as peri-implant marginal bone [3]. Continuous peri-implant marginal bone loss can threaten the longevity of the implant-supported prosthesis [4]. In particular, a substantial amount of bone loss occurs after the first year of function [5]. This early peri-implant marginal bone loss, is not only an important criteria for success of dental implant, but also a crucial factor for the subsequent progression of marginal bone [6].

Thus, minimal or no marginal bone loss after connecting the implant and abutment is a critical indicator for the long-term success of implant [7].

Many plausible hypotheses have been proposed about early peri-implant marginal bone loss, such as surgical trauma [8], biological width [9,10], occlusal overload [11] and microgaps [12]. However, there is little evidence for the apparent cause of early bone loss, and few studies have attempted to identify risk indicators while focusing on early bone loss. Prospective longitudinal studies and multivariate statistical analysis are required for identifying the true risk factors [13].

Several studies recent have focused on identifying specific host characteristics for peri-implant marginal bone loss in addition to those clinical and physiologic factors. Albrektsson et al. (2014) stated that the initial marginal bone loss of dental implants represents an imbalanced host response to the dental implant rather than a disease [14]. Other authors reported that certain host characteristics such as genetic polymorphism [15,16] or activities of molecules in the crevicular fluid [17] are correlated with peri-implant marginal bone loss. Several researchers have recently proposed that human alveolar bone-derived mesenchymal stromal cells (hABCs) are a key factor for the homeostasis of alveolar bone and regenerative strategy due to their differentiation potential in bone metabolism [18,19]. We therefore hypothesized that specific genes are involved in bone remodeling, the wound healing capacity, and early peri-implant marginal bone loss. To our knowledge, this is the first clinical study to evaluate the quantitative association between marginal bone loss and the mRNA expression of hABCs by using real-time

reverse-transcription polymerase chain reaction (RT-PCR) analysis.

The aim of the prospective cohort pilot study was to identify clinical risk factors relevant to the host and the implant-supported prosthesis and cellular responses of in situ human alveolar bone-derived mesenchymal stromal cells (hABCs) involved in early peri-implant marginal bone loss.

II. Materials and Methods

1. Study Design and participants

In total, 37 patients requiring implant-supported rehabilitation were recruited at the Department of Periodontology, Yonsei University Dental Hospital between December 2013 and November 2014. All patients who qualify for enrollment were participated in the study. The Institutional Review Board of Yonsei University reviewed and approved the clinical research protocol before the clinical trial begins (Yonsei IRB No. n2-2013-0037). All patients were led to write the informed consent before being enrolled in the study.

The following inclusion criterions were applied: (1) adult male or female (≥18 years) in good systemic health, (2) completely or partially edentulous patients needing dental implantation, (3) patients with pristine periodontal health or well-maintained clinical periodontal health, (4) patients having sufficient alveolar bone volume that could receive dental implants and (5) patients having stable occlusal relationship. The exclusion criteria were (1) uncontrolled systematic diseases (e.g., heart disease or metabolic disease), (2) medications known to interfere with bone metabolism, (3) pregnancy, (4) current smoker or history of smoking within the past 10 years or (5) extraction sockets with a previous bone grafting procedure. The study followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for prospective cohort studies [20].

2. Implant placement and prosthetic treatment

All patients were treated by 8 skilled surgeons working at the Department of Periodontology, Yonsei University Dental Hospital using the same implant system (Luna, Shinhung, Seoul, Korea). The implant used had a surface with sand-blasted/acid-etched, double thread and internal conical seal design; the diameter of abutments is smaller than that of implant platform (platform switching). All of the implants were installed in accordance with the manufacturer's instructions based on appropriate radiographic planning before the surgery. The surgery involved low-speed drilling (50 to 200 rpm) to obtain bone particles as outlined previously [21]. The bone particles were harvested from a single randomly selected site on patients with multiple operation sites (one sample from each patient). A guided bone regeneration procedure was performed using autogenous bone or xenografts, with resorbable collagen membrane if necessary.

The patients were referred to prosthetic specialists working at the Department of Prosthodontic, Yonsei University Dental Hospital to complete the final prosthesis 3 months or 4 months after implant surgery. A typical maintenance visit for patients was scheduled every 3 months to evaluate any changes in their oral condition and it lasted for at least 1 hour at every visit. All patients were given the rigorous oral hygiene instruction for cleaning the peri-implant soft tissue. Tooth and implant sites that showed easy bleeding on probing were carefully cleaned by using rubber cups in conjunction with antiseptic oral rinses.

3. Study variables

The host-related variables were listed as follows: (1) gender (male or female), (2) age (≤60 or >60 years), (3) involved jaw (mandible or maxilla), (4) implant location (posterior [premolar and molar] or anterior [incisor and canine]), (5) bone quality determined by the surgeon who performed the surgery registered in the clinical trial (classification according to Lekholm and Zarb as D2, D3 or D4; to standardize subjectivity of clinical bone quality judgement, all surgeons participated in the calibration meeting before the clinical trial.) and (6) relative mRNA expression levels of genes associated with bone remodeling and tissue healing.

The implant- and prosthesis-related variables were (1) implant diameter (narrow [3.5 mm], regular [4.0 mm or 4.5 mm] or wide [5.0 mm]), (2) implant length (short [7mm or 8.5mm] or long [10 mm or 11.5 mm]), (3) insertion torque (≤20 N or >20 N), (4) initial implant stability quotient (ISQ) (<70 or ≥70), (5) depth of implant placement (subcrestal [with the mesial or distal implant shoulder placed at least 0.5 mm to 1mm]) or equicrestal [with the mesial and distal implant shoulder placed within 0.5mm below the alveolar crest]) (6) presence of GBR procedure and (7) type of suprastructure (single crown, fixed partial denture or overdenture).

4. RNA extraction and quantitative RT-PCR

The isolation procedures performed in the laboratory were as follows: Bone chips

obtained by low-speed drilling were put in a 50-ml tube and placed in 20-ml medium comprising a-MEM, 15% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Invitrogen, Massachusetts, USA) and 100 μM L-ascorbic acid-2-phosphate (Sigma-Aldrich, St. Louis, MO, USA). A sequential digestion method with 2 mg/ml collagenase (Wako Pure Chemicals, Osaka, Japan) and 1 mg/ml dispase (Gibco, Invitrogen, Massachusetts, USA) was used for the immediate isolation of the hABCs. They were seeded into culture dishes(75T) (Thermo Scientific, Gibco, Invitrogen, Massachusetts, USA), incubated for 3 days at 37°C to allow for cell attachment. To remove any non-adherent cells, the dishes were washed twice with phosphate-buffered saline.

The stem-cell-like characteristics of hABCs at passage 2 or 3 were investigated according to Park et al. (2012) [21]. The primer set for each differentiation and the tissue-healing markers were designed using the software program (Primer software version 3.0, Applied Biosystems, Massachusetts, USA). Each cell was harvested using Trizol (15596018, Gibco, Invitrogen, Massachusetts, USA), and total cell RNA was prepared. cDNA synthesis from the isolated total RNA was carried out using the High Capacity RNA-to-cDNA kit (4387406, Applied Biosystems, Massachusetts, USA). The subsequent PCR was conducted using the PCR system (StepOne real-time PCR system, Applied Biosystems, Massachusetts, USA) and the PCR kit (Power SYBR Green PCR Master kit, Applied Biosystems, Massachusetts, USA) under the following conditions: an initial polymerase activation step at 95°C for 10 min, followed by 40 amplification

cycles comprising denaturation at 95°C for 15 s and annealing and extension at 60°C for 60 s. The relative levels of mRNA expression were quantified by comparison with an internal standard (glyceraldehyde 3-phosphate dehydrogenase). With the same total DNA, each analysis was performed in triplicate. The bone-remodeling- and tissue-healing-associated genes in alveolar bone were grouped into the following categories (Table 1): (1) osteogenesis, (2) osteoclastogenesis, (3) adipogenesis and (4) tissue healing.

5. Radiographic evaluation of peri-implant marginal bone loss

To obtain standardized periapical radiographs, a long-cone parallel technique and a digital imaging software system immediately after implant placement and at 3-month and 1-year follow-up visits (Figure 1). Care was taken with XCP film holding device (Dentsply Rinn, Elgin, IL, USA) to locate the X-ray film parallel to the long axis of the implant and clearly show the threads on both sides of the fixture. Two researchers, who were not involved in the surgery, independently measured the radiographs (DJ Kim and JK Cha). To avoid introducing any bias into the observations, all of the measurements were made on images at the same magnification using image-processing software (Photoshop 12.0, Adobe Systems, California, USA). For each implant, the loss of marginal bone height was determined as the distance between the first visible bone-to-implant contact and the fixture shoulder. If marginal bone was seen above the fixture shoulder, the measurement was recorded as 0 in order to avoid introducing bias into the

results. To adjust for any magnification error, the measured distance was calibrated based on the ratio of the measured implant length and the actual implant length. All measurements were made at the mesial and distal surfaces of each implant. The intraclass coefficient was calculated to evaluate the consistency between the two examiners; it was found to be 0.95 (p=0.002). The amount of peri-implant marginal bone loss over 3-month and 1-year were determined.

6. Statistical analysis

The incidence of peri-implant marginal bone loss greater than 1mm was normalized to the total number of implants placed. Differences in the incidence of peri-implant marginal bone loss greater than 1mm between the categories of each risk factors (age, gender, involved jaw, implant location, bone quality, implant diameter, implant length, insertion torque, initial ISQ, implant placement depth, GBR procedure and type of suprastructure) were analyzed by the chi-square test or Fisher's exact test. Statistically relevant risk factors were identified by performing multiple logistic regression analyses. Variables with a probability value of 0.2 or less in the univariate analysis were entered into the model using backward selection with the significance level for removal set at 0.1 [22]. To facilitate precise interpretations, the odds ratio (OR), the standard error of the mean (SEM) and 95% confidence interval (CI) for each risk factor are presented.

The statistical analysis of mRNA expression of bone-remodeling- and tissue-healing-associated genes was performed at the patient level. The mRNA data of each group are

shown as median and interquartile values due to their highly non-Gaussian distribution. Differences in the expression level between with and without marginal bone loss subgroups were tested using the non-parametric Mann-Whitney U-test for unpaired data.

All statistical analyses were performed using the statistics software (IBM SPSS Statistics (version 21), IBM Corporation, Chicago, IL, USA) with the significance level set to $\alpha=0.05$.

III. Results

1. Patients/Dropouts

The 1-year follow-up was completed in 31 of the 37 (86.5%) patients with 98 implants. The six dropouts were due to personal reasons; withdrawal of consent before surgery (n=5) and not attending the clinic on a scheduled visit (n=1). The included-subject pool comprised of 17 males (54.8%) and 14 females (45.2%). The age of the patients ranged from 22 to 74 years, with a mean of 53 years. The follow-up time was 12.4±0.7 months (mean±SD).

2. Implant survival and overall peri-implant marginal bone loss

100 dental implants were eligible for examination after 3 months, and 98 implants after 1 year. None of the implants failed among patients evaluated over the entire study period, representing a 100% survival rate. All of the prostheses (53 single crowns, 13 fixed partial denture, 5 overdenture) survived and remained stable without any complications.

The mean peri-implant marginal bone loss (n=98) was 0.05±0.13 mm at the 3-month follow-up and 0.30±0.60 mm at the 1-year follow-up. Overall, nine implants (9.2%) showed more than 1.0 mm of the mean peri-implant marginal bone loss (1.91±0.74 mm).

3. Clinical factors affecting peri-implant marginal bone loss

Table 2 presents the results of the univariate analysis of the incidence of early peri-implant marginal bone loss (>1mm) of predictor variables. The rate of peri-implant marginal bone loss was highest for implants supporting overdentures (50.0%).

The rate of early peri-implant marginal bone loss differed significantly with implant location (p=0.005), bone quality (p=0.048) and type of suprastructure (p<0.001), but not with age, gender, involved jaw, implant diameter, implant length, insertion torque, initial ISQ, implant placement depth or GBR procedure.

From the backward logistic regression analysis of the involved jaw, implant location, bone quality, implant length and type of suprastructure, the ORs for early peri-implant marginal bone loss were significantly higher for overdenture implants (OR=116.90, SEM=1.20, 95% CI: 11.09-1232.85, p=0.001) and those placed in the maxilla (OR=12.83, SEM=1.26, 95% CI: 1.08-152.26, p=0.043). The other variables such as implant location, bone quality and implant length were excluded from the final model.

After evaluating the amount of peri-implant marginal bone loss relative to both the involved jaw and type of suprastructure variables (Figure 2), the early peri-implant marginal bone loss was highest in the maxillary overdenture group (1.72±0.84 mm) and lowest in the mandibular single-crown/fixed-partial-denture group (0.10±0.18 mm). Statistically significant differences were present between (1) the mandibular overdenture group and the single-crown/fixed-partial-denture groups (both in the mandible and maxilla (0.14±0.29mm)) and (2) the maxillary overdenture group and the mandibular overdenture group (0.49±0.33mm).

4. Association between early peri-implant marginal bone loss and relative mRNA expression of genes

Among the 31 samples, quantitative RT-PCR was not performed in two bone samples due to the failure of RNA extraction and cDNA synthesis, thus, 29 eligible samples were analyzed.

The results of multivariable analysis showed that type of suprastructure (overdenture) was the strongest prognostic clinical factor. In order to adjust for the confounding effects of environmental factors such as type of suprastructure, the difference in mRNA expression was analyzed in subgroups divided according to the type of suprastructure. Intragroup comparisons in the overdenture subgroup were not performed due to smallness of the sample (n=2).

Table 3 and Figure 3 indicate that there was an association between early peri-implant marginal bone loss and the mRNA expression of bone-remodeling- and tissue-healing-associated genes for single crowns and fixed dental prosthesis (n=27). Intragroup comparisons were based on the presence or absence of marginal bone loss including minor bone change. There were statistically significant differences in the runt-related transcription factor-2 (Runx-2) (p=0.041) and bone morphogenetic protein-2 (BMP-2) (p=0.032) osteogenesis-related genes, and in the peroxisome proliferator-activated receptor gamma-2 (PPARγ-2) (p=0.025) adipogenesis-related gene and the receptor activator of nuclear factor κ ligand (RANKL)/osteoprotegerin (OPG) ratio (p=0.041)

between the groups with and without marginal bone loss. No significant differences were found in any other bone-remodeling- or tissue-healing-associated genes.

IV. Discussion

This study focused on identifying clinical factors and cellular responses of in situ human alveolar bone-derived mesenchymal stromal cells (hABCs) for early peri-implant marginal bone loss. To remove the influence of brand-specific characteristics on marginal bone level, we used a uniform implant system having an internal conical connection and platform switching design.

The survival rate was perfect during the first postimplantation year in the present study, with no implant or prosthesis complications. Most of the implants (90.8%) showed minimal or no bone loss. These results are consistent with those of previous short-term (6-months after loading or 1-year after insertion) studies using the same implant–abutment connection system [23-25].

This study represents the first attempt to determine the quantitative association between the mRNA expression of hABCs and early peri-implant marginal bone loss. Runx-2 is essential for osteoblastic precursor cell differentiation, and the null mutation of Runx-2 affects osteoblast differentiation in vivo [26]. BMP-2 has also been found to play an important role in osteoblastogenic differentiation, belonging to the transforming growth factor-β superfamily [27]. The decreased mRNA expression level of these two osteogenesis-related genes in the presence of marginal bone loss may be related to a low potential for bone formation capacity, and it seemed to affect the early marginal bone loss. PPARγ-2 is the key factor driving the adipogenic differentiation of mesenchymal stromal cells, and

was also decreased in the presence of marginal bone loss.

This similar pattern of mRNA expression in Runx-2, BMP-2 and PPARγ-2 is particularly interesting because it is generally accepted that osteoblastic and adipogenic differentiation exhibit a reciprocal relationship in normal bone metabolism [28]. This can be explained by a dual role of BMP-2 in mesenchymal stromal cell differentiation, and it means that the determination of the osteoblast or adipocyte lineage from a mesenchymal stromal cell precursor is regulated by BMPs and their distinct types of receptors [29]. Both RANKL and OPG are produced by osteoblasts and play crucial roles in regulating osteoclastogenesis [30]. Their mRNA levels were shown to be correlated with altered bone resorption in response to physiologic stimuli [31]. This is consistent with the this study finding that the RANKL/OPG ratio was higher (with a high potential for osteoclastic activity) in the presence of marginal bone loss. There is no particular association between the other genes involved in tissue healing and early peri-implant marginal bone loss. Because there was no similar study, we were not able to compare these results with other studies.

The multivariate analyses identified the type of suprastructure and the involved jaw as risk indicators for early peri-implant marginal bone loss. It was found that the OR and the mean amount of early peri-implant marginal bone loss were significantly higher in implants with an overdenture than in implants with a single crown or fixed partial denture. This is consistent with a previous report of a significant association between greater bone loss and the presence of a removable prosthesis [32]. In contrast, the systematic review by

Bryant et al. (2007) found similar levels of crestal bone loss in the first postimplantation year for both fixed and removable prostheses [33]. In this review, however, the difference in prosthetic type did not reach statistically significant conclusion. Also there was a lack of research on maxillary removable design, so only a site-specific comparison was possible between mandible prostheses. In terms of prosthesis, the clinical influence of suprastructure on marginal bone loss needs high interest and a randomized-controlled clinical study with high number of patients should be performed to identify it.

Another factor associated with a higher OR for early peri-implant marginal bone loss was implant placement in the maxilla. This is consistent with the clinical findings of Vervaeke et al. (2015) [34]. The mean peri-implant marginal bone loss of the maxilla overdenture group was significantly higher than that of the mandible overdenture group. In detail, most of the implants with early bone loss were restored with maxillary implant overdentures. It is generally accepted that the maxillary implant overdenture is often correlated with reduced bone quantity/quality and vulnerable on biomechanical forces [35,36]. Destructive cantilever forces can be present in the anterior and premolar regions when dental implants collide with natural teeth [37].

Some limitations of present study need to be considered when interpreting the results. Its prospective cohort pilot design meant that relatively few patients were treated. Although significant differences were found in the Mann-Whitney U-test, the statistical power is lower for non-parametric tests than for parametric tests. Further clinical studies involving larger samples are necessary to properly assess the significance of the results of

the present study. Another limitation of this study was the use of standardized digital periapical radiographs to evaluate peri-implant marginal bone loss. The disadvantage of using periapical radiographs is that these are only two-dimensional images (in the mesial and distal directions) that might not accurately represent the morphology of the defect site. However, periapical radiography is a widely accepted method used for assessing the interproximal crestal bone changes of dental implants and evaluate the peri-implant health [4].

V. Conclusion

Within the limitations of this prospective single-cohort study, the present data demonstrate that some genes involved in bone remodeling (Runx-2, BMP-2 and PPARγ-2) and the RANKL/OPG ratio are correlated with early peri-implant marginal bone loss. The suprastructure supporting the implant and the involved jaw were found to be significant clinical risk factors for early peri-implant marginal bone loss.

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Figure Legends

Figure 1. Intraoral radiographs of a patient with implants placed in molar regions. A. after implant placement, B. after 3-month follow-up and C. after 1-year follow-up.

Figure 2. Peri-implant marginal bone loss in relation to the involved jaw and type of suprastructure at the 1-year follow-up. Different symbols indicate statistical significant difference. Higher bone loss tendency was observed in the overdenture group than in the other suprastructure group. In addition, higher bone loss tendency was observed in maxilla than in mandible. Mn indicates mandible; Mx, maxilla; SC, single crown; FPD, fixed partial denture; OD, overdenture.

Figure 3. Box plots showing the median, quartiles, and minimum and maximum relative gene expression levels of runt-related transcription factor-2 (Runx-2), bone morphogenetic protein-2 (BMP-2) and peroxisome proliferator-activated receptor gamma-2 (PPARγ-2), and receptor activator of nuclear factor κ ligand (RANKL)/osteoprotegerin (OPG). The bone loss (+) subgroup has lower level of Runx-2, BMP-2, and PPARγ-2 and higher RANKL/OPG ratio than bone loss (-) subgroup. All differenced were statistically significant.

Tables

Table 1. Primer sequences and specific parameters of the real-time RT-PCR.

Gene type	Gene	Primer sequence		Annealing temperature (℃)	GenBank no.	Product size (bp)
		Forward (5′-3′)	Reverse (3′-5′)			
Osteogenesis-related	Osteocalcin	CAGGAGGGCAGCGAGGTA	GGCTCCCAGCCATTGATACA	62	X53698	60
	OPG	AGGCACTTGAGGCTTTCAGT	ACCCTGTGGCAAAATTAGTCA	60	NM_002546.3	120
	ALPL	ATTGACCACGGGCACCAT	CTCCACCGCCTCATGCA	61	NM_000478.4	57
	Runx-2	AGCCCTCGGAGAGGTACCA	TCATCGTTACCCGCCATGA	60	NM_004348.3	57
	BMP-2	AAAGGGCATCCTCTCCACAA	AGGCGTTTCCGCTGTTG	58	NM_001200.2	62
	BSP	GCGAAGCAGAAGTGGATGAAA	TGCCTCTGTGCTGTTGGTACTG	59	NM_004967.3	65
	Osteopontin	CCTGCCAGCAACCGAAGT	CTCGGCCATCATATATGTGTCTACTG	60	NM_001040058.1	62
Osteoclastogenesis-related	RANKL	CAGCCTTTTGCTCATCTCACT	TTATGGGAACCAGATGGGAT	55	AF019047.1	112
	ICAM-1	TGAGCAATGTGCAAGAAGATAGC	CCCGTTCTGGAGTCCAGTACA	60	NM_000201.2	104
	M-CSF	GAGCTGCTTCACCAAGGATTATG	CCCGTTCTGGAGTCCAGTACA	60	NM_172211.3	92
	IL-1	GATCCACACTCTCCAGCTGCA	CAACCAACAAGTGATATTCTCCATG	62	XM_017030279.1	152
	IL-7	TGCCCTAATCCGTTTTGACCATGGT	ACACGAACTTTAGCTGCATCCTCCA	62	NM_000880	97
Adipogenesis-related	LPL	TGGACTGGCTGTCACGGGCT	GCCAGCAGCATGGGCTCCAA	64	NM 00237.2	167
	FABP4	GCGTCATGAAAGGCGTCACT	GTCAACGTCCCTTGGCTTATG	59	NM_001442.2	63

	PPARγ-2	TCAGGGCTGCCAGTTTCG	GCTTTTGGCATACTCTTGATCTC	61	U79012.1	61
Tissue healing-related	VEGF	GAAGTGGTGAAGTTCATGGATGTC	CACCAGGGTCTCGATTGGAT	59	NM_001025366.2	63
	TGF-β	GGGAAATTGAGGGCTTTCG	AGTGTGTTATCCCTGCTGTCACA	61	X03205.1	61
	HGF	TCTGGTTCCCCTTCAATAGCA	GGTCAAATTCATGGCCAAATTC	56	NM_000601.4	63
	SDF-1	GCCCGTCAGCCTGAGCTA	TGGCAACATGGCTTTCGAA	61	NM_199168.3	56
	EGF	CAAAACGGAGGCTGTGAACA	CGACACGAACACCAAGCAGTT	58	NM_001963	62
	IL-6	GCTGCAGGCACAGAACCA	GCTGCGCAGAATGAGATGAG	61	NM_000600.3	58
Housekeeping	GAPDH	AGGCTGTGGGCAAGGTCAT	GGAAGGCCATGCCAGTGA	62	NM_002046.4	57

OPG indicates osteoprotegerin; ALPL, alkaline phosphatase; Runx-2, runt-related transcription factor-2; BMP-2, bone morphogenetic protein-2; BSP, bone sialoprotein; RANKL, receptor activator of nuclear factor κ ligand; ICAM-1, intercellular adhesion molecule-1; LPL, lipoprotein lipase; FABP4, fatty-acid-binding protein-4; PPARγ-2, peroxisome proliferator-activated receptor gamma-2; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; SDF-1, stromal cell-derived factor-1; IL, interleukin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TGF-β, transforming growth factor-beta; M-CSF, macrophage colony-stimulating factor

Table 2. Implant and treatment characteristics according to peri-implant marginal bone loss (>1 mm) (Univariate analysis; n=98).

Variable type	Variable	No. of implants	Implants with peri-implant bone loss	p
Host-related	Age (years)			
	≤60	64	4 (6.25%)	0.274
	>60	34	5 (14.71%)	
	Gender			
	Male	53	4 (7.55%)	0.728
	Female	45	5 (11.11%)	
	Involved jaw			
	Mandible	41	1 (2.44%)	0.075
	Maxilla	57	8 (14.04%)	
	Implant location			
	Posterior	82	4 (4.88%)	0.005*
	Anterior	16	5 (31.25%)	
	Bone quality			
	D2	41	4 (9.76%)	
	D3	39	1 (2.56%)	0.048†
	D4	18	4 (22.22%)	
Implant- or prosthesis-related	Implant diameter			
	Narrow	3	1 (33.33%)	0.253
	Regular	95	8 (17.22%)	
	Implant length			
	Short	35	1 (2.86%)	0.151
	Long	63	8 (12.70%)	
	Insertion torque (N)			
	>20	85	9 (10.59%)	0.602
	≤20	13	0 (0%)	
	Initial ISQ			
	≥70	75	8 (10.67%)	0.681
	<70	23	1 (4.35%)	
	Implant placement depth			
	Crestal	27	4 (14.81%)	0.255
	Subcrestal	71	5 (7.04%)	
	GBR procedure			
	No	81	7 (8.64%)	0.653
	Yes	17	2 (11.76%)	

	Type of suprastructure			
	SC	53	0 (0%)	
	FPD	29	1 (3.45%)	<0.001‡
	OD	16	8 (50.00%)	

*,†,‡Statistically significant

†D4 vs D2, D3

‡overdenture (OD) vs single crown (SC), fixed partial denture (FPD)

Table 3. Relative mRNA expression levels of bone-remodeling- and tissue-healing-associated genes in patients with or without early peri-implant marginal bone loss.

Gene type	Gene	Multiplier	Without marginal bone loss (n=15)	With marginal bone loss (n=12)	p
			Median (IQR)	Median (IQR)	
Osteogenesis-related	Osteocalcin	10^{-5}	0.57 (0.48–1.52)	0.80 (0.47–1.20)	0.94
	OPG		17.46 (1.31–40.00)	4.02 (0.92–9.97)	0.20
	ALPL	10^{-5}	1.26 (0.21–4.03)	2.31 (0.41–10.01)	0.26
	Runx-2	10^{-5}	**7.07 (5.32–13.50)**	**3.65 (2.43–7.93)**	**0.04***
	BMP-2	10^{-5}	**0.22 (0.15–0.71)**	**0.17 (0.08–0.20)**	**0.02***
	BSP	10^{-5}	0.25 (0.06–0.50)	0.14 (0.08–0.52)	0.76
	Osteopontin	10^{-5}	0.90 (0.70–2.33)	2.16 (0.78–6.15)	0.24
Osteoclastogenesis-related	RANKL		0.04 (0.01–0.09)	0.08 (0.05–0.25)	0.08
	ICAM-1	10^{-3}	5.74 (2.85–17.85)	6.12 (3.50–9.83)	0.76
	M-CSF		0.06 (0.02–0.09)	0.07 (0.05–0.16)	0.43
	IL-1	10^{-3}	1.38 (0.31–2.67)	1.91 (1.38–3.21)	0.26
	IL-7		233.23 (9.13–407.10)	199.03 (58.77–445.66)	0.55
	RANKL/OPG		**0.00 (0.00–0.03)**	**0.05 (0.00–0.08)**	**0.04***
Adipogenesis-related	LPL	10^{-5}	0.14 (0.00–0.89)	0.18 (0.01–0.55)	0.98
	FABP4	10^{-5}	0.12 (0.02–0.50)	0.14 (0.05–0.28)	0.91
	PPARγ-2	10^{-5}	**0.76 (0.44–1.22)**	**0.39 (0.24–0.64)**	**0.03***
Tissue	VEGF	10^{-5}	14.93 (10.57–17.95)	17.75 (12.73–22.04)	0.18

healing-related	TGF-β	10^{-5}	35.24 (32.40–48.14)	34.38 (30.11–51.22)	0.91
	HGF	10^{-5}	11.22 (5.98–21.68)	13.61 (9.59–35.08)	0.26
	SDF-1	10^{-5}	60.15 (41.37–86.45)	68.48 (52.82–110.68)	0.17
	EGF	10^{-5}	0.17 (0.06–0.56)	0.43 (0.12–1.05)	0.11
	IL-6	10^{-5}	3.11 (1.67–5.71)	1.61 (0.75–3.85)	0.26

*Statistically significant

IQR indicates interquartile range.

Figures

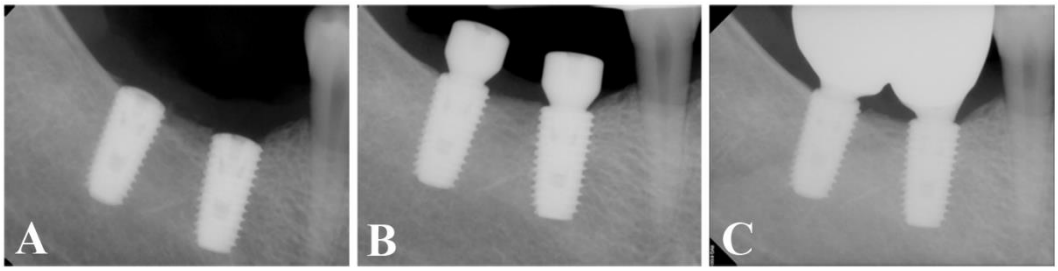

Figure 1

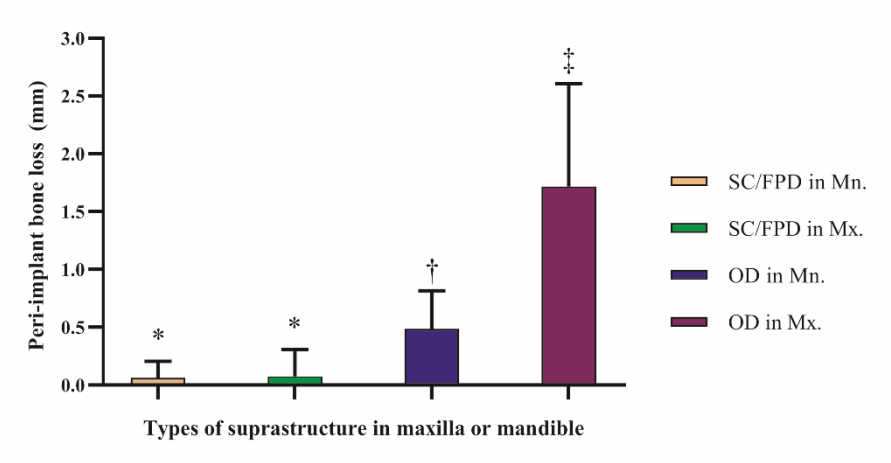

Figure 2

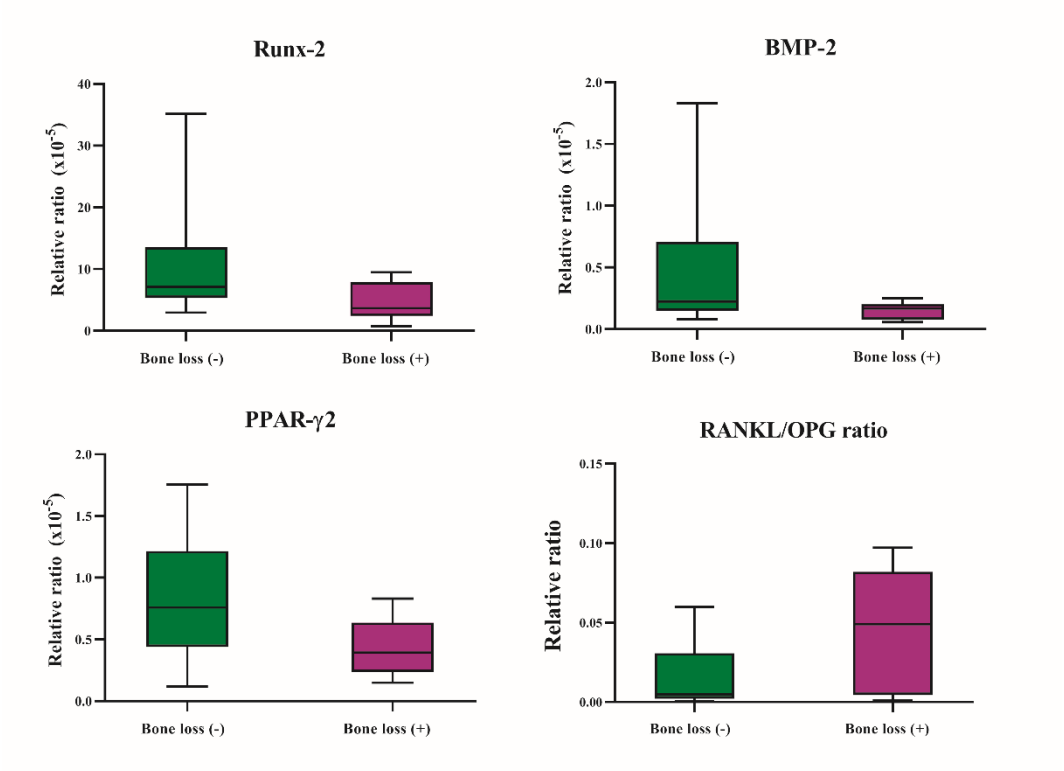

Figure 3

국문요약

초기 임플란트 변연골 소실과 연관된 임상적 요인 및 치조골 유래 중간엽 줄기세포의 세포 반응 : 전향적 코호트 예비 연구

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초기 임플란트 변연골 소실은 임플란트의 성공을 평가하는 중요한 기준일 뿐만 아니라 이후의 변연골의 변화에 결정적인 요인이다. 그 원인에 대한 많은 가설들이 제시되었으나, 임상적 요인을 찾는 연구는 많지 않으며 전향적이고 수직적 연구가 필요하다. 또한 최근의 연구는 임플란트 변연골 소실에 대한 임상적 요인뿐만 아니라, 숙주 특성에 초점을 맞추고 있다. 우리는 골 개조 및 조직 치유에 중요한 요소인 치조골 유래 중간엽 줄기세포 내의 특정한 유전자 발현이 초기 임플란트 변연골 소실과 연관이 있을 것이라고 가정하였으며, 이에 대한 정량적인 관계를 평가하고자 한다.
본 연구는 초기 임플란트 변연골 소실과 연관된 임상적 요인 및 치조골 유래 중간엽 줄기세포의 세포 반응을 연구하는데 목적을 둔다.

37 명의 완전 또는 부분 무치악 환자들이 본 연구에 등록되었다. 치근단 방사선 사진이 임플란트 수술 시, 3 개월 추적 관찰, 그리고 1 년 추적 관찰 시에 촬영되었다. 1mm 이상의 변연골 소실과 연구 요소들과의 연관성을 조사하기 위해 단변량 분석과

다중 로지스틱 회귀 분석이 시행되었다. 21 개의 골 개조 및 조직 치유에 연관된 유전자들의 mRNA 발현량은 하위 그룹으로 나누어 분석되었다.

총 31 명의 환자와 98 개의 임플란트가 추적되었다. 전체 연구 기간동안 실패한 임플란트는 없었으며, 1 년 추적 시 평균 변연골 소실량은 0.30±0.60mm 이었다. 단변량 분석 결과, 임플란트 식립 위치(p=0.005), 골질 (p=0.048) 및 임플란트 상부구조물의 종류 (p<0.001)가 유의한 요인으로 나타났으며, 다중 로지스틱 회귀 분석 결과 임플란트 상부구조물의 종류와 식립된 악궁이 최종 모델에 포함되었다. 피개의치 임플란트의 골소실 발생률 (OR=116.90, SEM=1.20, 95% CI: 11.09-1232.85, p=0.001) 과 평균 골소실량이 다른 보철물 임플란트보다 높았으며, 상악 임플란트의 골소실 발생률 (OR=12.83, SEM=1.26, 95% CI: 1.08-152.26, p=0.043)과 평균 골소실량이 하악 임플란트보다 높았다. 임플란트 상부 구조물의 종류에 따른 그룹내 분석 (단일 수복물 및 고정성 부분의치)에서, 골소실이 발생한 하위 그룹이 골소실이 발생하지 않은 하위 그룹보다 더 낮은 Runx-2 (p=0.041),BMP-2 (p=0.032), PPARγ-2 (p=0.025) mRNA 발현량을 보였으며, 더 높은 RANKL/OPG 비율 (p=0.041)을 보였다.

본 연구의 한계 내에서, 임플란트 상부구조물의 종류와 식립된 악궁이 유의한 임상적 요인이며 골 개조에 관여하는 특정 유전자들 (Runx-2, BMP-2, PPARγ-2)과 RANKL/OPG 비율이 초기 임플란트 변연골 소실과 연관되어 있다.

핵심되는 말 : 초기 골 소실, 임상 연구, 로지스틱 회귀, mRNA 발현