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Research Article

The influence of biological width violation on marginal bone resorption dynamics around two-stage dental implants with a moderately rough fixture neck: A prospective clinical and radiographic longitudinal study

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Abstract

Purpose: In the present study, biological width is considered a specific concept, revealing the relationship between its vertical dimension and structure and its ability to provide internal environment integrity and thus protect the underlying tissues and prevent marginal bone resorption. The study aims to assess the dynamics of marginal bone resorption depending on biological width violation.

Materials and Methods: Forty-three patients with 97 implants were included in this study. The marginal bone level and biological width dimension were evaluated based on clinical and radiographic examinations performed after implant placement and every follow-up thereafter. The biological width violation was diagnosed when the distance between the marginal bone level and free mucosa margin was less than 3 mm and/or connective attachment was absent. The clinical data were processed using linear mixed-effects model statistics at the patient level.

Results: The mean change in the marginal bone level after three years in function was –0.36 ± 0.57 mm, and –0.13 ± 0.42 mm taking implant and prosthesis placement baselines, respectively. The highest marginal bone resorption was determined in implants with the strongest violated biological width, both due to their peri-implant mucosa insufficient dimension (< 3 mm) and the absence of effective connective tissue attachment. In contrast, a significant decrease (p = 0.0002) in marginal bone resorption was observed in implants where biological width was fully respected in terms of a peri-implant mucosa sufficient dimension (> 3 mm) and the presence of an effective connective tissue attachment.

Conclusion: This study demonstrated that direct contact of connective tissue with the structured, bioactive, moderately rough neck surface of a two-stage fixture during (supra-, equi-crestal) placement significantly reduces marginal bone resorption during biological width restoration in patients with a thin and thick gingival biotype. A positive correlation between biological width violations and marginal bone resorption was documented.

Keywords

endosteal dental implant, biological width violation, connective tissue attachment, junctional epithelial attachment, peri-implant mucosa, marginal bone resorption, gingival biotype, moderately rough fixture neck, transmucosal implant part.

Declaration of Conflicting Interest

The author[s] declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledegements

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Introduction

Rehabilitation of dental function using endosteal dental implants has become an integral part of conventional dental treatment in the last twenty years. Today's dental implantology is characterized by two-piece titanium intraosseous screw implants healing by osseointegration placed in two stages submucosally[1], i.e. when isolating the fixture from the oral microflora. Furthermore, two-piece or one-piece single-stage implants healed by osseointegration, transmucosally[2], i.e. upon contact of the fixture with the oral cavity bacterial environment. Two-stage fixtures are typically placed to the alveolar ridge level (bone level) and single-stage fixtures to the mucosal sheath level (tissue level). Both of these types of osseointegrated implants, mainly due to the specific surface treatments of the intraosseous implant part[3-7] show increased osseoconductive properties, good biocompatibility with the human body, and a high success rate[8-10]. However, with the increasing lifespan of osseointegrated implants and with the new current requirements for advanced implant procedures, new aspects of their failure are emerging. Periimplantitis[11] can be considered as one of the main causes of current dental implants failure determined from long-term observations, with the prevalence being estimated at 28-56%[12].

Periimplantitis, as inflammatory changes of the peri-implant mucosa in the neck implant part accompanied by radiologically evaluable bone loss, is referred to as a typical multifactorial disease. Currently, bacterial infections and/or mechanical overload of the implant are stated as the main etiological factors. Bacterial infection occurs due to the infiltration of oral environment components, where the soft tissue around the transmucosal implant part does not create a sufficiently effective barrier against the penetration of oral cavity bacterial microflora, pathogens, and toxins into the space between the implant and the bone.

The anatomical structures of the gingiva around the natural tooth and the mucosa around the intraosseous implant, creating the so-called **biological width** (BW)[13-16] show a certain analogy. Significant parallel appears mainly in the tissue components, i.e. **sulcular and junctional epithelium** (SE, JE) and supra-alveolar **connective tissue** (CT)[17-19]. Some similarities can also be found in their composition and dimensions[20-24] .

In two-piece implants, a micro-gap between the fixture and the abutment is also considered a significant source of bacterial contamination due to the accumulation of bacterial plaque. As a result of the micro-motions of the joint, the tissue in the nearest area of the gap is infiltrated with inflammation. This infiltration may, again, increase the risk of marginal bone resorption[25-27].

A number of experimental studies demonstrated that the colonization of the dental implant surface by the oral cavity bacterial microflora and the accumulation of bacterial plaque occurs to a greater extent on the implant rough surfaces as compared to smooth and polished surfaces[28-30] . Therefore, the surface of the neck part of most dental implants is smooth, highly polished, and non-porous to avoid stronger plaque accumulation, further bacterial spread, and further progressive marginal bone resorption due to an initial exposure of the rough neck[31].

However, some studies remain to demonstrate that implants with a conventionally smooth neck show greater marginal bone loss compared to implants with a rough neck part[32-37]. According to one working hypothesis, the reason may be that the structured and rough surface of the neck part may align better to the connective tissue of the mucosal barrier cuff[38-44], than in the case of smooth neck, and thus, to some extent, act as a connective tissue attachment as one component of the biological width.

However, as pointed out in our recent studies [45,46] the mutual position of the surfaces of thetransmucosal implant part individual components and the mucosal barrier cuff adjacent tissue components (biological width) is closely related to the issue of functional connective attachment in all follow-up phases, i.e. from inserting the fixture during implantation, during healing and functional loading.

Nevertheless, in the present study, biological width is considered a specific concept, revealing the relationship between its vertical dimension and structure and its ability to provide internal

environment integrity and thus protect the underlying tissues and prevent marginal bone resorption. Therefore, the study aims to assess the dynamics of marginal bone resorption depending on biological width violation.

The tested null hypothesis proposed by the authors assumes that the biological width violation does not affect marginal bone resorption.

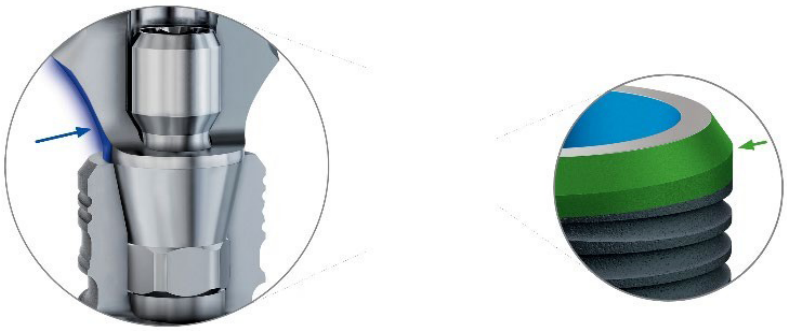
Materials and Methods

Patient Selection

Eligible patients who met the predetermined selection criteria were included in the prospective study to receive an implant treatment at a clinical workplace "Dental Practice, Radhošťská 4, Prague 3, Czech Republic".

Inclusion criteria: Male and female patients aged 18 years and older, sufficient alveolar bone volume (minimum alveolar width in the vestibular direction 5.5 mm, necessary usable bone height greater than 10 mm, at least 1 mm of bone surrounded by the selected implant from lateral and apical sides), alveolus without significant horizontal and/or vertical bone defect without the need for an augmentation procedure such as sinus lift or controlled bone regeneration, implantation into the healed alveolar site at least 5 to 6 months following extraction (3 to 4 months for a single-root tooth), bone density D1 to D4 (according to the Lekholm-Zarba classification modified by Misch)[50], non-infected alveolar site. The treated patients received full information about the treatment and the advantages and disadvantages of the chosen treatment. The patients confirmed their agreement to participate in the clinical trial via signed informed consent. The study was conducted in line with the Declaration of Helsinki (1964, 2008), and the study protocol was approved by the Ethics Committee of the University Hospital Hradec Králové (201806 S13 PM).

Exclusion criteria: Patients with general health contraindications of oral surgery, age below 18 years, and smoking (more than 10 cigarettes per day) were excluded from the study.



(a) (b)

Figure-1 Characterization and localization of contact surfaces of the BioniQ® implant transmucosal part. (a) Smooth, bioinert and hydrophobic contact surface in the distant part of the abutment intended mainly for the junction epithelium attachment (blue arrow); (b) Structured, bioactive, moderate rough, hydrophilic BIO (MR) fixture neck surface intended mainly for connective tissue attachment (green arrow).

Characteristics of Used Implants

The study used the BioniQ® system (LASAK s.r.o., Prague, Czech Republic) self-tapping screw implants of cylindrical (straight) or conical (tapered) shape with diameters of 3.5, 4.0 and 5.0 mm and lengths of 8, 10 and 12 mm. The surface of the intraosseous implant part (fixture) is provided with an osseoconductive[47] surface (BIO surface)[48]. The neck part of the fixture is provided with a BIO (MR) surface[41]. The characteristics of the transmucosal implant part surfaces and the fixture abutment junction (FAJ), which is realized by means of a conical joint Q-Lock®, are shown in **Figure-1**.

Surgical Protocol

The implant placement was performed in two stages with a shortened healing period (early loading)[49]. Bone bed preparation and implant placement were performed according to the procedure set out in the BioniQ® LASAK Surgical Manual. The recommended placement of fixtures into the alveolar ridge level (bone level) was verified by a radiographic evaluation immediately after the implantation. According to the measured vertical position of the fixture margin with respect to the alveolar bone ridge, the fixtures were categorized into two groups: 1) fixtures inserted sub-crestally (CTCPp (IM) > 0); and 2) supra-/equi-crestally (CTCPp (IM) ⩽ 0). Bone density was evaluated according to the subjective feeling of the surgeon during the bone bed preparation and differentiated according to the Lekholm-Zarba

classification modified by Misch into four classes, D1 to D4[50]. The implants were closed with a cover screw using a torque of 5-10 Ncm. The implants were placed using a final insertion torque from 15 to 70 Ncm with a mean value of 41±17 Ncm. Chlorhexidine gel was applied to the cover screw thread prior to screwing it into the fixture. Throughout the healing period, the fixtures were covered with soft tissue. The healing period was not less than 48 hours and more than 3 months in both the mandible and maxilla, with an average healing period of 2.6 months for all placed implants (n = 97)[49]. After this period, the second stage of surgical implantation (2SI) was initiated. The cover screw was removed from the implant fixture and replaced by a healing abutment. After two to four weeks, the healing abutment was replaced by an appropriate definitive abutment when the mucosal canal was formed. The definitive abutment was attached to the internal thread of the fixture using a screw and tightened using a torque of 25 Ncm. A chlorhexidine gel was applied to the screw thread prior to being screwed into the fixture. For all implants, an intraoral radiograph was performed immediately after the implantation (IM) and after the second surgical stage (2SI), and the stability of the implant was measured using resonance frequency analysis (RFA; Osstell AB, Gothenburg, Sweden).

Marginal Bone Level (MBL) Measurement

For the X-ray diagnostics, an orthopantomogram Planmeca Promax with calibrated imaging was used. The intraoral imaging was performed via the paralleling technique using Super-Bite Senso and Endo-Bite Senso holders (KerrHawe SA, Switzerland). Marginal Bone Level (MBL) was determined from radiographs perpendicular to the central axis of the implant on both sides of the implant (mesial MBLm and distal MBLd), always in relation to the reference level (RL) of the implant shoulder **(Figure-2a)**. The value, MBLp, was calculated as an average of the mesial MBLm and distal MBLd values. The length of

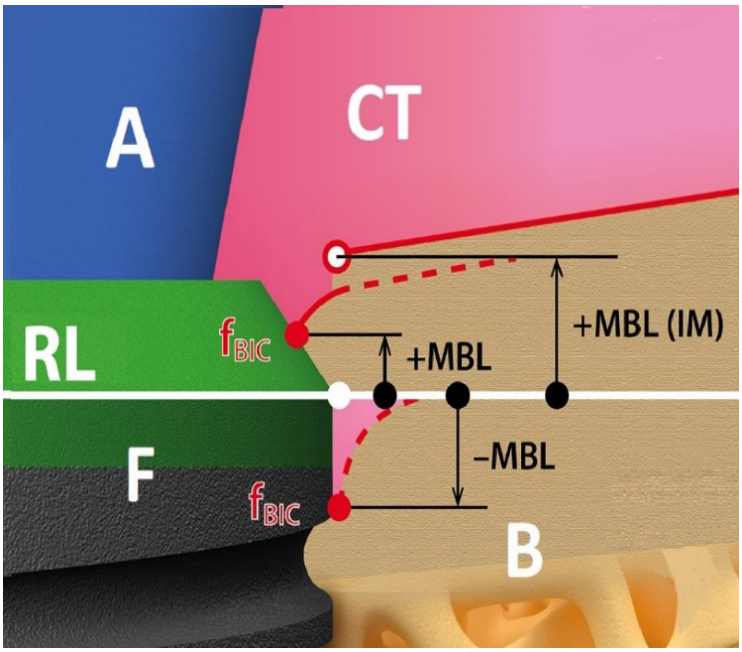


Figure-2a Illustrative scheme for determining the marginal bone level (MBL), given by the vertical distance of the first bone-implant contact (f_{BIC} – red points) from the reference level (RL) given by the implant shoulder (white point, line). If the measured level of marginal bone (red dot) is below the RL, i.e. in the apical direction, the MBL value is denoted as negative (–MBL), otherwise as positive (+MBL). The value of the marginal bone level upon implantation MBL(IM) (red circle with a white fill) corresponds to the vertical distance of the margin of the alveolar bone from the RL, measured immediately after implant placement.

the implant was used as a reference dimension. MBLm and MBLd were measured immediately after the implantation, i.e. after the placement of the implant MBL(IM), and further at time intervals during the second surgical phase of MBL(2SI), at the time of placement of final dental prosthesis MBL (DP), after 3, 6, 12, 24 and 36 months – MB(DP_{3mo}), MBL(DP_{6mo}), MBL(DP_{1y}), MBL(DP_{2y}), MBL(DP_{3y}] – from the placement of the definitive dental prosthesis, i.e. of the implant in service.

The changes in the marginal bone level over time ΔMBLm, ΔMBLd, ΔMBLp were determined as the differences in values measured at the individual time intervals in relation to the baseline at the time of implant placement MBL(IM) or at the time of dental prosthesis placement MBL(DP).

Determining Connective Tissue Contact Position (CTCP)

The connective tissue contact position CTCP is determined by the vertical (apical/coronary)

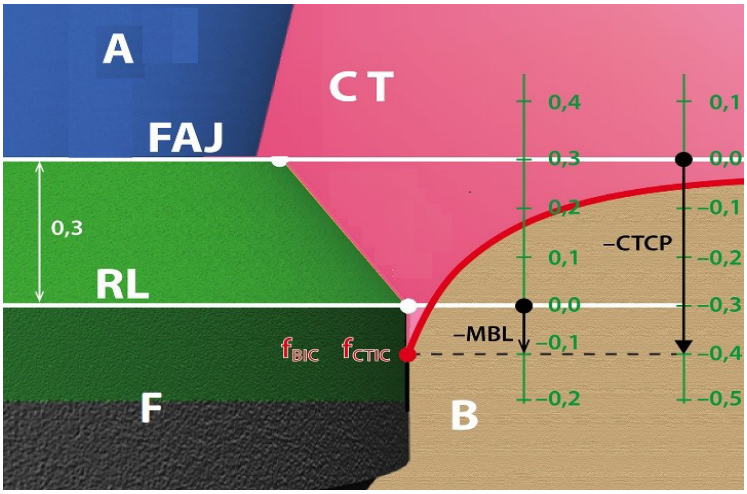


Figure-2b Illustrative scheme of determining the positions of the first connective tissue implant contact (solid arrow). The FAJ reference level for CTCP for BioniQ® implants is always 0.3 mm distant from the RL reference level for MBL, therefore $CTCP_{FAJ} = MBL_{RL} - 0.3$ mm. All values are given in millimetres

distance of the first connective tissue implant contact (f_{CTIC}) from the reference level of the FAJ (fixture abutment junction). CTCP values were determined from the corresponding experimental MBL data by conversion to the FAJ reference level using the transformation relationship $CTCP_{FAJ} = MBL_{RL}$ –0.3mm; **Figure- 2b**. Negative CTCP values (CTCPp <0) indicate the presence of CT contact with the BIO (MR) fixture neck surface and numerically indicate its vertical dimension. Positive CTCP values (CTCPp> 0) indicate the absence of CT contact with the fixture neck and numerically indicate the bone fixture submersion depth. CTCPp is the mean value of the position of the first connective tissue implant contact calculated as the average of the mesial and distal positions.

Peri-Implant Mucosa (PMv)

The vertical dimension of the peri-implant mucosa vestibular aspect (PMv) was determined by measuring the distance between the marginal bone level MBLp and the free mucosal margin position [51]. The free mucosal margin position was transformed to an intraoral image as the distance of the crown occlusal plane to peri-implant mucosa margin measure clinically on the midline of the crown vestibular side [52].

The PMv represents the vertical dimension of the SE + JE + CT, which is consistent with its BW but does not include the SE dimension. Measurements were performed upon the placement of provisional and/or definitive prosthetics and after 3, 6, 12, 24 and 36 months of the implant being in service.

Periodontal Biotype

The patient's gingival biotype was determined and categorized into two groups: thin biotype and thick biotype[53]. A black periodontal probe was used to determine the biotype. The probe was placed in the sulcus on the mid-vestibular side of the adjacent tooth. If the probe was visible through the gingiva, the biotype was categorized as "thin," otherwise as "thick".

Prosthetic Protocol

The prosthetic work was performed following the binding procedures of the BioniQ® LASAK system prosthetic manual. To achieve a high-quality and stable implant neck closure, the prosthetic treatment of the BioniQ® implants was initiated approximately 3 weeks after the stage-two surgery. For

Dental prosthesis type	Single crown	Connected crown	3-membered linear bridge	Multiple bridge	Splinted bridge	Anchor dentures	LOCATOR attach.	Total number
Maxilla	11(11)	3 (6)	2 (4)	3 (8)	1 (6)	1 (4)	-	21 (39)
Mandible	12(12)	4 (8)	7 (14)	1 (1)	4 (21)	-	1 (2)	29 (58)
Total number	23(23)	7 (14)	9 (18)	4 (9)	5 (27)	1 (4)	1 (2)	50 (97)

**Note: Supervised implants are given in parentheses*

Table-1 Number of Dental Prosthesis Types and Supervised Implants*

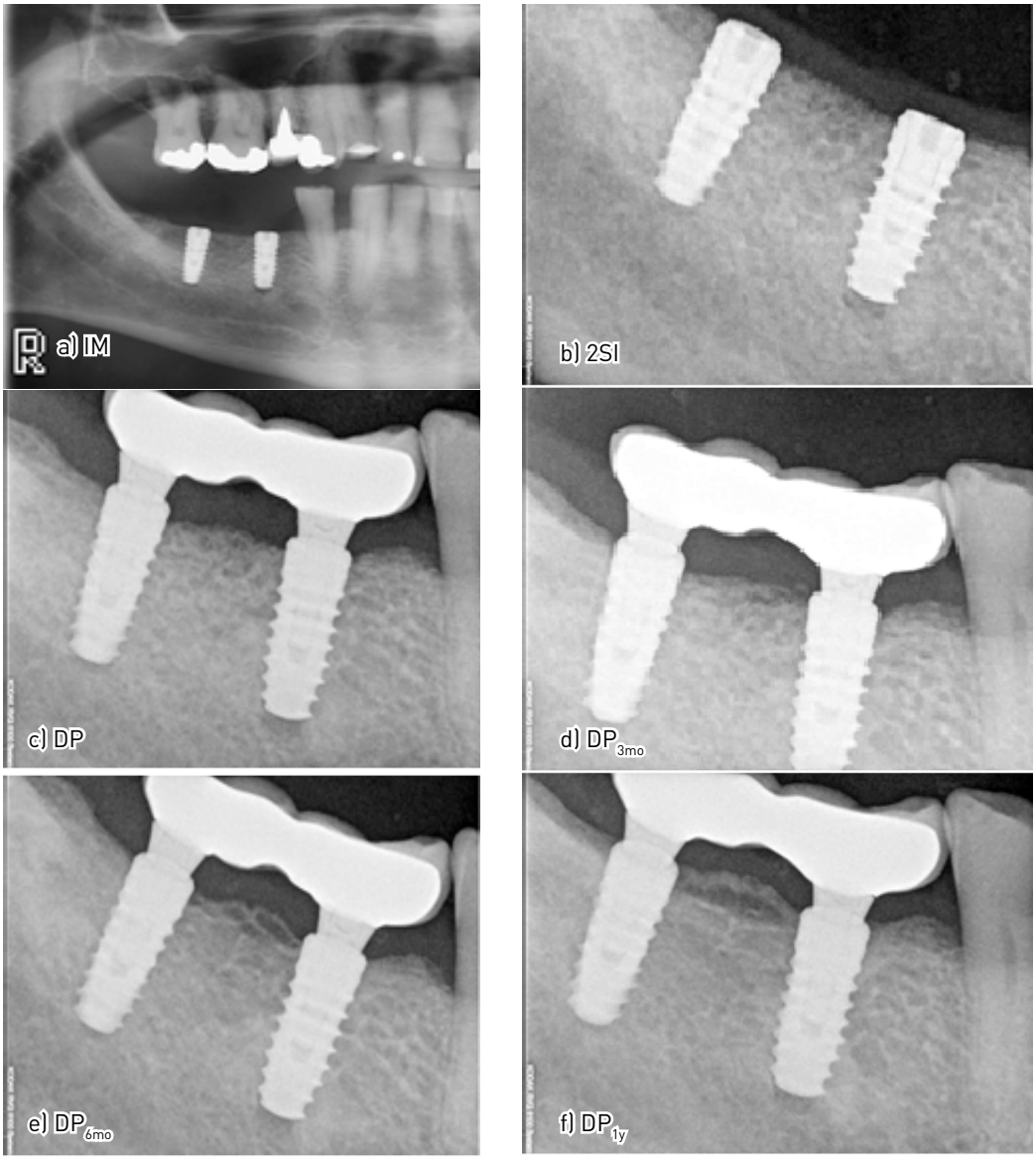
single-tooth implants, the crowns were predominantly positioned 0.5 to 1 mm below the gingival margin and cemented.

Multiple implants were mostly drilled and treated with screw-retained or cemented restorations with a passive fit and free articulation. Toothless arches were treated by inserting two implants in the mandible in the canine region, provided that there was a complete removable prosthesis in the maxilla. The LOCATOR retention system was used where matrix fixation was performed directly in the patient's mouth. The representation of the individual dental prosthesis types used in the present study and the number of inserted implants bearing prosthetic prostheses are shown in **Table 1**.

Statistical Analysis

Descriptive statistics were used to evaluate the collected data. Since not all data sets showed a normal distribution (Kolmogorov-Smirnov test), the median, maximum and minimum values and quartiles were included in the descriptive statistics in addition to the mean and SDs values. A linear mixed-effects model was performed to evaluate the effects of parameters on marginal bone changes during treatment, as some patients received more implants. The patients were considered as a random effect (independent statistical unit). Life-table analysis was used to determine the interval and cumulative success of implants.

Sample size was calculated by comparing the mean marginal bone loss for implants with contact (0.2 mm, SD: 0.4) and absence of contact (0.68 mm) of connective tissue with the fixture neck upon insertion, after two years in function determined by the previous study[46]. Using a test power of 0.9 at the significance level of p = .05, the sample size equalled 17 independent statistical units (implant,



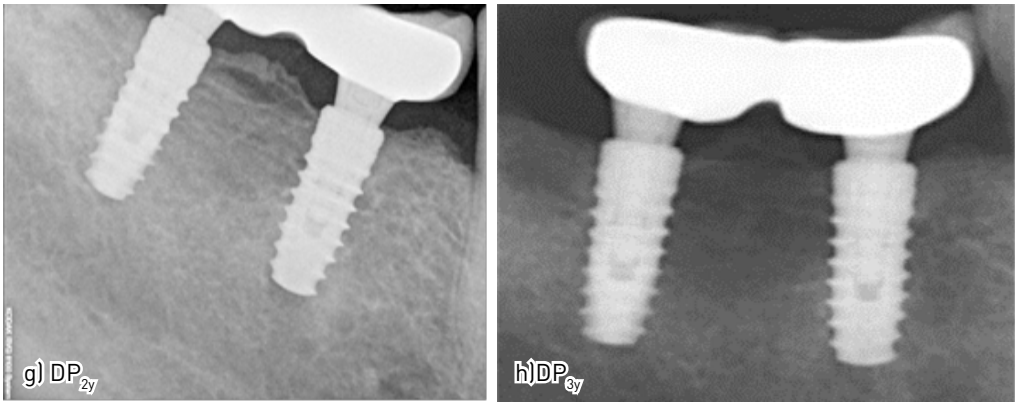


Figure-3 X-ray images of implant pair inserted into the mandible (positions 46, 47); (a) immediately after the implantation; (b) in the second surgical stage; (c) after the implementation of a definitive prosthesis; (d) three months after loading; (e) six months after loading; (f) one year after loading; (g) two years after loading; h) three years after loading;

patient) with a difference in bone loss.

Statistical analyses were performed using the statistical software Statistica 12. A p value smaller than .05 was considered to be statistically significant.

Results

From June 18, 2014, to March 9, 2015, a total of 97 implants (BioniQ®, LASAK s.r.o., Prague, Czech Republic) were used in 43 patients with a mean age of 57.6 years (range: 18 to 75 years). Of these, 39 implants were implanted into the maxilla and 58 implants into the mandible. All patients were treated by the same clinician (Z.N.).

All implants were successfully healed, and all patients showed up for the follow-up examinations immediately after the implantation (IM), during the second surgical stage (2SI), at the delivery of definitive dental prosthesis (DP), and after 3, 6, 12, 24 and 36 months (DP_{3mo}, DP_{6mo}, DP_{1y}, DP_{2y}, DP_{3y}) from the definitive dental prosthesis placement **(Figure-3)**.

After three years in function, all evaluated implants and prosthetic replacements were rated as successful, stable, and functional. According to the predetermined criteria[10], no implant was evaluated as unsuccessful. The cumulative success rate of implants after the 3rd year of functional loading was 100%. Bone resorption greater than 1,5 mm and less than 2.5 mm after 3 years in function was shown by 7 implants (7.2%) from the implantation and 1 implant (1,0%) from the prosthesis delivery.

MBL and its Changes ΔMBL at Individual Follow-Up Points

Time point	No. of patients[a] (implants)	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
IM	43 (97)	0.44	0.30	−0.25	2.30	0.00	0.75	0.49
2SI	43 (97)	0.35	0.30	−0.35	1.80	0.00	0.50	0.44
DP	43 (97)	0.21	0.05	−0.75	1.55	0.00	0.30	0.43
DP_{3mo}	43 (97)	0.11	0.00	−1.70	1.55	−0.05	0.30	0.50
DP_{6mo}	43 (97)	0.06	0.00	−1.20	1.55	−0.25	0.30	0.52
DP_{1y}	43 (97)	0.09	0.05	−1.35	1.55	−0.20	0.30	0.55
DP_{2y}	43 (97)	0.07	0.00	−1.25	1.55	−0.20	0.30	0.52
DP_{3y}	43 (97)	0.08	0.05	−1.25	1.30	−0.20	0.30	0.49

[a] The data were processed at the patient level.

Table-2 Mean Marginal Bone Level MBLp [mm] at Individual Follow-Up Time points

Time interval	No. of patients[a] (implants)	Average	Standard deviation
2SI – IM	43 (97)	–0.09	0.28
DP – IM	43 (97)	–0.23	0.42
DP_{3mo} – IM	43 (97)	–0.33	0.51
DP_{6mo} – IM	43 (97)	–0.37	0.57
DP_{1y} – IM	43 (97)	–0.35	0.56
DP_{2y} – IM	43 (97)	–0.36	0.55
DP_{3y} – IM	43 (97)	–0.36	0.57

[a] The data were processed at the patient level.

Table-3 Mean Change in the Marginal Bone Level ΔMBLp [mm] during the Individual Phases after the Implantation

Time interval	No. of patients[a] (implants)	Average	Standard deviation
DP_{3mo} – DP	43 (97)	–0.10	0.32
DP_{6mo} – DP	43 (97)	–0.14	0.31
DP_{1y} – DP	43 (97)	–0.12	0.39
DP_{2y} – DP	43 (97)	–0.13	0.39
DP_{3y}–DP	43 (97)	–0.13	0.42

[a] The data were processed at the patient level.

Table-4 Mean Change in the Marginal Bone Level ΔMBLp [mm] 3, 6, 12 and 24 Months after the Dental Prosthesis Placement

The statistically processed mean MBLp at individual follow-up points are given in **Table 2**. Mean changes in the ΔMBLp at individual follow-up time intervals with respect to the baseline at implant and prosthesis placement are provided in **Tables 3 and 4**, respectively.

The main bone loss during the treatment process occurs between implant and dental prothesis placement ΔMBLp (DP–IM) = –0.24 ± 0.42 mm (p = 0.000007) and during the first three months of functional implant loading ΔMBLp(DP_{3mo}–DP) = –0.11 ± 0.32 mm (p = 0.021). Marginal bone level changes from three months of functional loading to one year and two years were not found to be statistically significant ΔMBLp(DP_{1y}–DP_{3mo}) = –0.01 ± 0.51 mm (p = 0.93); ΔMBLp (DP_{2y}–DP_{3mo}) = –0.03 ± 0.51 mm (p = 0.68), respectively.

PMv during the Follow-up for Thin and Thick Gingival Biotype

	Thin biotype (PMv(2SI,DP)<3mm)		Thick biotype (PMv(2SI,DP)>3mm)	
Follow-up phase	PMv[a] ± Sm. Ch. (mm)	p-value[b]	PMv[a] ± Sm. Ch. (mm)	p-value[b]
2SI	(2.46c)		(3.24c)	
DP	2.74 ± 0.10	p = 0.01	3.26 ± 0.15	p = 0.43
DP_{3mo}	2.33 ± 0.16		3.25 ± 0.17	
DP_{6mo}	3.00 ± 0.14		3.18 ± 0.17	
DP_{1y}	3.30 ± 0.16		3.22 ± 0.13	
DP_{2y}	3.33 ± 0.16		3.36 ± 0.15	
DP_{3y}	3.21±0.15		3.47±0.16	

a PMv value includes sulcular epithelial dimension (SE+JE+CT)
b The data were processed at the patient level; Statistically significant difference p <0.05;
c Extrapolated data

Table-5 Mean Vertical Dimension of the Vestibular Peri-Implant Mucosa (PMva) during Individual Follow-Up Time Points

PMv values at the time following the second surgical stage were reduced (< 3 mm) for implants in patients with a thin biotype (PMv (2SI)$_{thin}$ = 2.46 mm; PMv (DP)$_{thin}$ = 2.74 ± 0.10 mm), while for implants in patients with a thick biotype, the values were greater than 3 mm (PMv (2SI)$_{thick}$ = 3.24 mm; PMv (DP)$_{thick}$ = 3.26 ± 0.15 mm). In patients with a thin biotype, there was a statistically significant increase in PMv over time in the DP to DP$_{1y}$ time interval (p = 0.01), while in patients with a thick biotype, a statistically significant change in PMv over time in the DP to DP1y interval could not be demonstrated (p = 0.43), **Table-5**. Statistical calculation of measured values of the PMv at individual follow-up time points (DP, DP$_{3mo}$, DP$_{6mo}$, DP$_{1y}$, DP$_{2y}$, DP$_{3y}$) for implant categories in patients with thin and thick gingival biotype are given in **Table 5**.

Implants with PMv (2SI, DP)> 3 mm (thick biotype) showed significantly lower mean marginal bone loss after two years in function, ΔMBLp (DP$_{2y}$–IM) = –0.26 ± 0.46 mm; n = 66 than implants with PMv

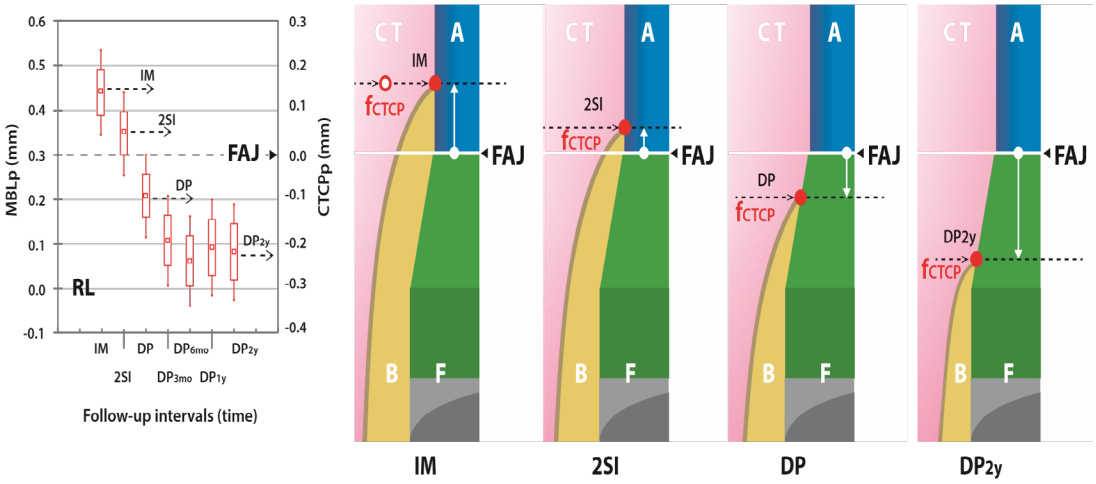


Figure-4 Mean marginal bone level MBLp (left axis of the box plot, RL-reference level) and mean position of connective tissue contact CTCPp (right axis of the box graph, FAJ-reference level) in individual follow-up phases. The right part of the figure visualizes the time evolution of the CTCPp dimension (full white arrows) and the localization of the connective tissue first contact with the implant transmucosal parts (f_{CTIC}) (red points) for the follow-up phases: IM, 2SI, DP and DP$_{2y}$. The actual vertical dimension of the implant corresponds to the CTCPp scale (mm); A – cover screw, healing or final abutment; F – neck fixture with BIO(MR) surface (green colour); FAJ – fixture- abutment junction, CT – connective tissue; B – alveolar bone; f_{CTIC} – first supra-alveolar connective tissue implant contact.

(2SI, DP) <3 mm (thin biotype), with ΔMBLp (DP$_{2y}$–IM) = –0.59 ± 0.66 mm, n = 31 (p = 0.006).

CTCP during Follow-Up

The development of the connective tissue contact position CTCP at the individual phases of follow-up is illustrated in **Figure- 4**. In these follow-up points, the observed positive CTCPp values during the implant placement (CTCPp (IM)> 0) and during the second surgical stage (CTCPp (2SI)> 0) document the absence of CT contact with the BIO (MR) fixture neck surface. In the next follow-up phases of DP, DP$_{3mo}$, DP$_{6mo}$, DP$_{1y}$, and DP$_{2y}$, the negative values of CTCPp found indicate the presence of contact of supra-alveolar CT with the BIO (MR) fixture neck surface.

Connective Tissue Contact with the Fixture Neck during Implantation and its Effect on the Marginal Bone Resorption

Implants with the absence of CT contact with the neck fixture during (sub-crestal) implantation (CTCP (IM) > 0) show significantly higher (p = 10–10) marginal bone resorption after two years in function calculated from implantation ΔMBLp (DP$_{2y}$ –IM) = –0.67 ± 0.67 mm (n = 33) than implants with direct contact of CT with the neck fixture during (supra- and equi-crestal) implantation (CTCP (IM) <0), where ΔMBLp (DP$_{2y}$–IM) = – 0.21 ± 0.40 mm (n = 64).

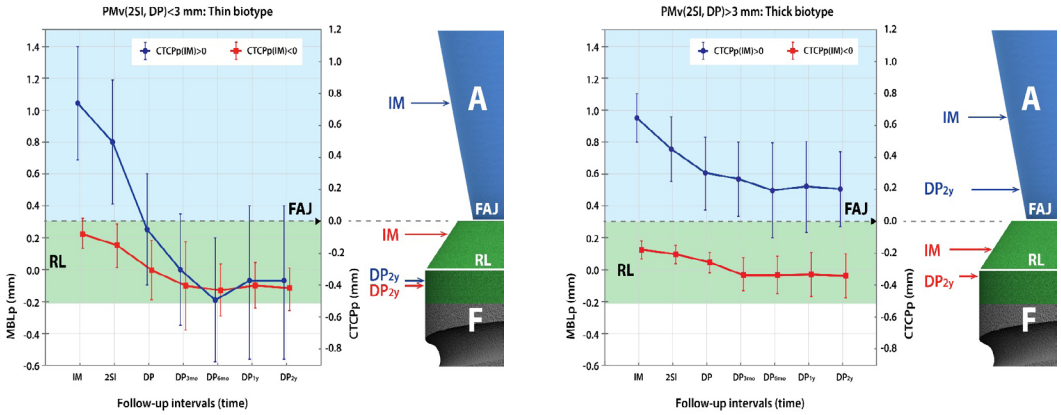
Connective Tissue Contact with the Fixture Neck during Implantation and its Effect on the Marginal Bone Resorption Dynamics in Patients with Thin and Thick Biotypes

In patients with a thin biotype **(Figure- 5a)**, the edge of the marginal bone gets stabilized when CT contact with the fixture neck is reached at CTCPp ≈ –0.4 mm, i.e., about 0.4 mm apically from the FAJ, regardless of the contact or absence of CT contact with the fixture neck during implant placement. However, in implants with an initial absence of CT contact with the fixture neck (CTCP (IM)> 0), the edge of the marginal bone gets stabilized later (DP$_{6mo}$ to DP$_{1y}$). Stabilization is preceded by significant marginal bone resorption occurring during biological width restoration in the time interval 2SI to DP$_{6mo}$ (ΔMBLp (DP$_{6mo}$–2SI) = –0.98 ± 0.93 mm; p = 0.0029; **Figure -6**. While for implants with direct initial

contact of CT with the fixture neck (CTCP (IM) <0), the edge of the marginal bone stabilized earlier (DP_{3mo}), and marginal bone minimal resorption was observed in the interval 2SI to DP_{6mo} (ΔMBLp (DP_{6mo} – 2SI) = –0.25 ± 0.45 mm; p = 0.27; **(Figure - 6)**.

However, the marginal bone total resorption after two years in function calculated from the implant placement in patients with a thin biotype with the absence of initial CT contact with the fixture neck is approximately 3.5 times higher (p < 0.05) than in patients with direct initial contact of CT with the fixture neck (CTCPp > 0: ΔMBLp (DP_{2y}–IM) = –1.11 ± 0.80 mm; n = 11 vs. CTCPp < 0: ΔMBLp (DP_{2y}–IM) = –0.31 ± 0.34 mm; n = 20).

In patients with a thick biotype **(Figure 5b)**, the edge of the marginal bone stabilizes at CTCPp



a) b)

Figure-5 Mean marginal bone level (MBLp) (left axis) and connective tissue contact position (CTCPp) (right axis) for a) thin biotype (PMv (2SI, DP) < 3 mm), b) thick biotype (PMv (2SI, DP) > 3 mm), during the individual follow-up phases with respect to the contact (CTCPp (IM) <0) and the absence of contact (CTCPp (IM)> 0) of the connective tissue with the fixture neck during the implant insertion. The vertical bars represent a 95% confidence interval.

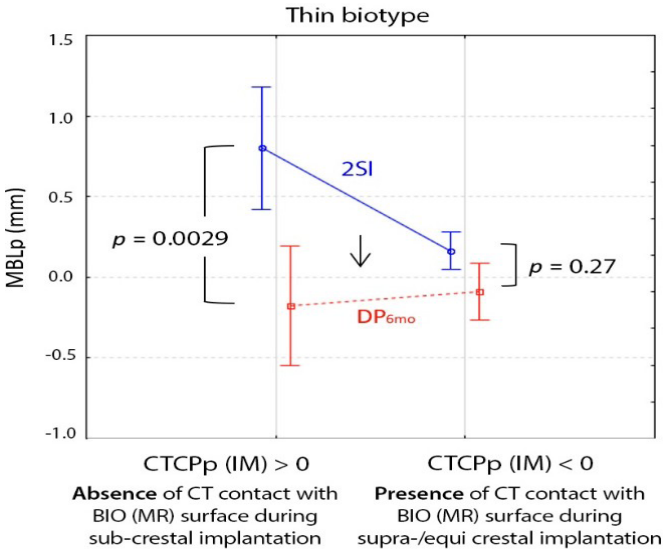


Figure-6 Marginal bone level (MBLp) for thin biotype at the beginning of the stage-two surgery (2SI) and six months after the delivery of the definitive prosthesis (DP_{6mo}) for implants with the absence (CTCP (IM)> 0) and the presence (CTCP (IM) <0) of CT contact with BIO(MR) surface of the fixture neck part during the insertion (Linear model – patient as a random effect). The arrow shows the time shift.

≈ 0.2 mm, i.e. about 0.2 mm in coronal direction from the FAJ in implants with the absence of initial CT contact with the fixture neck (CTCPp (IM)> 0).

In implants with direct initial CT contact with the fixture neck (CTCPp (IM) < 0), the edge of the marginal bone stabilizes at CTCPp ≈ –0.34 mm, i.e. about 0.34 mm apically from the FAJ.

Implants with the absence of CT initial contact with the fixture neck, the marginal bone

resorption after two years in function (from implantation) is almost three times higher (CTCP (IM) > 0: ΔMBLp (DP_{2y}–IM) = –0.44 ± 0.48 mm; n = 22; p < 0.05) than in implants with CT direct initial contact with the fixture neck (CTCP (IM) <0: ΔMBLp (DP_{2y}–IM) = –0.16 ± 0.43 mm; n = 44).

According to the results of this study, the null hypothesis must be rejected because the violation of biological width due to insufficient dimension of periimplantate mucosa (<3mm) and/or the absence of effective attachment of connective tissue, statistically significantly increases the resorption of the marginal bone.

Discussion

The results presented in this study demonstrate high success and predictability of BioniQ® implants, where the observed values of mean marginal bone loss in the first year after loading ΔMBLp (DP_{1y}–DP) = –0.12 ± 0.39 mm (N/n = 43/97) and for the second year of implants in the function ΔMBLp (DP_{2y}–DP_{1y}) = –0.02±0.21 mm (N/n = 43/97) are ten times lower than consensually accepted standard resorption values, maximum 1.0 – 1.5 mm in the first year after loading and maximum 0.2 mm per year from the second year after implant loading[54-56].

The finding from the present study regarding the marginal bone loss after two and three years in function from implant placement **(Table -3)** and from the prosthesis placement **(Table- 4)** are also in concordance with the best documented dental implant systems.

Wenstrom et al.[57] analysed Astra Tech® implants (Astra ST; TiO-blast®) inserted in two stages, where the ΔMBLp (DP_{2y}–DP) reached the value of 0.14 ± 1.06 mm (N/n = 38/43) after two years in function. A study conducted by Friberg et al.[58] focusing on a one-stage, smooth surface Brånemark system implants resulted in the ΔMBLp after one and three years from the insertion of the definitive prosthesis ΔMBLp (DP_{1y}–DP) = –0.26 ± 0.57 mm (n = 151) and ΔMBLp (DP_{3y}–DP) = –0.42 ± 0.72 mm (n = 135), respectively. A multicenter study by Hämmerle et al.[59] and Sanz et al.[60] documented two-piece Straumann® implants (Bone Level; SLActive) in two-stage insertion, with submucosal healing (referred to as "submerged implants") and in one-stage insertion, with transmucosal healing (referred to as "non-submerged implants"). These implants showed marginal bone loss ΔMBLp (DP_{3y}–IM)sub = –0.68 ± 0.98 mm and ΔMBLp (DP_{3y}–IM)trans = –0.58 ± 0.77 mm three years from the implant placement. Moreover, Sanz et al.[60] also determined the frequency of implants with a marginal bone loss greater than 1,5 mm three years after implant placement for submucosal healing with 19% and for transmucosal healing with 18%. These values are almost three times higher than the value of 7.2% reported in this study for submucosally inserted BioniQ® from implant placement up to three years after loading.

Sanz et al.[60] also documented that almost half of the total bone loss occurred during the first six months after the IM, i.e. before the implants were put into function, which agrees with the results of this study, where approximately 2/3 of the total bone loss occurred from the IM to the DP (ΔMBLp (DP–IM) = –0.23 ± 0.42 mm; p = 0.000002; **Table- 3**). Marginal bone level changes were evaluated as minimal in BioniQ® implants after 3 months of functional loading **(Table- 2)**. These statistically insignificant year-on-year values of marginal bone resorption (ΔMBLp (DP_{1y}–DP_{3mo}) = –0.01 ± 0.51 mm; p = 0.93 and ΔMBLp (DP_{2y}–DP_{1y}) = –0.02 ± 0.21 mm; p = 0.68; ΔMBLp(DP_{3y}-DP_{2y})= –0,01±56mm; p=0,91) indicate the stabilization of hard and soft peri-implant tissues and the achievement of a steady state of bone remodelling under functional loading with BioniQ® implants.

Because the mean values of MBLp and ΔMBLp are of limited clinical significance, the marginal bone stabilization dynamics were further analysed in the context of the BW restoration process. Two aspects were considered important. One aspect entails the overall vertical dimension of the peri-implant mucosa (PMv). The second includes the connective tissue attachment CTA, or more precisely, the creation of direct contact between the CT and BIO(MR) fixture neck surface as a precursor of the CTA. The biological width expressed as the mean vertical dimension of the vestibular peri- implant mucosa found in this study (PMv = 3.35 ± 0.16 mm) for two-stage BioniQ® implants after three years in function is comparable to the histometric dimensions of two- stage implants found in many studies (PMv = 3.11 to 3.80 mm)[20-24,51].

However, in patients with a thin biotype after the second-stage surgery, the present study revealed a reduced dimension of the peri-implant mucosa PMv(2SI), PMv(DP), and its subsequent growth after the next 6 months **(Table- 6)**. This growth in implants with the absence of CT direct initial contact with the fixture neck occurred at the expense of the receding marginal bone **(Figure - 5a, Figure- 6)**.

These results are consistent with the findings reported by Berglundh et al.[20], Cochran et al. [21] and Abrahamsson et al.[22], who observed that a minimum 3 mm vertical dimension of the peri-implant mucosa, which effectively protects the underlying tissues, is necessary to restore the physiological dimension of the stable epithelial and connective attachment.

Berglundh et al.[20] demonstrated that if the mucosal height is surgically reduced to about ⩽ 2 mm around Bränemark implants prior to the abutment, the peri-implant mucosa dimension grows apically at the expense of the resorbing bone. After 6 months, the vertical dimension of the epithelial barrier reached a thickness of 2 mm and the connective tissue zone 1.3 mm, total PMv = 3.3 mm.

However, the results of the present study show that no statistically significant marginal bone

resorption occurred during the restoration of reduced WB in patients with a thin biotype with BioniQ® implants with a direct CT contact with the fixture neck during the insertion (CTCPp (IM) < 0), as was the case for implants without direct initial CT contact with the BIO(MR) surface (CTCPp (IM) > 0; **Figure- 6**).

Based on the presented clinical data, it can be assumed that early, ideally immediate, contact of the connective tissue with the structured BIO (MR) fixture neck surface **(Figure-5a)** stimulates the CTA as an effective component of BW, which is further able to protect the underlying tissue to avoid marginal bone resorption **(Figure - 6)**. Restoration of biological width **(Table- 6)** without remission of marginal bone **(Figure - 6)** then represents the growth of its vertical dimension in the coronary direction (tissue rebound). The created high-quality CTA is, at the same time important prevention of epithelial downgrowth.

On the other hand, the initial absence of CT contact with the moderately rough neck of the fixture **(Figure -5a)** does not allow effective CT attachment. Biological width restoration is realized only by its vertical dimension growth **(Table- 6)** as a response to the insufficient ability of the original, reduced peri-implant mucosa (PMv(2SI) < 3 mm; **Table - 6**) to protect the underlying bone tissue and avoid its resorption **(Figure- 6)**. Marginal bone is stabilised later (DP_{1y}; **Figure - 5a**) only when the BW physiological dimension is reached, i.e. when the vertical dimension of the peri-implant mucosa reaches at least 3 mm (PMv (DP_{1y}) = 3.30±0,16 mm , **Table - 6**) and an adequate morphology[61] and representation of effective CTA (CTCPp ≈ –0.4 mm) and JEA.

The results consistent with the results of this study are also reported by Jung et al.[35] in experimental work on an animal model in two-part rough surface implants (SLA) along the entire length of the fixture. In implants inserted supra-, equi- and sub-crestally (+1; 0; –1 mm) during submucosal and transmucosal healing, the authors observed changes in the level of crestal bone after six months from loading. The greatest vertical bone loss was observed for implants with the absence of CT contact with the fixture neck during the IM, i.e. for implants that were inserted sub-crestally (–1 mm) for submucosal healing –1.32 mm and for transmucosal healing –1.40 mm. Significantly lower bone loss was found in implants with direct CT contact with the fixture neck during the IM, i.e. in implants that were inserted supra-crestally (+1 mm). There was a slight increase of +0.17 mm in submucosal healing, while transmucosal healing showed a slight decrease of –0.20 mm.

In implants with a thick biotype, the present study demonstrated the existence of a peri-implant mucosa with a vertical dimension greater than 3 mm already at the time of the second-stage surgery PMv (2SI) = 3.26 mm **(Table- 6)**. The achieved BW dimension, which no longer changes over time **(Table- 6)**, sufficiently protects the underlying tissues, minimizes resorption, and enables marginal bone stabilization in the DP_{3mo} to DP_{6mo} interval both for implants with contact and absence of CT contact with the fixture neck during the IM **(Figure-5b)**.

Implants with CT contact with the fixture neck during (supra-/equi-crestral) insertion make it possible to create an effective subepithelial CTA with a BIO(MR) surface, at the implant insertion and in all other follow-up phases **(Figure-5b)**. In vitro tests and tests on an animal model[62] showed that already 14 days after implantation, the nano- and micro- structured, bioactive, moderately rough BIO(MR) surface formed a firm attachment with CT through the ingrowth of CT collagen fibres into prominences and concave and porous contact surface structures, both in the form of perpendicularly adhering fibre bundles and individual fibrils integrated into the surface CaP deposit formed by the reaction of body fluid with the alkali-modified BIO (MR) surface [62,47].

On the contrary, in the absence of CT contact with the fixture neck during (sub-crestal) insertion, the soft tissues rest only on the smooth surface of the abutment transmucosal part at all follow-up stages **(Figure- 5b)**. Therefore, in the unavailability of BIO(MR) surface contact with CT, the BW restoration is preferably realized by effective JEA on the smooth surface of the titanium abutment through hemidesmosome binding [63,64]. However, the connective tissue with a smooth surface creates a loose contact with a completely missing attachment to the smooth surface of titanium.

The process of BW restoration and marginal bone stabilization is thus clearly influenced by the morphology of the transmucosal surfaces. Glauser et al.[39] demonstrated a longer length of the junctional epithelium (2.9 mm) and a smaller length of connective tissue (0.7 mm) formed on a smooth (machined) transmucosal surface, and conversely, a smaller length of the junctional epithelium (1.4 mm) and a longer length of connective tissue (2.6 mm) formed on a structured (acid-etched or oxidized) surface. Values were determined by histometric analysis of the vertical morphology of peri-implant soft tissues of human biopsies after two months of healing [39].

In addition, the marginal bone stabilization in the thick biotype occurred independently of the relative position and distance of the micro-gap from the marginal bone level **(Figure -5b)**. This result signals that the biological width with a vertical dimension of the peri-implant mucosa greater than 3 mm is, in this case, the predominant factor controlling the marginal bone resorption to the micro-gap acting as a source of bacterial propagation. Thus, bacterial action is, in fact, secondary, and the primary reason for marginal bone stabilization is the biological width sufficiency to protect the underlying tissues. Conversely, the biological width violation (insufficiency) leads to inflammation (as the immune reaction of the organism to a foreign body) and to the loss of alveolar bone due to mechanical or bacterial

action. Sichr [15] and Gargiulo et al.[16] defined biological width as a functional unit sharing the different functional characteristics of their two components of epithelial and connective tissue. However, if we look at biological width as a barrier, its violation must be considered from both the physiological and morphological points of view.

The positive correlation between the degree of biological width violation (mucosal barrier) and the bone tissue response (marginal bone loss) for BioniQ implants is evidenced by the results of the present study for the tested groups of implants presented in **Table-6**. The biological width violation was diagnosed when the distance between the MBL and the loose mucosa margin was less than 3 mm (PMv < 3 mm) and/or the contact between CT, and the BIO(MR) surface was no created, indicating the absence of effective CTA.

The highest marginal bone resorption (ΔMBLp (DP_{2y}–IM) = –1.11 ± 0.80 mm) was determined in implants with the strongest violated biological width, both due to their peri- implant mucosa insufficient

Tested groups of implants		Evaluation of biological width violation		Bone tissue response
Group designation	Implant categorization Gingival biotype / position of fixture insertion	Vertical dimensions of mucosal barrier[a] PMv (2SI) (mm) (at the beginning of BW restoration)	Connective tissue attachment[b] CTA Presence/Absence (time interval)	Mean change in marginal bone level after two years in function since implantation ΔMBLp (DP_{2y}-IM) (mm)
A	Thin biotype /sub-crestally	PMv (2SI) < 3	Absence of CTA (IM to DP) Presence CTA (DP toDP_{2y})	–1.11 ± 0.80 ($p=10.10^{-12}$)[c]
B	Thick biotype /sub-crestally	PMv (2SI) > 3	Absence CTA (IM to DP_{2y})	–0.44 ± 0.48 ($p=7.10^{-8}$)[c]
C	Thin biotype /supra-, equi-crestally	PMv (2SI) < 3	Presence of CTA (IM to DP_{2y})	–0.31 ± 0.32 (p=0.006)[c]
D	Thick biotype /supra-,equi-crestally	PMv (2SI) > 3	Presence of CTA (IM to DP_{2y})	–0.16 ± 0.43 (p=0.005)[c]

[a] Vertical dimension of Mucosal barrier = PMv(2SI) peri-implant mucosa SE+JE+CT (at 2SI)
[b] Attachment of connective tissue with BIO(MR) surface upon reaching their contact .
[c] Data were related to the number of patients N (linear model, patient as a random effect).

Table-6 Biological Width Violation and Bone Tissue Response for Tested Groups of Implants

dimension (PMv (2SI) < 3 mm), and the absence of effective connective tissue attachment. In contrast, a statistically significant decrease (p=0,0002) in marginal bone resorption (ΔMBLp (DP_{2y}–IM) = –0,16 ± 0,43 mm) was observed in implants where biological width was fully respected (maintained) in terms of a peri-implant mucosa sufficient dimension (PMv (2SI) > 3 mm) and the presence of an effective connective tissue attachment (CTA).

The bone tissue response to biologic width violation only by the absence of effective CTA or only by an insufficient mucosal barrier dimension (PMv (2SI) < 3 mm) is represented by marginal bone loss of implant group B: –0.44 ± 0.48 mm and C: –0.31 ± 0.32 mm (Table - 6).

The above-presented study results processed in relation to the biological width restoration process show that BioniQ® two-stage implants can be predictably used for patients with a differentiated offer of hard and soft tissues (thin/thick gingival biotype), reaching high success rate, significant marginal bone stability, and optimal long-term functional and aesthetic result of the treatment.

The benefit of the present study is also the clinical verification of the two-zone transmucosal implant part with vertically differentiated surface morphology principle, enabling both effective connective tissue attachment and effective junctional epithelial attachment. Moreover, the smooth surface of the epithelial zone provides a proper function of peri-implant sulcus as the first barrier against bacterial invasion. At the same time, the connective zone structured surface, located on the neck part of the fixture, reduces or eliminates the disadvantages of most current two-stage fixtures, where the CT implant contact is positioned on the distant part of the abutment. This leads to the

traumatization of transmucosal attachment and to complete amputation of connective tissues due to repeated repositioning of impression and prosthetic parts. The findings of the present study revealed that two-zone transmucosal part implants (implant group C and D; **Table- 6**) show significantly lower marginal bone resorption compared to implants where the transmucosal part of the implant forms only a smooth surface of the abutment distant part (implant group A and B; **Table 6**).

Nevertheless, the presented results of the study have some limitations. For further research, it would be beneficial to use histometric evaluations of human biopsies to determine the structure of the peri-implant soft tissues vertical morphology to obtain more accurate information, particularly about the temporal development of the connective and epithelial attachment. The study also did not optimize the vertical position of supra-crestal fixture insertion, although the coronal direction is probably limited by the risk of dehiscence (and reaching the emergence profile while maintaining biological width) and the apical direction by insufficient CT contact area with BIO(MR) surface. However, the vertically situated fixture margin of the two-stage implants , which is located approximately in the middle of the zone of adjacent connective tissue (CTCPp≈ –0.5 mm), was verified in principle by this study and represents a significant impetus for the development of a new connective tissue – level implants.

Conclusions

The results presented in this study demonstrate the high success and predictability of two-stage BioniQ® dental implants with a structured, bioactive, moderately rough BIO(MR) fixture neck surface. Direct contact of connective tissue with the moderately rough BIO (MR) fixture neck surface during (supra-/equi-crestal) insertion prevents marginal bone resorption during biological width restoration in patients with thin and thick gingival biotype. The positive correlation between biological width violations and marginal bone resorption was documented.

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