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***Original Article***

# Effect of Bovine Milk on Implant Osseointegration: an *in vivo* Study

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**Abstract**

The safety and success of an implant after surgery depend on many factors, some related to the implant's biocompatibility, properties, surface modification, design, and other factors related to surgical procedures, implant bed preparation, and drilling techniques. It is recognized that the success of implant dentistry depends on several factors that may be related to biochemical properties and modification in mechanical properties. The present study aimed to assess the effect of using bovine milk as an irrigant solution on implant osseointegration. The implant socket was prepared by drilling bone holes in 20 femurs of the rabbits at steady rotation speeds with different irrigate solutions (normal saline / commercial pasteurized bovine milk). Mechanical tests and histological investigation were performed to estimate the removal torque record and implant contact area, BIC. Findings illustrate that implant contact area (BIC) and removal torque mean values are higher in experimental compared to control with more bone apposition and maturation at 4&8 week measured periods. Osseointegration is accelerated by using bovine milk in irrigation and rinsing of implant socket.

**Keywords:** Bone Healing, CpTi, Bovine Milk, Osseointegration, Dental Implant

## 1. Introduction

The safety and success of an implant after surgery depend on many factors, some related to the implant's biocompatibility, properties, surface modification, design, and other factors related to surgical procedures, implant bed preparation, and drilling techniques (1-3). All these factors Participate in accomplishing the reactions that helps in osseointegration. Recent research has used the modification in the implant-related factors to get maximum osseointegration and shorten the healing time (4-6).

Implant bed preparation is an essential factor in surgical techniques that influence osseointegration. Drilling the implant bed may increase the temperature in the bone tissue adjacent to the implant surface and cause thermal and mechanical damage to the bone. Furthermore, it may influence the initial stability of the implant (7, 8).

Many studies reported that milk is an effective agent in maintaining cell survival and their proliferative ability in the periodontal ligament of avulsed tooth and was recommended as storage media and essential for successful reimplantation (9-11). Although no studies were found related to using of milk with implant, therefore the present work is designed to show the effect of using bovine milk as an irrigate material during surgical implant procedure to reduce the healing period of implantation and increase the bone-implant interface that induces Improvement in osseointegration.

## 2. Materials and Methods

### 2.1. Preparation of Implant

Forty implant screws were prepared from the rods of pure titanium (CpTi), about 30 mm in length and 5 mm in diameter, by using a lathe machine. The set screw is characterized by length (8mm), threaded (5mm) and smoothness (3mm), and diameter (3 mm). The head of the screw had a slit about (1.5mm) in length .cleaning of screws was done by application of ultrasonic cleaner.

### 2.2. Animals

Twenty healthy males (New Zealand rabbits) with a weighting of (2-2.5 kg) and ages ranging from 10-12 months have been enrolled in this study. The animals were kept in the National Center of Drug Control and Research /Iraq / animal department at a constant temperature of 23°C and humidity, followed by the National Council's guide about laboratory care of animals.

### 2.3. Surgical Procedure

Before the operation, the animals were injected intramuscularly with anesthetic solutions (Xylazine (0.1 ml: 6 mg)/kg and Ketamine 50 mg/kg). The skin of the operation regions was shaved and scrubbed with a piece of damped cotton with 2% povidone-iodine and 75 % alcohol solution for 5 minutes. The skin and fascia flap were dissected in the femoral region, then the underlying subcutaneous tissue and muscular tissues were bluntly dissected, reflecting and exposing the bone surface. Two holes were made (3 mm diameter and 5 mm depth). The hole was performed using intermittent drilling with an angled handpiece and a steady speed of 1400 rotations per minute and 35 N.cm torque. The holes were expanded progressively with a 2.0 mm spherical drill, 2.0 mm twist drill, and 2.8 mm twist drill. After preparation, one hole was irrigated and rinsed with 0.9 % w/v saline solution, considered a control group, while the other hole was irrigated and rinsed with commercial pasteurized bovine milk with a pH of 6.5-7.2 and considered the experimental group. External irrigation for both irrigate materials was done at room temperature.

The implant was inserted with the bone ridge using the manual torque meter; the torque value was standardized at 10 N.cm. Furthermore, the implants' entire (5) mm threaded part was inserted and fixed to the bone. After 4 and 8 weeks of implantation, the rabbits were sacrificed (control, experimental). Ten rabbits for histology investigation and bone-implant contact (BIC) were done (five rabbits for each period of 4, 8 weeks).

The percentage of bone-implant contact (BIC) was determined by measuring the length of bone tissue in direct contact with the surface of the screw. The mean measurements from both implant sides in three sections were recorded (12).

Additional ten rabbits were used for mechanical testing and both periods. The torque was measured with the torque wrench device (OsstellTM; Savedalen, Sweden).

### 2.4. Statistical Analysis

ANOVA tests with P value were used to analyze the differences in removal torque test and implant contact area (BIC) for different groups at 4&8 week measured periods.

## 3. Results

Histological features for the control group at a 4-week duration show the formation of sparse bone trabeculae at the implant bed with ill-defined threads, while for the experimental group, the specimen showed more obvious bone trabeculae that filled most of the implant bed. At eight weeks of post implantation period, the control specimen showed threads with trabeculated bone defined at the threads' apex, while the implant bed's base was filled mostly with immature bone. On the other hand, the experimental specimen showed well-defined threads with an implant bed filled with mature bone, Figure 1A, 1B, 1C, and 1D).

Results show that the torque value in the experimental implant was higher than the control for both study periods (4 and 8 weeks) and with a significant value.

Moreover, the findings illustrated a difference in the mean value for each group for both periods and were higher in the 8th week compared to the 4th week.

Results for BIC mean value record a significant difference for the experimental specimens compared to control in both periods. Also, mean BIC showed a higher record value in the 8th week compared to the 4th, tables 1, 2 and 3.

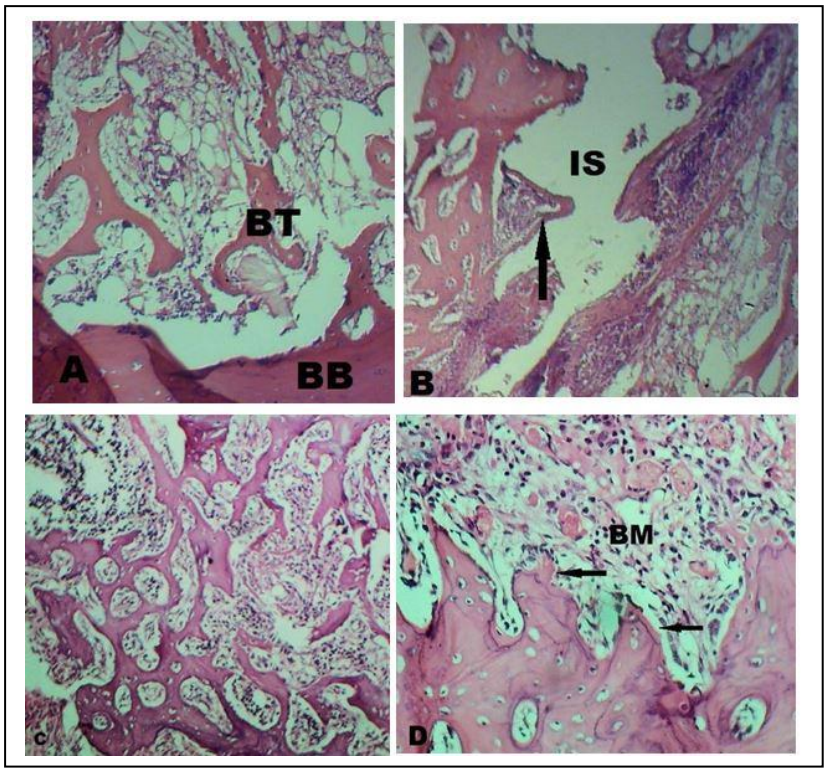


**Figure 1.** Microscopic views for CpTi implant with different irrigate materials at 4 &8 weeks A. Control specimen (with saline irrigation) shows basal bone (BB) and newly formed bone trabeculae (BT) At 4 weeks duration. H&E X10. Control at 8 weeks duration shows threads (arrow) around the Implant space (IS). H&E X4. Experimental specimen (with bovine milk irrigation) shows that bone Trabeculae filled the implant bed at 4 weeks. H&E X4. Experimental at 8 weeks duration shows well-developed threads (arrows) with bone marrow (BM) H&E X10

**Table 1.** Statistics analysis for Removal Torque test (Ncm) in different groups at 4&8 week measured periods.

| Period | Group | No. implant | Mean | Std.Dev | Std.Error | 95% Confidence Interval for mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper bound | | |
| 4 week | Control | 5 | 14.22 | 0.77 | 0.34 | 12.54 | 16.23 | 12.11 | 16.44 |
| | Experimental | 5 | 18.56 | 0.88 | 0.42 | 16.54 | 19.88 | 16.32 | 20.50 |
| 8 week | Control | 5 | 19.54 | 0.91 | 0.79 | 16.92 | 21.56 | 17.22 | 22.22 |
| | Experimental | 5 | 26.34 | 0.60 | 0.31 | 24.32 | 28.44 | 23.79 | 28.98 |

**Table 2.** Statistics analysis for BIC test in different groups at 4&8 week measured period.

| Period | Group | No. Implant | Mean | Std.Dev | Std.Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| 4 week | Control | 5 | 11.22 | 0.67 | 0.32 | 9.54 | 12.23 | 9.11 | 13.44 |
| | Experimental | 5 | 22.56 | 0.48 | 0.32 | 20.34 | 23.88 | 20.12 | 23.59 |
| 8 week | Control | 5 | 21.54 | 0.71 | 0.42 | 19.92 | 23.56 | 19.22 | 24.22 |
| | Experimental | 5 | 25.34 | 0.70 | 0.30 | 24.32 | 26.44 | 23.79 | 27.36 |

**Table 3.** LSD after ANOVA test for removal torque and BIC between groups at 4&8 week measured period

| Parameter | Period | Group | Mean Diff. | Sig. | CS (*) |
|---|---|---|---|---|---|
| Removal torque | 4 week | Control/experimental | -5.1 | 0.000 | HS |
| | 8 week | | -5.04 | 0.000 | HS |
| BIC | 4 week | Control/Experimental | -6.22 | 0.000 | HS |
| | 8 week | | -1.60 | 0.011 | S |

## 4. Discussion

The success of dental implants depends on the biocompatibility of materials used within and during the surgical procedure and on the osseointegration resulting from implantation (13, 14). The present study uses commercial pasteurized bovine milk for irrigation and rinsing of implant sockets during surgical preparation and records the direct association between bovine milk and tissue composition (15). The histological evaluation shows bone formation (bone trabeculae) at 4 weeks, the mature bone at 8 weeks, and reports to be more than control. The area where the implant is in contact with hard tissue is responsible for establishing a kind of anchoring of the implant in its bed. The present result records a high BIC value for experimental compared to control, which results in high osseointegration with a high removal torque value.

Moreover, many factors are explicitly related to the benefit of using bovine milk; the important one is the presence of calcium and phosphorus in the chemical composition of the milk that is shared in the maturation of the newly formed bone (16, 17). Furthermore, milk acts as a chemical bond between titanium implants and bone tissue that maintains the hydration of the cells and prevents their death (18, 19) during and after the surgical operation more than saline does.

Milk also contains essential nutrients with specific growth factors that stimulate migration and proliferation and accelerate the osteogenic differentiation into specialized bone cells that adhere to the implant surface (10, 11). In addition, using milk as an isotonic liquid (20) with a pH of 6.5- 7.2, a neutral pH that may act as supportive physiological conditions, help in osseointegration. In the present study, the milk is used at room temperature as external irrigation during drilling that provides sufficient cooling and tries to keep the temperature below the conclusive value and away from harm to surrounding tissues (21).

Although, several authors evaluated the benefits of using milk in dentistry and reported using the milk as a storage medium for avulsed teeth, and studied the ability of the milk to prevent cell death (22-24). However, our study is the first to use milk as irrigated material that helps in the osseointegration of implants.

Using milk as external irrigate material appears to be an effective agent to enhance bone formation in the implant bed and around the surface and records more

removal torque value compared to saline irrigation. Therefore, we recommended using milk in the surgical procedure of implant preparation.

## Authors' Contribution

Study concept and design: L. K. L. and A. Y. A.
Acquisition of data: A. Y. A.
Analysis and interpretation of data: O. M. C.
Drafting of the manuscript: B. F. A.
Critical revision of the manuscript for important intellectual content: O. M. C.
Statistical analysis: L. K. L.
Administrative, technical, and material support: A. Y. A.

## Ethics

The ethical approval from was obtained from the ethical committee of the Al-Mustaqbal University College (license No: 069222) for all of the experimental approaches.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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