Evaluation of osseointegration of dental implant with and without primary stability: An experimental study on sheep

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Background and objective: Primary implant stability is a prerequisite for reaching and maintaining osseointegration. This study aimed to check the amount of osseointegration of dental implant when placed with and without primary stability, clinically, radiological, and histologically.

Methods: The experimental study includes four male sheep with a weighted mean of 50 kg and 2 to 4 years old. Forty implants placed, divided into two groups, 20 implants without primary stability as a study group and other 20 implants with primary stability. After intramuscular sedation and local anesthesia, the implant site performed in the inferior border of the basal bone of mandible drilling to 4.3 mm in diameter and 8 mm in length. Forty implants were inserted, 20 implants in the study group (3.3 mm diameter and 8 mm length) and 20 implants in the control group (4.3 mm diameter and 8 mm length), after 3 months the 4 sheep were sacrificed and the universal torque ratchet and Periotest was used to measure the stability of the dental implants clinically. Radiologically, Cone Beam Tomography (CBCT) was taken using ImageJ software for measuring density in both groups. Histologically also ImageJ software used for measuring thread width, the distance between threads and amount of cortical bone at bone-implant contact.

Results: Non-significant difference between both groups. Forty implants successfully tolerated a 30 N/cm reverse torque test, and the results of the Periotest were non-significant. Cone Beam Tomography (CBCT) showed no sign of bone radiolucency, and the density result was non-significant. The histological evaluation confirmed the formation of bone around dental implants in both groups and ImageJ software measurement showing no significant difference between study and experimental groups in depth of threads, the distance between threads and amount of cortical bone in the area of bone-implant contact.

Conclusion: Dental implants had the same chance to Osseointegrate in the absence or presence of primary stability at three months follow up.

Keywords: Dental implant, primary stability, osseointegration.

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Introduction

Osseointegrated dental implants measured as one of the main treatment options for restoring missing teeth dentulous patients over the last three decades.[1] The osseointegrated term was defined by Branemark (1985) as "a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant".[2]

There is some principle factor to reach successful osseointegration which includes; using biocompatible materials, a traumatic surgery, the presence of primary implant stability and uninterrupted healing phase.[3]
Primary stability is a prerequisite for achieving and maintaining osseointegration.[4] Primary implant stability is defined as the absence of dental implant movement in the bone bed, immediately after insertion.[5] The concept of primary stability is considered essential to determine the loading time; that is immediate, early or delayed loading.[6]
The absence of primary implant stability has been displayed to harm the process of osseointegration.[7] Micromotions above 50–100 micrometers may negatively influence osseointegration and bone remodeling by forming fibrous tissues and inducing bone resorption surrounding the dental implant.[8] Therefore, high primary stability is essential for successful osseointegration of dental implants, while, in cases of poor bone structure, biomechanical overloading, and bone resorption at the contact, the primary stability is insufficient.[9]
However, attaining bone stability around dental implants depends on several factors that may or may not be directly affected by the implantologist, such as quality of the bone as a local one and age, bone metabolism, systematic chronic diseases, and lifestyle (e.g., smoking habits) as systemic ones.10 On the other hand, treatment-related factors, such as augmentation material, implant design (macro and micro), surgical procedures, and loading protocols, are also involved. [10]
Many studies showed that it is essential to assess implant stability at different time-points ensure successful osseointegration[7, 11], however, another study found that implant can osseointegrated without primary stability, (which 95% of implants successfully osseointegrated without primary stability.)[3] This experimental study aimed to evaluate the result of dental implants with and without primary stability clinically, radiological and histologically.

Material and method

This experimental study includes four male sheep with a mean 50 kg in weight and 2 to 4 years old. All surgical procedures are done at the operation theater (Qushtapa Veterinary Center-Erbil-Iraq). Forty implants placed in two groups, 20 in the study group which placed without primary stability and other 20 in the control group with primary stability.

Anesthesia and surgical procedure. The surgical procedures performed under intramuscular sedation and local anesthesia. The animals first took sedation using Ketamine hydrochloride 5mg/kg (KETALROM-50, S.C ROMVAC company, Ilfov,Romania) and xylazine 0.2mg/kg (xyla; metaalweg 8,CG ventery, the Netherland), and 0.2 mg/kg local anesthesia consisting of Lidocaine Hydrochloride 2% and Epinephrine 1:100,000 (Lignospan, Louisville CO,80027 by Novocol Pharmaceutical of Canada, Ontario, Canada) are administered in the surgical area. Then the surgical area was clipped, shaved, washed, and disinfected with povidone-iodine (Betadine). The incision is made by blade number 15 in the inferior border of the mandible, surgical site exposed. The implant site performed according to the manufacturer's guidelines of implant system (implant Swiss) drilling to 4.3 mm in diameter and 8 mm in length. Twenty dental implants placed as a study group with (3.3 mm diameter and 8 mm length), and other 20 implants placed as a control group (4.3 mm diameter and 8 mm length). All implants submerged about 1 mm in the marginal ridge of prepared implant bed and the cover screw installed, then surgical site sutured and the sheep followed up for three months later sacrificed (Figure 1. A,B).The mandible was separated from skull, cleaned then fixed in formalin aldehyde and underwent clinical, radiological and histological evaluation.

Postoperative care. The animals were followed up, the surgical area disinfected by iodine and antibiotics amoxicillin ith clavulanic acid (betamox LA) 0.1 ml/kg once daily with analgesic (NP ANALGIN-Vietnam) contain Analgin 0.1 ml/kg 1 time daily for seven days postoperatively. They take the same food as the other sheep in the farm postoperatively during the three months of follow-up.

Primary stability measurement. Primary stability of dental implants measured us-

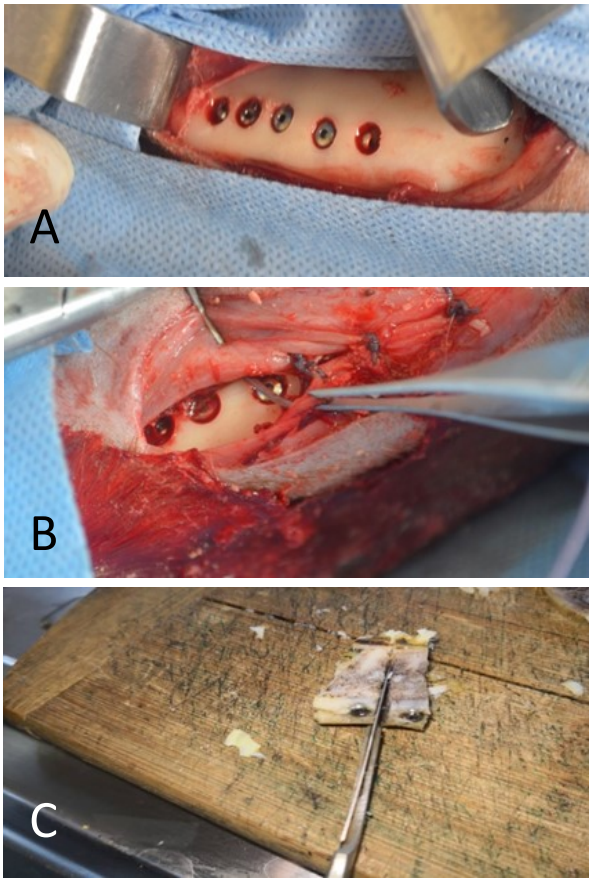

Figure 1: A. Implant placement in the inferior border of the mandible. B. Suturing in layers. C. showing cutting of the bone around the implant after its softening.

ing Periotest, the control group mean was (-3.8) while in the study group was so loose and hadn't any stability, so we couldn't measure.

Characteristic of implant Swiss. Implant Swiss is a special implant design with a cylindrical (straight) design on the neck area and conical (tapered) design on the apical of the implant. This dental implant, following calcium phosphate sanding, the surfaces are roughened using double acid etching. The special thread design provides ideal primary stability on every type of bone without transferring stress to the bone. The implant can guide itself in the bone with its self-cutting edges.

Clinical, radiological, histological evaluation. Clinically reverse torque test using universal torque ratchet and Periotest for measuring the secondary stability of dental implants of both groups three months after osseointegration.

Radiologically cone-beam computed tomography (CBCT) (Cone Beam CT Newtom, model: Giano. Italy) used with using ImageJ software (National Institutes of Health, Bethesda, MD, USA) for measuring the density of the bone around dental implants in both groups.

Later the mandible placed in formaldehyde for fixation and transferred to the laboratory, then placed in the bone solvent solution (10% hydrochloric acid) for five days for demineralization and softening of the bone. When the bone becomes soft, the dental implants removed a traumatically from the bone (figure 1.C), later the block and the slides fabricated and underwent histological investigation by light microscope, and ImageJ software (National Institutes of Health, Bethesda, MD, USA) for measuring depth of threads, distance between threads and cortical amount of formed bone at bone-implant contact.

Results

The results of the clinical, radiological, and histological examinations for the experimental operated sheep were collected throughout three months of follow-up, respectively.

Clinically, there was bone formed over the dental implants, no sign of peri-implant marginal bone loss was observed. The bone removed, and universal torque ratchet placed after removal of healing screw, all the dental implants of both groups successfully tolerated a 30 N/ cm reverse torque test comprising (100%) as shown in Figure 2.A and B.

Periotest also was used for measuring the stability of dental implants three months after osseointegration. The cover screw was removed and gingival former placed, then Periotest device placed in both groups (Figure 2.C). The mean of the implants in the control group was -3.5, whereasin the test group was -2.40. The result was not significant p value> 0.05 as shown in Table 1.

Radiological evaluation. Cone-beam computerized tomography (CBCT) was used to evaluate the dental implants radiologically using new tom (Figure

Table 1: Descriptive statistics of the mean and standard deviation of perio test measuring the secondary stability of the dental implant

	Control group	Study group	T-test	P-value
N	20	20		
Mean	-4.02	-3.85	0.845	0.204
SD	0.58	0.309		

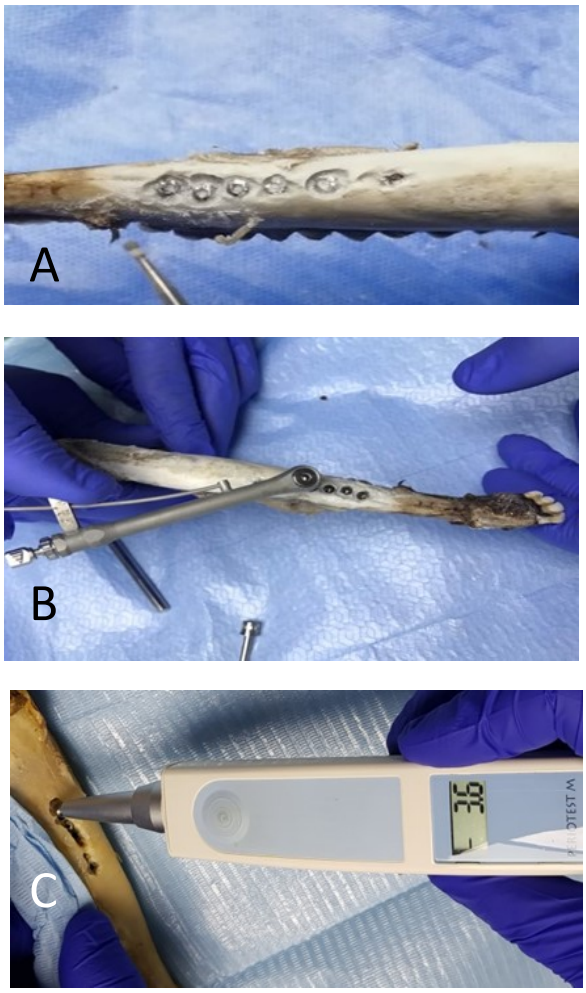

Figure 2: A. Shows osseointegrated implants bone formation over the healing screw. B. Reverse torque test. C. using a perio test for measuring secondary stability

3). There was no sign of marginal bone loss, and bone radiolucency in both groups and implants was stated in their position.

The bone density around the dental implant was measured using ImageJ software of both groups. The mean of the control group is slightly higher than the test group, in which the means were 124.68, 115.84 pixels respectively, and the p-value is (0.299). The result is not significant because ($p > 0.05$) as shown in Table 2.

Histological evaluation. Three months after implantation in the study group, the bone interface appeared like an exact

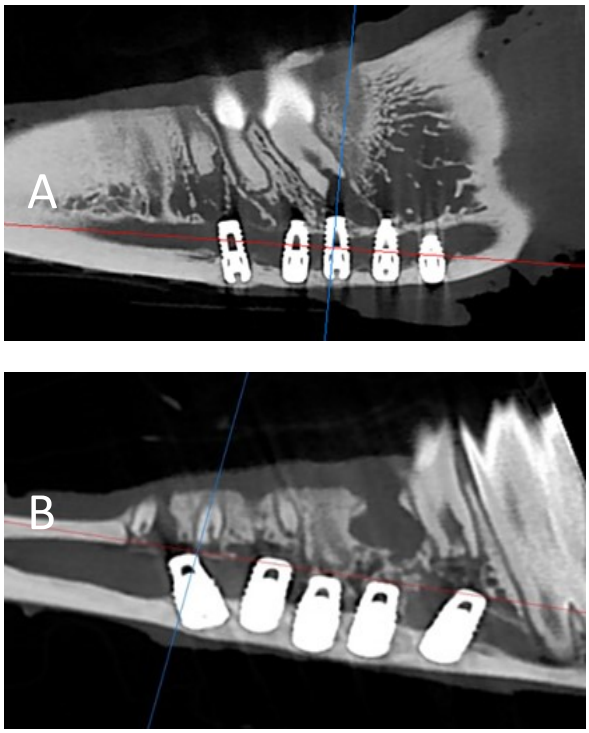

Figure 3: A. CBCT of the study group, B. CBCT of the control group

imprint of the implant, and the outlines of the implant threads were fully visible on the bone surface. The external surface of the bone seemed compact, verifying that a dense cortical bone was formed at the bone-implant contact to create a continuous osseointegrated interface Figure 4.

Table 2: Descriptive statistics of the mean and standard deviation of density measurement of bone-implant contact on cbct by ImageJ Software.

	Control group	Study group	T-test	P-value
N	20	20		
Mean	124.68	115.84	0.530	0.299
SD	50.27	52.49		

Figure 4: Bone-implant contact after removal of the dental implant from the bone.

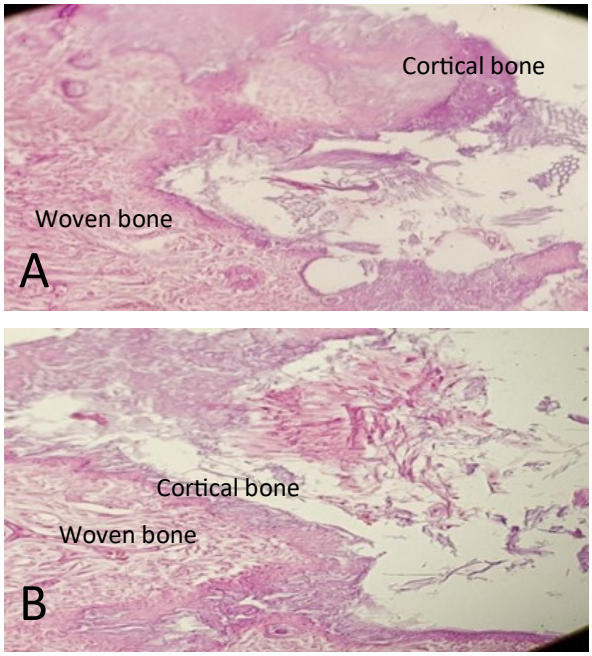

Figure 5: A.experimental group. B.control group

The light microscope showed compact bone formation surrounding the dental implant in the area of bone-implant contact three months after osseointegration. The surrounding area shows granulation tissue (GT), woven bone (WB) and areas of vascular congestion (VC). It indicates that bone formed but complete mineralization didn't occur and need more time. (Figure 4.A) (H and E 400x).

In Figure 4.b, light microscope showing bone formation around dental implant three months after osseointegration. The injured bone replaced by newly formed bone, especially in the area of bone-implant contact in which cortical bone formed. Woven bone is seen in a pinkish area surrounded by the area of vascular congestion and granulation tissue formation, indicate that bone formed but complete mineralization didn't occur and need more time (H and E 400x).

On the other hand, ImageJ software showed no significant difference in the depth of threads, the distance of threads and the width of cortical bone in the bone-implant contact in which the p-value was 0.39, 0.249 and 0.092 pixels respectively and these results non-significant ($p > 0.05$). This result displayed a similar amount of bone formation around dental implants three months after osseointegration. As shown in Table 3.

Discussion

It had dependably been reported that primary stability is related to the successful dental implant restoration.[4] Our primary objective for doing this study was to determine the amount of osseointegration in implant within three months of the time of placement when installing without primary stability, loosely inserted into the bone and other implants that have primary stability.

Table 3: Descriptive statistics of the mean and standard deviation of the width of thread and the distance

Groups	Control		Study		T-test	P-value
	Mean	SD	Mean	SD		
depth of threads	186.33	4,35	186,89	3.98	-0.282	0.390
Distance btw threads	196	7.37	194	6.32	0.689	0249
Amount of cortical bone	168	7.85	163	6.01	139	0.092

The concepts of primary stability are considered essential to determine because this can serve as a guide about the time of loading; either immediate, early or delayed loading.[6] In this study, all the dental implants in the control group which placed with primary stability were successfully osseointegrated three months after placement.

While there are some situations in which lack of primary stability may occur when the bone mineralization lessened, and the bone, does did not offer adequate anchorage, additional clinical condition where lack of primary stability may result is the immediate placement of an implant in extraction socket.[27] Inexpert clinicians may either over prepare the osteotomy site, or apply an excessive level of torque, breaking the bone around the implant.

Impaired primary implant stability had been shown to hazard the process of osseointegration.[7] whereas, in our study, all the dental implants in the study group, which placed without primary stability, were successfully osseointegrated three months after placement.

There were limited studies regarding the absence of primary stability of the dental implant. The result of our study is in disagreement with that of Lioubavina-Hack (2006) who did it on sixteen, 6-month-old male rats.A rigid non-porous hemispherical teflons capsule fixed to the ramus with four mini-screws. In stud group implants placed without primary stability, in such a way that had no contact with mandibular ramus, while in the control group placed with primary stability and concluded that primary stability is a prerequisite for successful osseointegration, moreover, primary instability of dental implants resulted in fibrous encapsulation of the implants.[12]

The data of the present study is agreed with that of Shihab (2017) which was enrolled on two adult sheep 3–4 years old in the same way as ours and concluded that dental implants have a chance to osseointegrate even in the lack of primary stability.[13]

Our result is also in the same line with a cohort study of Cobo-Vázquez (2018) when dental implants placed without primary stability. Out of the 92 implants in a 12-month follow-up, only three dental implants were loosed which represent 3, 3% and 89 implants remained (96, 7%), and concluded that poor primary stability was not statistically significant in the loss of dental implants.[14]

Numerous aspects may play a role in achieving implant osseointegration. It had been informed that the implant design is a significant parameter for getting primary stability.[15] In our study, the used implants share good design (tapered) and special thread design and this also may aid in getting a high success rate.

A microscopically rough surface on the implant is more favored than a smooth surface as it increases bone anchorage and strengthens the biomechanical interlocking of bone with the implant.[16] Rough implant surfaces existing a larger surface area and allow a firmer mechanical link to the surrounding tissues.[17] Numerous reports had been demonstrated that implants with the roughed surface have rapid and increased bone accru-

al or bone implant contact.[18-20] The information that these implants can promote osseointegration at different levels has already been an unquestionable fact.[21,22] In our study, the used implants have roughened surface; this also may aid in getting the high success rate.

Excessive trauma during surgery may affect the bone-to-implant interface and lead to lessening the osseointegration.[16] Internal or external irrigation by cool saline with intermittent pressure on the drills, every 3 to 5 seconds, using new drills and a gradual drill sequence can promote osseointegration.[23] The same protocol we followed in our study what may help in getting such results.

The process of osseointegration is started by homeostasis, which begins as a result of surgical trauma of drilling and continues after dental implant insertion, this staging period may vary from a few minutes to several hours.[24] Bleeding of Injured vessels would cause fibrinogen, polymerization and later extracellular matrix formation in the bony defect.[25] Following platelet activation, clot formation occurred.[26] We suppose that, in addition to the quality of the bone, implant design and surfaces, surgical technique, the primary blood clot formation may play a main role in the process of healing of dental implants, which played an important role in networking and stabilizing the loose dental implant and consequently secondary stability and osseointegration.

Conclusion

Dental implants had the same chance to Osseointegrate in the presence or absence of primary stability at three months follow up.

Clinical relevance

On the basis of our results, we indeed assured that the primary stability is important for successful dental implant osseointegration, especially for immediate loading implants, but even in the absence of primary stability, there is a high possibility of success and ossointegration after three months.

Conflicts of interest

The author reported no conflict of interests.

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