"TO EVALUATE THE OSTEOGENESIS AND OSSEOINTEGRATION ON TITANIUM IMPLANTS SURFACE TREATED WITH CISSUS QUADRANGULARIS HYDROGEL: AN IN-VIVO ANIMAL STUDY".

By

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Dissertation

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of the requirements for the degree of

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In

PROSTHODONTICS AND CROWN & BRIDGE (BRANCH – I)

Under the guidance of

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KAHERV.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI, KARNATAKA.

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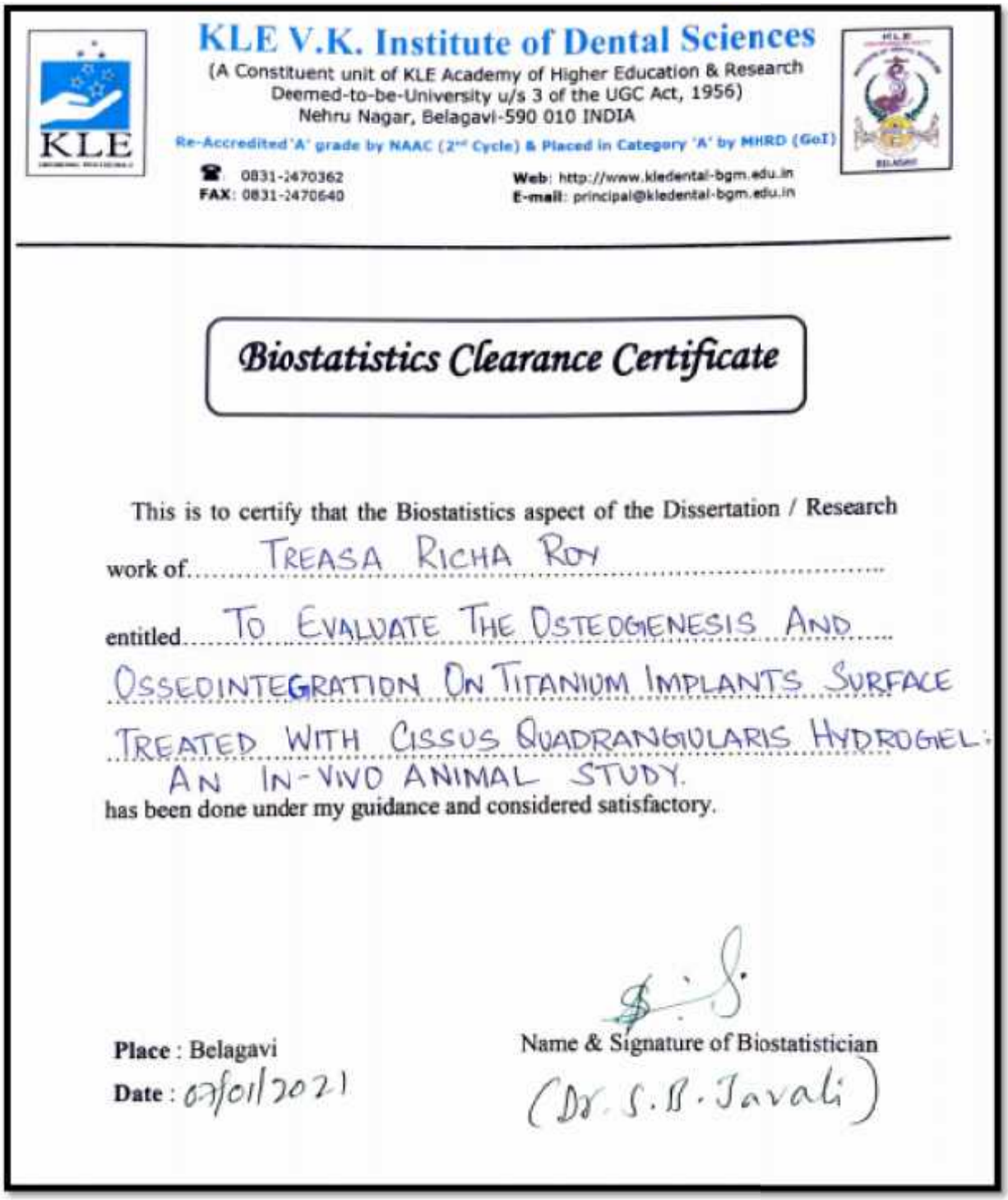

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To

My family

And

My guide

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LIST OF ABBREVIATIONS

Cq	-	*Cissusquadrangularis*-
Ch	-	Chitosan
Cq-Ch-H	-	*Cissusquadrangularis*-Chitosan Hydrogel
Ostn	-	Osseointegration
Ti	-	Titanium
c.p.	-	Commercially pure
RFA	-	Resonance Frequency Analysis
ISQ	-	Implant Stability Quotient
RTQ	-	Removal Torque Quotient
RT	-	Removal torque
SfM	-	Surface modification
BIC	-	Bone to Implant Contact
ECM	-	Extracellular matrix
GAG	-	Glycosaminoglycans
ALP	-	Alkaline phosphatase
SEM	-	Scanning Electron Microscope
Wks	-	Weeks
°C	-	Degree Celsius
µl	-	Microlitre
ml	-	Millilitre

mg	-	Milligrams
nm	-	Nanometer
hrs	-	Hours
mins	-	Minutes
SPSS	-	Statistical package for social science
%	-	Percentage
Fig.	-	Figure
IM	-	Intramuscular
IV	-	Intravenous
SC	-	Subcutaneous
O_2	-	Oxygen
RVG	-	Radiovisiography
H & E	-	Haematoxylin and Eosin
MT	-	MassonsTrichrome

ABSTRACT

Background:

The challenge in implant placement remains to reduce the time taken for bone healing following dental implant placement. Surface modified implants have gained attention as a factor to stimulate osseointegration. In the present study a novel hydrophilic hydrogel is made using Cissusquandrangularis (Cq) taking into consideration the excellent osetoproliferative and osteogenic properties of cissus on a promising chitosan scaffold.

Purpose of the Study

Titanium implant systems, though considered as the gold standard for rehabilitation of edentulous spaces, have been criticized for certain inherent flaws. The tiring wait for optimum osseointegrationoftens makes the patient reconsider dental implant placement. Surface treated titanium implants are emerging as a promising alternative to machined Titanium implants for oral rehabilitation with superior osseointegration, early loading protocols and shortened period of edentulousness. This research aims to critically analyze and review the credibility of Cissusquadrangularis as a surface coating on a commercially pure dental implant surface.

Aim/Hypothesis:

The aim of the present study was to evaluate the effect of Cissusquadrangularis hydrogel on bone regeneration around titanium implants.

Materials and Methods:

24 BioLine implants (3.75mm x 6mm) were divided into 2 subgroups of 12 each. Testgroup consisted of commercially pure titanium implants surface treated with novel Cissusquandrangularis hydrogel and control groupconsisted of commercially pure titanium implants. Preparation of Cq-Ch hydrogel: Ch hydrogel was prepared. Pure, dry Cq extract was procured. The pure cissus extract was incorporated into the chitosan hydrogel to obtain 3% CqCh hydrogel. The novel hydrogel was then coated on the titanium implants using dip coating technique prior to placement in the rabbit tibia or femur. 24 commercially available BioLine Implants (BioLine Dental GmbH & Co. KG- Germany), 3.75mm x 6mm, were placed in the tibia of 12 rabbits. Implants were placed with conventional drilling technique. Hydrogel coated implants as the experimental group (n=12), and commercially pure implants as the control group (n=12). Resonance frequency analysis was conducted at 6 and 12 weeks. Simultaneously, after 6 and 12 weeks of healing, animals were sacrificed and subjected to removal torque tests and samples were retrieved en bloc and stained for histologic analysis.

Results:

After a healing period of 3 months, a significantly higher removal torque was demonstrated to unscrew the CqCh coated implants (average 52%increase) compared to the uncoated implants which showed an increase of 10%. An average of 22% increase was observed in implant stability quotient values in the test group implants when compared to 10% increase in the control group, uncoated implants. The collected data was subjected to statistical analysis using independent t test and dependant t test and results were found to be statistically significant. ($P < 0.05$).

Conclusions and Clinical Implications:

Study data concludes that hydrophilic implant surface not only increases bone to implant contact but also stimulate new bone formation. It demonstrates a hydrogel that is one of its kind being osteogenic, biomimetic in nature and enhancing the rate of osseointegation. The study holds clinical evidence that CqCh hydrogel coated implants can reach firm stability in bone and may offer promising results promoting early loading of implants.

Keywords: titanium implant, surface modification, osseointegration, cissusquadrangularis, chitosan, hydrogel, rabbit

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INRODUCTION

Dental implant placement for the replacement of missing teeth has increased steadily through the last 30 years. Although it was discovered in the early 1930's, through archaeological excavations that the Mayan civilization used dental implants, there is an ever increasing demand for novel implant innovations to this day.[1] The ultimate success and best long term prognosis of dental implant treatment depends primarily on the secured position of the implant in the jawbone, termed Osseointegration. Branemark stated that ostn is an adherent connection formed between the bone and the titanium implant which can be observed at light microscopic level.[2] Ostn, seen as the close contact between bone and implant, has been proved to be better in implants with SfM and so, the interest in SfM of dental implants has to be understood as a vital yet natural trend.[3] Even at the end of the ostn phase of 3-6 months, only about 60–70% of the implant surface is covered by bone. This is termed bone-to-implant contact (BIC) and is widely used in research to measure the degree of ostn.[4] BIC is crucial in the long-term success of dental implants. Numerous studies have concluded better bone formation seen surrounding surface modified implants when compared to machined titanium surfaces.[3,5,6,7]

Today, there is a demand from patients with missing teeth, for rehabilitation of masticatory function and aesthetic appearance, as well as to shorten the period of ostn of the implants, which takes a relatively long time of 3-6 months, depending on the available bone.[8] The rate of bone regeneration, meaning the quantity and quality, is directly related to implant surface properties. Modernization in implant material, design, and surface topography enhances the stability of the dental implant as well as shortens healing periods.

Over the years there has been comprehensive research into the physical, chemi cal and biological charcteristics of the dental implant surface in an effort to improve th e ostn process and achieve earlier loading of dental implants.[4]

Most surface treatments for implants aim to enhance the function of bone-forming cells and their mediators to increase the development of new bone and facilitate and promote earlier ostn and higher secondary implant stability. Surface treated implants showed advantageous characteristics for enhanced bone formation and has become the current drift in implant dentistry. In addition to microtopography, other biophysical variables also have an effect on bone formation, such as surface chemistry, surface charge, and wettability.[9] Compared to hydrophobic surfaces, hydrophilic surfaces appear to favour interactions with biological fluids and cells, and the hydrophilicity is influenced by the chemical composition of the surface. For protein adsorption and cell attachment, the structure and surface charges plays an important role. Following implant placement, its initial interaction with proteins and cells is influenced by the implant surface.[10] Implant surface treatments have been proven to enhance hydrophilicity of the implant surface.[11,12] Various techniques of surface treatments can broadly be divided as Additive and Subtractive methods. Additive approaches include treatments in which other materials are either superficially added or integrated onto the surface of the implant. Whereas in subtractive methods, removal of surface material by mechanical methods such as shaping/removing, grinding, machining, or blasting to generate surface roughness is included.[13] Furthermore, the biofunctionalization of implants surfaces, by adding different substances to improve its biological interactions has also been recently investigated. One of the SfM to further stimulate ostn is the coating of implant surfaces with biological components. In this context, many different types of surface

coatings have been analyzed in recent years. These involve coatings with proteins from the extracellular matrix (ECM), peptides, growth factors, lipids, and so on. Various types of organic hydrogel coatings, biopolymers, biomimetic and bioinspired films (e.g., components of the natural cell surroundings) have been explored on biomaterials used for dental implants, which can potentially influence cellular activity during peri-implant healing, and get an intimate BIC, promoting ostn by the proliferation of osteoblasts. Hydrogel coating has been shown to significantly increase the implant surface area invariably having a positive effect on the ostn.[6] Hydrogels are three-dimensional (3D) physically or chemically crosslinked networks of hydrophilic polymers with porous structures and high capacities for water absorption.[14] Either natural (chitosan, gelatin and polynucleotides) or synthetic polymers (poly(vinyl alcohol), poly(ethylene glycol) and poly(sulphobetaine)) can be made into hydrogels.[15] Due to their typical "soft and wet" properties, which are extremely similar to biological tissue, hydrogels have been widely used in various biomedical fields, such as tissue engineering, drug delivery, contact lenses, wound dressings and implantable devices.[16] Owing to its three dimensional structure and its ability to swell significantly in water hydrogel scaffolds help in spatially preserving wound healing sites. In tissue engineering, hydrogels have become a promising form of scaffold for simultaneous cell growth and drug delivery.[17] A wide range of animal models have been applied in testing repair with hydrogels in bone defects.[18,19,20] The use of animal models in oral health science has increased significantly over the past 20 years. In attempts to understand the onset and dissemination of different oral diseases and to identify and develop dental materials and methods suitable for the restoration of the damaged tissues, animal experiments are of fundamental significance. A particular animal model is selected because it is deemed suitable for the condition being investigated

and is believed likely to react to the suggested treatment for the character being investigated in the same way as humans. Valuable information can be retrieved from appropriately planned animal experiments, regardless of the particular animal models (rat, rabbit, sheep, dog, pig or non-human primate) or surgical sites. As measurable host/implant response indicators where different surface designs are compared, static and dynamic histomorphometric parameters plus biomechanical testing are recommended. In in-vivo animal studies, bone-to-implant contact (BIC) is the most frequently evaluated parameter, along with bone density and the amount and form of cellular material. The most often used and preferred animal models for investigations of the ostn of dental implants and the osteogenesis of different bone substitutes are the rabbit femur and tibia models.[21] Due to their size, easy handling, short life span, and economical aspects of obtaining and maintaining, rabbits appear as a first-hand option. At about 6 months of age, rabbits achieve their skeletal maturity shortly after sexual maturity.[22] Consequently, prior to testing in a larger animal, rabbits are primarily used for screening implant materials. In implant screening studies, the decisive factors to show ostn are elementary bone remodeling properties and a bone volume large enough for implantation. The rabbit fulfills these criteria, and thus there is no need to use higher-order animals for implant design screening tests. During the initial phase of implant testing, rabbits are often used to look for all potential components and systemic effects of the proposed construct, because a wide range of cells, sera, tissue structures, and organs of rabbits bear a near resemblance to humans.[22] The goals behind the development of implant SfM and subsequent testing in animal models are to improve clinical significance in cases with poor bone quantity or quality, to help accelerate bone healing, thus enabling immediate or early loading protocols, and also to stimulate bone growth to enable implant placement in sites that do not have

adequate residual alveolar ridge. This research has been conducted keeping in mind the need for a new potential coating material which is a biopolymer and biomimetic in nature, comprised of Cissus quandrangularis (Cq) in combination with Chitosan(Ch). Chitosan (1-4,2- amino-2-deoxy-D-glucan) is the de-acetylated derivative of chitin, a linear polysaccharide found primarily in the exoskeletons of arthropods.[23] It has a polycationic carbohydrate structure and its cationic nature provides a suitable substrate for cell adhesion.[24] The distinct structural similarities of chitosan to mammalian glycosaminoglycans (GAGs), a family of mammalian heteropolysaccharides found predominantly on the cell surface and in the extracellular matrix (ECM).[25] Chitosan in tissue engineering, potentiated the differentiation of osteoprogenitor cells, facilitated the formation of bone and inhibited fibroblast proliferation. Osteoconductive properties, enhanced wound healing and antimicrobial properties makes chitosan, a biopolymer, attractive as a biocompatible and bioactive coating for dental implant surfaces.[26] Minimal inflammatory reactions have been observed in tissues which have been in contact with the chitosan coated pins; while the healing sequence of bone remains typical. Thus, chitosan coatings have shown that dental implants can develop adequate ostn.[27] Chitosan also increases the vascularization of blood vessels and stimulates budding tissues.[28] The spongy nature of chitosan hydrogel supports the proliferation of osteoblastic cells which in turn activates osteoblasts thereby accelerating osteogenesis. Studies have interestingly reported that the bone density in extraction sockets treated with chitosan was 98.2% of maximum mandibular bone density, which was 29.3% more than that of untreated sockets.[29] In animal models, chitosan has a high degree of biocompatibility and can be conveniently adapted for implant biomaterial growth. Similarly researchers have also studied the beneficial effects of chitosan compounds on animal bone repair.[30] Cissus quadrangularis

(Vitaceae), a rambling shrub, characterized by a thick quadrangular, fleshy stout stem, the plant is commonly referred to as the "Bone Setter" in Sanskrit as "Asthisamdhani" and in Hindi as "Hadjod" because of its ability to join bones.[31] It is assumed that a phytogenic isolated steroid is the primary constituent of Cissus quadrangularis. The pharmacological properties of Cissus quadrangularis include excellent antioxidant properties, free radical scavenging potential, antibacterial activity, antiosteoporotic activity, antitumor activity, analgesic activity, antipyretic activity, and bone fracture healing activity.[32,33] Fracture healing studies indicate that this elusive anabolic steroid may be able to function on bone estrogenic receptors. Efficacy of Cissus quadrangularis on early ossification and bone remodelling has been documented and Cissus quadrangularis has been shown to perform by stimulating metabolism and increasing the osteoblast's absorption of the minerals calcium, sulphur and strontium in fracture healing.[34,35] Studies report the use of C. quadrangularis in reducing post-operative inflammation, pain and swelling, as well as fracture mobility and reported an accelerated healing of fractured jaw bones. Studies have shown that Cissus tends to reduce the immobilization duration and early recovery period.[36] There is evidence from previous literature that supports the fact that osteogenesis is triggered by Cissus quadrangularis and that bone diseases such as osteoporosis can be used as preventive/alternative natural medicine.[37] Studies suggest that Cissus quadrangularis extract may regulate osteoblastic activity by enhancing Alkaline Phosphatase activity and mineralization process.[38] A research was conducted to determine the effect of Cissus quadrangularis extract on the healing process of the dog's experimentally broken radius-ulna. It was found that animals treated with cissus quadrangularis showed faster initiation of the healing process and on the 21st day of fracture, healing was almost complete.[39]

Thus, this technique aims to facilitate the ostn mechanism with faster and stronger bone formation, to provide greater stability during the healing process, thereby allowing the implant to be loaded more quickly.

NEED FOR THE STUDY

In the past twenty years, optimization on titanium implant surfaces has been advocated for hastening the ostn process. This aims to impact certain clinical situations with reduced mineral density of the alveolar bone or where rapid healing is required for early loading protocols. Numerous methods related to surface modifications or coatings of titanium implants are being studied extensively in order to facilitate bioactivity and boost bone integration.[40] Several techniques have been developed to modify implant surfaces, such as plasma spray, grid blasting, acid etching and anodization, which can lead to topography and chemical composition alterations in order to favor ostn.[41] More research on the peri-implant tissue reaction to novel coatings or surface alteration of implants is justified, however, as the optimum dynamics of ostn of implants are still elusive. The positive outcome of the in-vivo treatment of titanium implants is also focused on the bio-response of these implants to osteogenic cells, i.e. osteoblasts during the healing period.[40] Nowadays, the majority of clinically tested implant surfaces are hydrophilic, following studies showing beneficial results for the same. Wettability, protein adsorption and deposition of new cells across the treated surface are improved by covering the implant with a hydrogel. Hydrogel scaffolds have a three-dimensional structure and greatly swell in water so that wound healing can be preserved spatially. These are being designed to encourage cell anchorage, migration, proliferation and the maintenance of cell-specific functions, to support osteoconduction, vascular invasion, and timely bone formation.[42] It has been claimed that osseous tissue regeneration in rabbit research models is very rapid, and all healing-related events will last for about six weeks. [2, 44]

Thus, in the present study, preparation of an osteogenic, anti-inflammatory and economical novel hydrogel coating on the surface of titanium implant and its subsequent in vivo bio-response in rabbit models have been explored. To our knowledge, this is the first detailed and elaborate in vivo study to establish the correlation between the coating of novel Cq hydrogel around the titanium implant surface and its subsequent effect in prompt in vivo ostn.

HYPOTHESIS

Null Hypothesis:

There is no difference in bone regeneration between the titanium implants surface treated with *Cissus quadrangularis* hydrogel and the untreated titanium implants

Research Hypothesis:

There is bone regeneration around the titanium implants surface treated with *Cissus quadrangularis* hydrogel as compared to the untreated titanium implants

AIM AND OBJECTIVES

AIM OF STUDY

- To evaluate the effect of *Cissus quadrangularis H*ydrogel on bone regeneration around titanium implants.

OBJECTIVES

- To evaluate and compare the bone regeneration around titanium implants surface treated with *Cissus quadrangularis* hydrogel and untreated titanium implants.
- To evaluate and compare the removal torque of titanium implants surface treated with *Cissus quadrangularis* hydrogel and untreated titanium implants.

REVIEW OF LITERATURE

1) **Johannsson CB et al in 1991** conducted a study in which commercially pure (c.p.) niobium and c.p. titanium implants were inserted in rabbit bone. After a healing period of 3 months, a significantly higher RT was demonstrated to unscrew the niobium implants (average 32.9 Ncm) compared to the c.p. titanium implants (average 25.3 Ncm). In the histomorphometric part of the study, there were no significant differences in bone- to- metal contact between the 2 implant materials. An average of 41.1% bony contact was demonstrated for the niobium screws compared to an average of 37.2% for the c.p. titanium ones. The study concluded that a similar quantitative bone response was seen in c.p. niobium and c.p. titanium implants. [46]

2) **T. Kawakami et al in 1992** conducted a study in which an experimental bone substitute composed of a Ch-bonded hydroxyapatite paste was prepared. Thirty-seven female adult Japanese white rabbits were used for this experiment. 0.5g of the paste was applied directly on to the surface of the shin bone of each animal after removal of the osteomembrane. Two types of specimens were used for control, one in which only the periosteum was removed, and the other that received no operation at all. After 2 wk, radiographic examination revealed the presence of a bone-like irregular radiopacity in the region of the implant, and this radiopacity increased in size over time. Histopathologically the 2 wk specimens showed the newly formed bone was related directly to the embedded paste. The data suggests that the paste has osteoconductive properties, and may, therefore, prove clinically useful as a bioactive bone substitute. [47]

3) **D.K. Deka et al in 1993** conducted an investigation in which an attempt was made to study the effect of CQ on experimentally fractured radius-ulna of dog by radiological, histological and bio-chemical parameters pertaining to serum calcium level. CQ treated animals revealed faster initiation of healing process than the control animals on radiological and histopathological examinations. Healing was almost complete on 21stday of fracture in the treated animals and remained incomplete in the control animals.[39]

4) **Jue-Yeon Lee et al in 2004**[48] led a study that attempted the development of Ch useful as a scaffolding device for the purpose of obtaining high bone forming efficacy. Porous Ch matrices, Ch–poly(L-lactide) (PLLA) composite matrices and Ch coated on PLLA matrices were dealt with in this research. The hydrophobic surface of PLLA matrices was modified by Ch to enhance cell affinity and wettability. Overall results in this study demonstrated the usefulness of Ch as drug releasing scaffolds and as modification tools for currently used biomaterials to enhance tissue regeneration efficacy. These results may expand the feasibility of combinative strategy of controlled local drug delivery concept and tissue engineered bone formation in reconstructive therapy.

5) **Albrektsson T. et al in 2001** conducted a study explaining the basic process of osteoinduction, osteoconduction and ostn; the three most important factors towards healthy healing and bone formation. Osteoinduction is the process by which osteogenesis or new bone formation is induced. Seen regularly in any type of bone healing. Whereas Osteoconduction is when bone grows on a surface that acts as the conductor for bone formation. In the case of implants, bone conduction is not only dependent on conditions for bone repair, but also

on the biomaterial uses and its reactions. Bone conduction is not possible on certain materials such as copper and silver, whereas high biocompatibility is seen in commercially pure (c.p.) titanium. The most important factor though, is, Ostn which is the stable anchorage of an implant achieved by direct bone to implant contact.[40]

6) **Trisi P. et al in 2003** directed a study in which the loading protocol originally proposed by Bränemark and others states that in order to achieve a predictable and lasting bone integration, implants should heal submerged and not be loaded until after 3 months in the mandible and 6 months in the maxilla was evaluated. It evaluated the 2-month bone-implant contact for dual acid-etched and machined implant surfaces to determine if this criteria is met. Custom manufactured implants (2x5mm), having on one side a machined surface and on the other side a dual acid-etched surface, were placed in the posterior maxilla of 11 patients, allowed to integrate for 2 months, then removed using a 4 mm internal diameter trephine with irrigation. Sections were processed and stained for histologic and histomorphometric analysis. Histomorphometric analysis showed that after 2 months of healing, the 47% BIC% on the dual acid-etched side was statistically higher than the 19.00% BIC% on the machined side. Based on the histomorphometric results of this study, sufficient bone for functional loading of the implant exists on a dual acid- etched surface after 2 months of healing in the posterior maxillary arch.[49]

7) **Annie Shirwaikar et al in 2003** evaluated the ethanol extract of *Cq* for its anti-osteoporotic activity in ovariectomized rat model of osteoporosis at two different dose levels of 500 and 750 mg/kg per day. Healthy female albino rats were divided into five groups of six animals each. First group was sham

operated and served as control. All the remaining groups were ovariectomized. Group 2 was fed with equivolume of saline and served as ovariectomized control. Groups 3–5 were orally treated with Raloxifen (5.4 mg/kg) and ethanol extract of *Cq* (500 and 750 mg/kg), respectively. The findings assessed on the basis of biomechanical, biochemical and histopathological parameters showed that the ethanol extract of the plant had a definite antiosteoporotic effect.[50]

8) **Ambarish Sanyal et al in 2005** led a study that analysed the extract of the stem of *C. quadrangularis* from the point of view of its putative ability to promote mineral growth. The stem extract contains a high percentage of calcium ions (ca. 4% by weight) and phosphorus, both essential for bone-fracture healing. Furthermore, we show that calcite crystals of interesting morphology may be obtained by simple reaction of the calcium ions present in the extract with CO2 bubbled directly through the extract. In conclusion, we have shown here the formation of truly biogenic CaCO3 crystal of exquisite morphology by simply bubbling CO2 into the aqueous stem extract of an indigenous medicinal plant, *C. quadrangularis*. The plant contains a high percentage of calcium probably due to its thick cell wall, which makes it suitable for growth of mineral crystals. The presence of phosphorous in the plant can also be exploited for synthesizing hydroxyapatite, thus utilizing the traditional knowledge of bone-fracture healing in advanced technique of new material synthesis.[51]

9) **Sennerby L et al in 2005** conducted a study in which they investigated histologically and biomechanically the bone tissue response to zirconia implants with two different surface modifications in comparison with

machined, non modified zirconia implants and oxidized titanium implants. Threaded zirconia implants with a diameter of 3.75 mm with either a machined surface (Zr-Ctr) or one of two surface modifications (Zr-A and Zr-B) were manufactured. The implants were characterized with regard to surface topography. Twelve rabbits received 96 implants, of which the implants in six rabbits were subjected to RT (RTQ) tests after a healing period of 6 wks. The implants in the remaining six animals were removed en bloc for light microscopic analysis. The Zr-A implants showed the highest surface roughness, followed by the Zr-B implants and, finally, the Zr-Ctr implants. The nonmodified ZrO2 implants showed statistically significant lower RTQs than all other implants. The study concluded by confirming a strong bone tissue response to surface-modified zirconia implants after 6 wks of healing in rabbit bone.. The findings suggest that surface- modified zirconia implants can reach firm stability in bone.[52]

10) **Park YS et al in 2005** led a study that aimed to evaluate the possibility of microtomography as a tool for assessing ostn. Twenty-four titanium dental implants were installed in the tibia of New Zealand white rabbits, and retrieved with the surrounding bone after 3 months. The specimens were analyzed by three-dimensional microtomogram images and compared with the conventional histomorphography. The correlation coefficient was found to be statistically significant. To predict the histomorphometric data using microtomographic data, a linear regression model was applied. These results showed that microtomograms can be used for non-invasive ostn assessment.[53]

11) **Duenpim Parisuthiman et al in 2008** directed a study in which the effects of ethanol extract of C. quadrangularis (CQ-E) on osteoblast differentiation and

function were analyzed using murine osteoblastic cells.Cq Linn. has been implicated as therapeutic agent for enhancing bone healing. Alkaline phosphatase (ALP) activity and the extent of mineralized nodules were significantly increased in treated cells compared with controls. These results suggested that CQ-E may regulate osteoblastic activity by enhancing ALP activity and mineralization process, and the increased ALP activity effect of CQ-E is likely mediated by MAPK dependent pathway.[54]

12) **Klein MO et al in 2008** conducted a study the purpose of which was the first intra-oral in vivo assessment of alveolar crest UTV values of both upper and lower edentulous jaws in a patient collective. A total of 108 patients, partly or fully edentulous adults were enrolled. Six different measurement points were defined. Ultrasound transmission velocity values were measured bicortically (in bucco-oral direction) and correlated to sex, age, measurement site and history of osteoporosis or radiation therapy. Assessment of alveolar-ridge UTV might offer the possibility to identify critical bone quality before implantation or to monitor bone healing after augmentation procedures. [55]

13) **Bhagath Kumar Potu et al in 2009** led a study where the effects of petroleum ether extract of CQ on bone marrow mesenchymal stem cell proliferation and osteogenic differentiation were evaluated. This study also aimed to determine the additive effect of osteogenic media and Cq on proliferation, differentiation and calcification. MSCs were cultured in media with or without Cq for 4 wks and were then stained for alkaline phosphatase. Extracellular matrix calcification was confirmed by Von Kossa staining. marrow mesenchymal stem cells cultures in control media and osteogenic media supplemented with Cq extract (100, 200, 300 microg/mL) were also

subjected to a cell proliferation assay (MTT).Treatment with 100, 200 or 300 microg/mL petroleum ether extract of Cq enhanced the differentiation of marrow mesenchymal stem cells into ALP-positive osteoblasts and increased extracellular matrix calcification. Treatment with 300 microg/mL petroleum ether extract of Cq also enhanced the proliferation rate of the marrow mesenchymal stem cells. Cells grown in osteogenic media containing Cq exhibited higher proliferation, differentiation and calcification rates than did control cells. CQ extract induces differentiation of MSCs into osteoblasts. MSCs grown in the basal media and treated with CQ extract (300 µg/mL) showed calcium deposition in the extracellular matrix as early as 15 days after the beginning of the treatment. [37]

14) **Degidi M et al in 2009** led a study aimed to compare the insertion torque and the bone to implant contact ratio of human implants retrieved after a 4-8 week period. The implants were divided into three groups: those retrieved after 4 wks, after 5–6 wks and after 7–8 wks.In this study on human retrieved implants, no statistically significant correlation was found between the IT values and BIC. The limitations of the present study must, however are limited sample size, different implant types and geometries and different retrieval times. All these factors could influence IT and BIC. The present results could be due to a lack of relationship between bone structure and IT, or to the fact that primary stability may not only be influenced by bone volumetrical density or bone trabecular connectivity but also by the thickness and density of the cortical layer. [56]

15) **Koh JW et al in 2009** conducted the study in which the biomechanical reaction influenced by modified implant surface is evaluated. Three test

groups were prepared: sandblasted, large-grit and acid-etched (SLA) implants, anodic oxidized implants, and anodized implants with Ca-P immersion. Each rabbit received 4 implants in the tibia; one each from all test groups and one machined implant from the control group. Resonance frequency values were measured at the time of implant insertion, 2 wks and 4 wks of healing and RT values (RTV) were measured 2 and 4 wks after insertion. The RFA (RFA) showed increase in the ISQ (ISQ) values of implants during 2 wks of healing period although the test and control groups showed no significant differences. The test and control implants also showed significantly higher ISQ values during 4 wks of healing period. No significant differences, however, were found among all the groups. Within its limitations, this study concludes that neither anodic oxidation nor Ca-P immersion techniques have any advantage over the conventional SLA technique with respect to implant stability.[57]

16) **Veltri M et al in 2010** led a study that aimed to evaluate the correlation between insertion torque and quantitative ultrasound analysis in an ex vivo rabbit femur model. : Implants were planned at diaphyses (group 1) and epiphyses (group 2) of 16 rabbit femurs where amplitude-dependent speed of sound (Ad-SOS) was measured. The insertion torque from 7-mm-long implants placed at planned sites was recorded. The correlation between cutting torque and Ad-SOS was evaluated using Spearman's coefficient. Results showed A negative correlation between insertion torque and Ad-SOS. The study concluded that In the rabbit bone model investigated, quantitative ultrasound correlates inversely with implant insertion torque. Although this correlation remains to be verified in humans because rabbit femur does not convincingly represents different human bone qualities, it seems that

ultrasound could convey potentially useful, pre-surgical, site-specific, non-invasive information on bone mechanical characteristics therefore deserving further research efforts.[58]

17) **Park IP et al in 2011** directed a study that aimed to evaluate initial ISQ values in relation to BIC% using rabbit model ISQ (ISQ) values have been supposed to predict implant stability. However, the relationship between ISQ values and bone-to-implant contact ratio (BIC%) which is one of the predictors of implant stability is still unclear. Four New Zealand white rabbits received a total of 16 implants in their tibia. Immediately after implant placement ISQ values were assessed. The measurements were repeated at the time of sacrifice of the rabbits after 4 wks. Peri-implant bone regeneration was assessed histomorphometrically by measuring BIC% and bone volume to total volume values (bone volume %). The relationships between ISQ values and the histomorphometric output were assessed, and then, the ostn prediction model via the initial ISQ values was processed. Initial ISQ values showed significant correlation with the BIC%. The bone volume % did not show any significant association with the ISQ values. In the limitation of this study, RFA is a useful clinical method to predict the BIC% values and examine the implant stability.[59]

18) **Martin- Monge E et al in 2011** conducted a study that aimed was to validate an osteoporotic animal model for analysis of poor-quality bone. Sixteen female New Zealand rabbits, each 6 months old and weighing 4 to 5 kg, were used in this study. The animals were anesthetized, and an in vivo densitometric analysis was performed by dual-energy x-ray absorptiometry (DEXA) to measure bone mineral density (BMD) in the calvaria, cervical

spine, and tibia. Ovariectomy was then performed, and animals were fed a low-calcium diet that featured 0.07% calcium, rather than the 0.45% calcium of a standard diet, for 6 wks. After this period, new densitometric measurements were carried out and results were statistically significant. Ovariectomy and a low-calcium diet were able to induce a quick decrease in BMD, as measured at 6 wks by DEXA. This decrease was statistically significant in the calvaria and the cervical spine but not in the tibia. Study cocluded that ovariectomy and a low-calcium diet are able to induce experimental osteoporosis in rabbits in a short period of time.[60]

19) **ArawattiSiddaram et al in 2012** led a study in which clinical evaluation was done to check the efficacy of the Asthishrankhala (Cq Linn) for early mobilization in the management of Colle's fracture. Colle's fracture is a fracture at the distal end of the radius, at its cortico-cancellous junction with typical displacement. Colle's fracture is the commonest fracture in people above forty years of age, and is particularly common in women because of post-menopausal osteoporosis. 30 registered, clinically diagnosed and confirmed patients of Colle's fracture were selected for the present clinical trial from OPD/IPD of NIA, Jaipur. They were randomly divided in following three groups of 10 patients each, Group A treated with only external application, Group B-treated with only internal application and Group C-treated with both external and internal application of Asthishrankhala *(Cq* Linn.). At the end of study it was found that results were highly significant in group B & C (combined therapy) and can be concluded that asthishrankhala is effective in the management of colle's fracture as it is safe, cost effective and free from any side effects.[61]

20) RasalePrashantLingram et al in 2014 conducted a random controlled study with the aim to evaluate the effective remedial therapy to accelerate bone healing so as to rehabilitate the individual as early as possible. Fracture healing was assessed with biochemical parameters like serum calcium, serum phosphorus and a hormonal parameter PTH (parathyroid hormone) and their values were evaluated during fracture healing. From the assessed data it was found that bone healing process was accelerated as serum level of PTH Hormone have shown increasing trend during the period of 31 days and it was at its peak on 21st day from inception of administration of drug; but levels of calcium and phosphorus were remain maintained during period of fracture healing. In control group it was observed that serial values of Serum Calcium, Serum Phosphorus and PTH Hormone were not having significant fluctuations. This indicates that the process of fracture healing or osteoblastic activity was initiated earlier in trial group. Hence study concludes that the drug *Cq* is having influence on accelerating the fracture healing process and further it helps in reducing period of immobilization and early rehabilitation.[62]

21) Lee JH et al in 2014 conducted a study in which a β-TCP microsphere/ poloxamer 407-based hydrogel composite is generated to test its efficacy as an rhBMP-2 carrier and its effects on the bone fusion ratio of dental implants. The level of penetration and new bone formation of the composite carrying rhBMP-2 were evaluated by grafting dental implants into rabbit tibiae. Four wks later the percentage of new bone formed around the fixture, the percentage of bone directly associating with the implant, and the new bone quality were evaluated by radiography, histology and micro CT.The micro-CT results showed a significantly higher level of trabecular thickness and new

bone and peri-implant new bone formation in the experimental treatment compared to the control treatment. Histomorphometry revealed a significantly higher bone-implant contact ratio and peri-implant bone formation with the experimental treatment. The study concluded that the use of β-TCP/poloxamer 407 hydrogel composite as a carrier of rhBMP-2 significantly promoted new bone formation around the dental implant fixture and it also improved the quality of the new bone formed in the tibial marrow space.[63]

22) **Rozé J et al in 2014** led an investigation that aimed to study the stability and ostn of implants with different surface treatments. Titanium implants were either grit-blasted and acid etched (BE group), or coated with a uniform electrodeposited CaP layer, incorporating cAMP or Dex molecules in the CaP coatings. In order to assess the ostn, twenty four cylindrical titanium implants were inserted bilaterally into the femoral epiphyses of New Zealand White, female, adult rabbits for 4 wks. After 4 wks, the ISQs were measured by RFA and compared to primary implant stability on the day of implantation. Bone-to-implant contact (BIC) and bone growth around implant were determined from histology sections and correlated to implant stability. ISQs (ISQ) increased in each group after 4 wks of healing but were not significantly different between the groups.. In conclusion, the CaP coating enhanced bone formation around the implants, which was correlated to stability measured by RFA.[64]

23) **Hemal R. Brahmkshatriya et al in 2015** conducted a study the purpose of which was to evaluate the effect of *C. quadrangularis* in healing process of maxillofacial fracture. All the patients were treated by open reduction internal fixation method and in postoperative management, antibiotics, and analgesics.

Patients were divided into two groups. In Group 1, one capsule of *C. quadrangularis* (500 mg) thrice a day for 6 wks was administered (n = 5), and in Group 2 (control group), no supplementary medication was administered (n = 4). Pain, swelling, fragment mobility, serum calcium, and serum phosphorus were evaluated pre- and post-operatively on day-1, -21, and -45. Pain, swelling, and fragment mobility were low in Group 1 compared to Group 2. Serum calcium and serum phosphorus were also high, and healing of bone was clearly seen in Group 1 on day 21 as compared to control group, concluding that *C. quadrangularis* helps in reducing pain, swelling, and fracture mobility and accelerate the healing of fracture jaw bones. [36]

24) Salou L et al in 2015 led a study which aimedto compare the ostn of nanostructured surfaces with standard grit-blasted acid-etched and machined titanium alloy implants. Three types of surface, machined (MA), alumina grit-blasted and acid-etched (MICRO), and nanostructured (NANO), were prepared and characterised using profilometry, scanning electron microscopy and Raman spectroscopy. These 3 groups of implants were bilaterally inserted into rabbit femoral epiphyses. After a healing period of 4 wks, the pull-out test, bone-to-implant contact and newly-formed bone were measured and compared. Simple cylindrical implants were used instead of threaded implants in order to directly compare the effect of surface on the kinetic of bone integration. The study concluded that after implantation for 4 wks in rabbit femoral condyles, the bone anchorage of the NANO surface was similar to that of the MICRO implants, whereas its roughness was three times lower. The NANO surface presented higher but not significant bone integration than the other two surfaces regarding BIC and apical BG values.[65]

25) **Mayer L et al in 2015** evaluated the effects of low-level laser therapy (LLLT) on peri-implant bone regeneration by means of RFA and histologic analysis of bone-to-implant contact (BIC).Thirty-two male New Zealand rabbits were randomly divided into four groups of eight animals each, one control group (nonirradiated animals) and three experimental groups that received LLLT (group E5 = 5 J per session; group E10 = 10 J per session; group E20 = 20 J per session). The mandibular left incisor was surgically extracted in all animals, and a nanoparticle-treated-surface osseointegrated implant was placed immediately afterward. The experimental groups were irradiated with aluminum-gallium-arsenide laser diode every 48 hrs over a 13-day period for a total of seven sessions. ISQs (ISQs) were measured at the time of implant placement and 30 days after the last LLLT session. The animals were then euthanized and dissected, and histologic slides of the implant region were obtained for BIC evaluation.

Significant differences in ISQ were detected between groups before and after LLLT, with group E20 showing significantly higher values than controls. The percentage of BIC was also significantly higher in group E20 than in control animals. Hence, the study concluded that low level laser therapy can be used as an effective treatment.[66]

26) **Velasco E et al in 2016** conducted an investigationthat aimed to correlate the roughness and topography with the residual stress in order to analyze the different fatigue behavior and ostn, for four different types of surface treatments. The analyzed surfaces were as-machined (CTR), acid-etching (AE), spark anodization (SA) and grit-blasted (GB) cp titanium dental implants. Residual stresses were determined by means of X-ray diffraction.

The fatigue tests were carried out at 37ºC on 160 dental implants and the S-N curve was determined. The fatigue tests show that the gritblasting process improves the fatigue life. The current study also assessed the short- and midterm bone regenerative potential and mechanical retention of the implants in bone of New Zealand rabbits and compared them. The mechanical retention after 4, and 10 wks of implantation were evaluated with histometric and pull-out tests, respectively, as a measure of the ostn of the implants. The results demonstrated that the GB treatment produced micro-rough that accelerated bone tissue regeneration and increased mechanical retention in the bone bed at short periods of implantation in comparison with all other implants tested. The study concluded that these type of treated implants can have great potential in clinical applications. [67]

27) **Sohn SH et al in 2016** compared the biomechanical force for HA-blasted (RBM) implants with that of SLA implants in terms of RT and to ascertain the correlation between the surface roughness and the size of the blasting media. The implants were installed on both sides of rabbits' tibiae. Four wks after the implants were installed, the implant RT was measured using a digital torque device. The roughness of the implant surface was analyzed using field emission scanning electron microscopy and confocal laser scanning microscopy. Both groups of surface textures exhibited a regular porosity. No significant differences in RTs were observed between the control and experimental groups. The study concluded that there was no significant differences in ostn between hydroxyapatite-blasted and SLA implants. [68]

28) **Dundar S et al in 2016** conducted a study to evaluate the histomorphometric effects of local melatonin application on the BIC during surgical implant

placements in rabbit tibiae. the rabbits were divided randomly into three groups, Control group (CG): No treatment was applied, and dental implants were simply inserted rabbit tibiae. Melatonin Dose 1 (MLT D-1) group: 1.2 mg lyophilized powder melatonin was administered locally into the dental implant socket before implant placement in rabbit tibiae. 16,18 Melatonin Dose 2 (MLT D-2) group: 3 mg lyophilized powder melatonin was administered locally into the dental implant socket before implant placement in rabbit tibiae. Two implants for each tibia and four implants for each animal were integrated. Four wks after placement, their tibiae were dissected, fixed with formaldehyde, and embedded in methacrylate. Histologic and histomorphometric analyses were then performed under light microscopy. Following this, BIC, was detected histomorphometrically, and results were considered statistically significant. Results showed that the highest BIC percentage was detected in MLT D-2, as compared in group MLT D-1 and in CG. Similarly, the mean BIC percentage of the MLT D-2 group was the highest among the three. Within the limitations of this rabbit study, it appears that local melatonin application during implant surgery may improve BIC.[69]

29) **Boot W et al in 2017**conducted an animal study that investigated the effect of a commercially available hydrogel, either unloaded or loaded with 2% vancomycin. In 18 New Zealand White rabbits, an uncoated titanium rod, a rod coated with unloaded hydrogel, or a rod coated with 2% vancomycin-loaded hydrogel was implanted in the intramedullary canal of the left tibia. After 28 days, the bone volume fraction near the implant was measured. The study concluded that hydrogel coated on titanium implants, unloaded or loaded with 2% vancomycin, had no effect on the volume or timing of bone

apposition near the implant, and did not induce an inflammatory reaction in vivo, with the numbers available.[70]

30) Chan HL et al in 2017directed a studyto evaluate the accuracy of using ultrasound to measure facial crestal bone level and thickness. A commercially available medical ultrasound scanner, paired with a 14 MHz imaging probe was used to scan dental and periodontal tissues at the mid-facial site of each tooth on 6 fresh cadavers. The alveolar crest level in relation to the cemento-enamel junction and its thickness on ultrasound images were measured and compared to those on cone-beam computed tomography (CBCT) scans and/or direct measurements on a total of 144 teeth. The mean crestal bone level measured, The mean crestal bone thickness, the mean absolute differences in crestal bone height and thickness between ultrasound and CBCT were measured respectively. The study concluded that Ultrasound was as accurate in determining alveolar bone level and its thickness as CBCT and direct measurements.[71]

31) Chan HL et al in 2017 conducted a investigation in which the ultrasound to image soft tissue, hard tissue surface topography and specific vital structures was compared. A clinical ultrasound scanner, paired with two 14-MHz transducers of different sizes (one for extraoral and the other for intraoral scans), was used to scan the following structures on a fresh cadaver: (i) the facial bone surface and soft tissue of maxillary anterior teeth, (ii) the greater palatine foramen; (iii) the mental foramen and (iv) the lingual nerve. Multiple measurements relevant to these structures were made on the ultrasound images and compared to those on cone-beam computed tomography (CBCT) scans and/or direct measurements. Results showed that ultrasound imaging

could delineate hard tissue surfaces, including enamel, root dentin and bone as well as soft tissue with high resolution. The greater palatine foramen, mental foramen and lingual nerve were clearly shown in ultrasound images. Merging ultrasound and CBCT images demonstrated overall spatial accuracy of ultrasound images, which was corroborated by data gathered from direct measurements. The study concluded that ultrasound can be a real-time and non-invasive alternative for the evaluation of oral and dental anatomical structures relevant for implant and oral surgery.[72]

32) **Li X et al in 2018**led an study to quantitatively and qualitatively evaluate the osteointegration abilities of MAO-treated and smooth surface (SF) implants in vivo. In a rabbit model, a comprehensive histomorphological, osteogenic, mineralizational, and integrative assessment was performed using light microscopy, fluorescence microscopy, confocal laser scanning microscopy, and radiographic analyses. Compared with the SF groups, the MAO-treated groups exhibited more active contact osteogenesis, as well as distant osteogenesis, under fluorescence examination, the mineral apposition rate was found to be greater for all of the MAO-treated implants, and the osteointegration index (OI) value was greater in the MAO-treated groups at different times. The study concluded that the calcium-rich amorphous layer created by MAO provided a better environment for osteointegration, with more active contact osteogenesis, a more rapid mineral apposition rate and greater OI values.[73]

33) **Gehrke SA et al in 2018 compared** through biomechanical and histological analyses, the effects of aluminium (AlO2) and titanium dioxide (TiO2) microparticles for blasting used to produce the SLA surface treatment of two

commercially available titanium dental implants, using a rabbit tibia model. Forty-eight cylindrical dental implants were used for this study. They were divided into two groups of 24 implants each: a control group of implants with SLA surface that is produced using the AlO2 microparticles for blasting and subsequent acids conditioning and a test group of implants produced using TiO2 microparticles for blasting and subsequent acid conditioning. The implants were randomly installed in both tibias of eight rabbits. En block samples were removed 4 and 8 wks after implantation. Resonance Frequency Analyses were performed immediately after the implantation and at 8 wks. Twelve implants of each group were removed to measure the reverse torque. The remaining implants were used for histological analysis. In comparing the ISQ at the two time points, as well as the RT test at 8 wks after implant placement, no significant difference was found between the two groups. Histomorphometric analysis showed a high degree of bone organization in all samples with no significant difference between groups in the bone-to-implant contact. Within the limitations of this study, the results indicate that the media of surface blasting (AlO2 or TiO2 microparticles) did not show significant differences in the tested parameters for assessing the ostn of the implants.[74]

34) **Kumararama SS et al in 2018**evaluated the influence of different shapes of microthread in enhancing ostn in experimental animal study and supported by a numerical analysis. 24 implant prototypes were placed in rabbits tibia and femur, out of which 12 were with V-shaped microthreads and the other 12 were with Power-shaped microthreads. After 4 wks, all the study rabbits were sacrificed. The bone surrounding implants were retrieved enbloc and stored in 10% Formalin. Histomorphometric analysis was carried out of the

sections obtained. Hitomorphometry showed statistical significance difference in new bone volume (BV) and Total BV for V-shaped microthreaded prototype implant. Study concluded that V-shaped microthreaded dental implant design can be preferred over Power-shaped microthreaded dental implant for proper stress distribution and for promoting ostn.[75]

35) **Trento GD et al in 2019** conducted this in vivo study to evaluate, through histologic, histometric, and radiographic (microCT) assessment, the bone formation at different time periods, of two different titanium implant surfaces placed in bone defects, simulating fresh extraction sockets, either filled with biphasic ceramics of hydroxyapatite/β-tricalcium phosphate (HA/TCP), or BC.The animals were divided into two groups according to the type of implant surface. Before implant placement, a defect was created on both tibias of all the rabbits. The groups were divided according to how the created defect was to be filled: BC-N: the bone defect was filled with a BC, and implants with a porous surface; (control group), BC-A: the bone defect was filled with a BC, and implants with a porous-hydrophilic surface, HA/TCP-N: the bone defect was filled with biphasic ceramics of hydroxyapatite/β-tricalcium-phosphate, and implants with a porous surface, HA/TCP-A: the bone defect was filled with biphasic ceramics of hydroxyapatite/ β-tricalcium-phosphate, and implants with a porous-hydrophilic surface. In a rabbit tibia model, implants with porous-hydrophilic surfaces accelerated bone tissue formation in association with bone substitute material in created defects compared to porous surface implants.[76]

36) **Lee JB et al in 2019** led a study to evaluate the effect of UV photofunctionalization on implants with a machined surface compared to the SLA surface. Machined surface treated with UV light (M + UV) was compared to sandblasted, large-grit, acid-etched (SLA) surfaces. In a rabbit tibia model, the implants were examined to evaluate the bone-to-implant contact ratio and the bone area. In the M + UV group, we observed the lower amount of carbon, a 0° -degree contact angle, and enhanced osteogenic cell activities. The histomorphometric analysis showed that a higher bone-to-implant contact ratio was found in the M + UV implant at 10 days. The study concluded that the UV photofunctionalization of a Ti dental implant with M surface attained earlier ostn than SLA.[77]

37) **Gehrke SA et al in 2019** directed a study to compare titanium (machined and surface treated) versus zirconia implants inserted in tibias of rabbits after a period of 6 wks.50 commercially pure titanium implants grade IV; 25 implants with a machined surface (TiM group), 25 implants with a treated surface (TiT group) and, 25 implants were manufactured in pure zirconia (Zr group).The implants were placed in the tibia of 10 rabbits.Six wks after the implantation, 10 implants for each group were removed in counter-torque for analysis of maximum torque value. The remaining samples were processed, and cut to obtain non-decalcified slides for histomorphological analyses and histomorphometric measurement of the percentage of bone-implant contact.The results of RT values showed statistically significant difference between the groups. However, the BIC% presented similar values for all groups, with no statistical differences. Within the limitations of the study, the findings suggest that the quality of the new bone tissue formed around the

titanium implants present a superior density in comparison to the zirconia implants.[78]

38) Gehrke SA et al in 2019conducted an animal study that evaluated and comparedin vitro and in vivo the influence of a surface with the microgrooves modification plus microrugosities on the ostn, when compared to that of machined and treated implants without microgrooves.Thirty disks and thirty-six conical implants manufactured from commercially pure titanium (grade IV) were used in this study. Three groups were determined; Group 1 (G1)-machined samples; group 2 (G2)-samples were machined and had their surface treated to generate roughness; and test group 3 (G3), where the samples were machined with microgrooves and the surface was treated to generate the roughness. For the in vitro analysis, the samples were submitted to scanning microscopy (SEM), surface profilometry, the atomic force microscope (MFA) and the surface energy test. For the in vivo analyses, thirty-six implants were placed in the tibia of 9 New Zealand rabbits, after histological and histomorphometric analysis, to determine the level of contact between the bone and implant (BIC%) and the bone area fraction occupancy (BAFO%) inside of the threads. The in vitro evaluations showed different roughness patterns between the groups, and the G3 group had the highest values. In vivo evaluations of the BIC% showed significant statistical difference between the groups. In the BAFO% values, also showed statistical difference between the groups. The results obtained in the evaluations showed that the surface with microgrooves stimulates the process of ostn, accelerating the healing process, increasing the contact between the bone and the implant and the area of new bone formation.[79]

39) Pachimalla PR et al in 2020 led a study in which hydrophilic gel was applied on to the dental implant surface, to enhance bone to implant contact (BIC). In first part of this study, Acemannan and Moringa oleifera hydrogel formulated in different proportions were coated on the titanium disk and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done to evaluate cell viability. Cytotoxicity of aqueous extracts of two plants were tested against UMR106 cells. In second part of study, the prototype titanium implants were placed in tibia and femur of 8 male rabbits. Hydrophilic gel was coated on the study groups of implants. Histomorphometric analysis was carried out of the enbloc sections specimens. Student's unpaired t-test was used to compare mean values between the two groups. The alkaline phosphatase assay showed least cell inhibition for Acemannan and Moringa oleifera (2:1) as 4.45% and osteoblastic differentiation as 0.328 at 540 nm. Titamium disc coated with hydrogel of Acemannan and Moringa oliefera and seeded with Human MSC shows increased proliferation of osteoblast cells. Compared to study group implants, control group showed no new bone formation. Hence the study concluded that hydrophilic implant surface showed new bone formation with increased bone to implant contact. There was absent of degenerative changes, necrotic changes, fibrosis, and inflammation at the new BIC.[80]

40) Sanchez-Perez A et al in 2020 directed a present study to investigate the effects of ultraviolet C light (254 nm 75 applied for 15 minutes at a distance of 15 cm) on dental implants that were inserted in rabbit 76 tibiae after 8 wks of healing was evaluated in comparison between treated and non-treated implants. A total of 20 implants were inserted in five New Zealand rabbits,

with each animal receiving 2 implants per tibia (one photofunctionalized and one untreated). After 8 wks of healing, histological analysis was performed. The two groups showed no statistically significant differences in terms of bone-to-implant contact. Compared to control implants, the photofunctionalized implants showed improved wettability and more homogenous results. Within the limitations, the study concluded that the use of a 6-W Ultraviolet C42 lamp, for an irradiation time of 15 min at a distance of 15 cm, did not improve the percentages of bone-to-implant contact in rabbits at an ostn time of 8 wks in rabbits.[81]

41) **Nayak T et al in 2020**led a study that was aimed to understand if CQ provides a quantifiably early healing and to understand the value of adding CQ to a regular regimen of mandibular fracture treatment. In this study, two similarly treated sets of mandibular fracture patients were studied: one set that had been treated surgically with an addition of oral CQ and another set that had only been treated surgically. The study was conducted on 30 consecutive consenting patients who presented with isolated traumatic mandibular fractures. The CQ group included 15 patients who were prescribed capsules of C. quadrangularis (250 mg each) two capsules B.D. for 42 days post-trauma by an Ayurvedic practitioner. The control group was treated without the Ayurvedic drug. In this study, biochemical findings inferred a faster callus formation in patients who had consumed CQ; however, there was no significant radiographic or clinical improvement when compared to the control group. [82]

MATERIALS AND METHODS

This study was carried out for a period of 3 months between September 2020 and December 2020. Ethical clearance was obtained on august 5th 2020 from Central Animal Research Facility of Jawaharlal Nehru Medical College, KLE's Academy of Higher Education and Research. (Annexure II)

Source of data

1. KLE Academy of Higher Education and Research
    - The Department of Prosthodontics and Crown and Bridge, KLE Vishwanth Katti Institute of Dental Science- Belagavi.
    - Central Animal house, JNMC- Belagavi
    - College of Pharmacy- Belagavi
    - Department of Oral Medicine And Radiology
    - Department of Oral Pathology and Microbiology, KLE Vishwanth Katti Institute of Dental Science- Belagavi.

Sample size estimation

Since this is an animal study and the animals are sacrificed at the end of the study, the sample size has been maximally minimised according to CPCSEA guidelines.

Inclusion criteria

- 12 Healthy male, white New Zealand rabbits (2-2.5 kgs)
- 24 identical titanium implants (3.5mm x 6mm)

Exclusion criteria

- Diseased, underweight rabbits
- Female rabbits
- Rabbits below 6months of age
- Rabbits with the inability to comply with research procedures

TABLE 1: MATERIALS USED IN THE STUDY: (Fig 1, Fig 2, Fig 5, Fig 6)

MATERIALS	MANUFACTURER/BRAND
Cissus quadrangularis	Kshipra Biotech PVT. LTD, Indore
Chitosan	Pelican Biotech & Chemical Labs, Bangalore
Bioline dental implant	BioLine Dental Implants, Hessen, Sachsen-Anhalt, Germany
SURGICAL MATERIAL	**MANUFACTURER/BRAND**
Povidone iodine solution 5%	BETADINE-WIN MEDICARE CAS 25655-98-06
Silk sutures	ETHICON Lot No:149337301
Surgical gauze bandage 4 inches	Lot No:150715911
Suture needle 3/4th circle, round body	Lot No:155470650
Sterile gauze, sterile cotton	Lot No:147821020
Catgut sutures	UNIGUT CHROMIC CATGUT Lot No:A 200306

PRE AND POST OPERATIVE MATERIAL	**MANUFACTURER/BRAND**
Lactocalamine	Caladryl Mcure Pharma Pvt Ltd
Ketamine Hydrochloride Injection	Ketmin Themis Medicare LTD
Xylazine Injection	Xylaxin Indian Immunologicals LTD
Carboxymethylcellulose drops	Refresh Tears-Allergan
Normal Saline	Ipsol Healthcare Pvt Ltd. Item No:142064554
Povidone -Iodine Ointment	Betadine Item Code: 20383
Placentrex Ointment	Albert David Manufacturers Item code: 152677319
POST OPERATIVE MEDICATIONS	**MANUFACTURER/BRAND**
Taxim 125mg	Alkem laboratories Ltd.
Meloxicam	GMT Pharma International
Levocetrizine	Nimbles Biotech Private Limited Item No:145673614
Thiopentone Injection 500mg	Neon Laboratories LTD
5% Dextrose	Otsuka Pharmaceutical Pvt Ltd. Lot No: 1185988

TABLE 2: ARMAMENTARIUM USED IN THE STUDY: (Fig 7-11)

ARMAMENTARIUM	MANUFACTURER
Electric trimmer	Nova Electric Trimmer
2ml insulin syringes	Dispovan
Bard Parker blade No. 11	Lister
Bard Parker handle No. 3	GDC
Periosteal Elevators	Wilson & W. Wilson
Tissue retraction forceps	Wilson & W. Wilson
Physiodispenser	W&H Implantmed
Osstem Surgical Kit	Hiossen OneGuide surgical kit
Needle holder	Wilson & W Wilson
Surgical scissors	Wilson & W. Wilson
Resonance frequency analyser	Penguin
Customized electronic Removal torque wrench	Cresent Industries
Optical microscope	Olympus Pentahead

Method

Preparation of Chitosan Hydrogel (ChH)

Pure dry Cq stem extract was procured (Kshipra Biotech PVT.LTD:KBPL/PI156/20-21) (Fig-1 Annexure III). A mass of 1.0g Ch was suspended with blender in 1.0 L of 2.0% (w/v) acetic acid solution. 10.0% sodium hydroxide was slowly added into Ch solution till pH of the solution reached to 10–12. The obtained hydrogel was dialyzed against distilled water until the outer solution was neutralized. After the dialysis, the chitosan hydrogel was separated by centrifugation.

Preparation of CqChH

The Cissus extract alongwith the chitosan hydrogel was prepared in 1%w/v and 3%w/v concentrations using propeller stirrers. Prepared Cissus hydrogels were stored in airtight containers at 4 degrees temperature till further use. (Fig 3) The prepared hydrogel was made thixotropic in nature so that it remains stable when the specimen is dip coated in it. Thixotropic behavior of the prepared hydrogel was evaluated using vial inversion method. (Fig-4)

In vitro analysis

The in-vitro analysis of CqChH was analysed using MTT cytotoxic assay on MG63 (osteoblast like cells) cell lines procured from National Centre for Cell Sciences, Pune.

The prepared concentrations were tested for cytotoxicity and cell proliferation via MTT Assay.

MTT Solution preparation- 5mg in 1ml of Phosphate Buffer Saline (PBS- pH 7.4) Cytotoxicity Assay:

- In vitro growth inhibition effect of test compound was assessed by colorimetric or spectrophotomeric determination of conversion of MTT into Formazan blue by living cells.
- 50µl of $1x10^5$ cells/ml cell suspension was seeded into each well in a 96 well micro titre plate and final volume was made upto 150µl by adding DMEM media
- Dilutions of the test compounds were prepared in DMEM media
- 100 µl of the test compounds of different concentrations was added to the wells and incubated for 24hrs, in the presence of 5% carbon dioxide, at 37 degrees room temperature in an carbon dioxide incubator
- After 24hrs, 20µl of 5mg/ml MTT reagent was added to the wells. The plate was kept for 4hours incubation in a dark place, at room temperature. The plate was covered with aluminium foil since MTT reagent is photosensitive.
- The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100 µl of DMSO was added to dissolve the crystals formed.
- The optical density was measured at wavelength of 492nm. The study was performed in triplicates. The result represents the mean of three readings.

Formula:

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound} \times 100}{\text{Mean OD at control (untreated cells)}}$$

In Vivo Animal Study

At 3% concentration of Cissus hydrogel, maximum cell proliferation was observed and hence chosen to be the desired concentration in this study. (Table 3)

Animal Inclusion:

Animals were procured from Raghavendra Enterprises, Bangalore (841/PO/Bt/S/04/CPCSEA) and were maintained in the Central Animal House, JNMC. (627/PO/Re/S/02/CPCSEA) The animals were maintained throughout the study period in accordance with the Guidelines for the Care and Use of Laboratory Animals laid down by the CPCSEA, India. The temperature as well as the humidity was monitored and maintained daily. Twelve hour light and dark cycles were maintained. 12 healthy, New Zealand White, male rabbits (2-2.5 kgs) were kept in individual cages where paddy husk served as the bedding. Cages were fitted with stainless steel grill tops with facilities to provide filtered, purified water ad libitum.(Fig 12) All experimental animals received a standardised diet of commercially available rodent food (VRK Nutritional Solutions, Pune) twice daily as well as 100gms of fresh greens and vegetables each.

Animal preparation before surgical procedure:

One day prior to surgery, the tibia head and the femur head of each leg for every individual rabbit was measured using vernier calipers to ensure adequate bone is available. The leg of the animal was decided after measuring tibial and femur heads to quantify the amount of available bone. The chosen leg was trimmed and shaved for twice the size of the expected surgical field with an electric trimmer.(Fig-13) All loose hair and debris were removed from the animal. The surgical area was cleaned with sterile gauze dipped in 2% chlorhexidine solution to remove any debris from the surgical site and washed with povidone iodine antiseptic. Topically, lactocalamine was applied to avoid irritation to the exposed skin.

Implant selection

Twenty four commercially pure, tapered titanium implants (*BioLine Dental Implants*, Hessen, Sachsen-Anhalt, Germany), measuring 3.5 mm in diameter and 6 mm in length, were included in the study. (Fig-5) The implants were divided into the following 2 groups (n = 12); Test group i.e. the experiment group with surface treated implants (12 implants coated with novel CqChH) and control group, i.e. 12 commercially pure implants. Each rabbit received two implants, one from the Test group coated with the novel hydrogel and one from the control group.

Surgical Procedure

Twelve New Zealand white rabbits weighing between 2.5 and 3.0 kg were included in this study. The experimental protocol was evaluated and approved by the Ethics Committee for Animal Research Protocol JNMC, Animal House, KAHER, Belgaum. The guidelines of the CPCSEA were followed for all animal protocols.

The whole surgical procedure was performed by the gloved and gowned surgical team under sterile conditions. 20mins before being transported to the operating room the rabbits were sedated in the cage with 0.1 mg/kg medetomidine (SC). Rabbits were anesthetized with a combination of xylazine and ketamine, (50mg/kg+35mg/kg), given IM. Respiratory rate, heart rate, O2-saturation and body temperature was monitored during surgery. The rabbits eyes were kept moist using carboxymethylcellulose eyedrops and gently taped shut. The animal was draped and fixed with clamps and covered with a sterile, impermeable covering with an opening made exposing only the surgical site.(Fig-14) The rabbit was kept on a heat pad during surgery. Antiseptic skin preparation was done starting at the center of the surgical site towards the outside of the prepared area in a centrifugal manner. Three scrubs and three alternating rinses with 5% povidone iodine solution were performed. (Fig-15) A 3-cm-long incision, on the medial side of the limb over the femur head and the tibial head was made to gain access to the periosteum and a full thickness flap was reflected for exposure of the rabbits femur and tibia head. (Fig 16, Fig 17) Osteotomy site was prepared using a progressive sequence of drills, under constant irrigation with saline, according to the manufacturer's instructions. (Fig 18, Fig 19) All drilling procedures were conducted at 1200 rpm. Implants were placed by a blinded operator. The test group implant was uniformly immersed in the prepared novel hydrogel, for 3 mins before placement. (Fig 20) One test group implant dip coated in Cissus hydrogel followed by one commercially pure control group implant was randomly selected to be installed in the proximal head of the tibia and in the proximal head of the femur, at random. (Fig 21, Fig 22) Following the placement of two implants, the soft tissues were sutured in separate layers using catgut 4-0 for subcuticular, fascia closure and skin closure was done using silk 3-0 sutures. (Fig 23, Fig 24) Rabbits were placed

under a halogen bulb and observed until sitting up fully awake. (Fig 25) Post-operative pain control was achieved with administration of 250mg/ml Meloxicam (SC) every 24 hrs for 5 days. Taxim (SC) 125mg was administered every 12 hrs for 5 days post-surgery for infection prophylaxis. Rabbits were carefully observed on a daily basis through the entire term of the study of 3 months. Each rabbit was maintained in individual cages and received food and water ad libitum. (Fig 12) The appetite was closely monitored thrice daily and if the rabbit did not eat within 12 hrs post surgery it was fed with a syringe twice a day as well as administered with 5% Dextrose once daily, with care, in a dosage according to its weight.

Non- Invasive evaluation:

Radiovisiography as well as resonance frequency analysis evaluated the implant non invasively at baseline, 6 wks and 12 wks.

Radiographic Assessment

Radiographic assessment was carried out using Kodak radiovisiography software, soon after implant placement for each rabbit. Follow up radiovisiograph's were taken at 6 wks and 12 wks. (Fig 26) Isodensity values were noted surrounding the implants surface using the Kodak Radiovisiography software.

Resonance Frequency Analysis (RFA)

Each individual rabbit was evaluated for values of implant stability quotient (ISQ) after 6 and 12 weeks using a Penguin RFA unit. For every series of RFA measurements, the ISQ values were recorded using the device in all four directions. A transducer smartpeg was attached to the implant, and ISQ ranging from 1 to 100 was

recorded, until an audible signal confirmed that the measurement had been taken. (Fig 27)

Euthanasia

6 rabbits were sacrificed at 6 weeks and remaining 6 rabbits at 12 weeks. The rabbits were pre-medicated in the pen with 0.1 mg/kg medetomidine. Local anesthetic lignocaine jelly was applied on the ear and euthanasia solution, Thiosol 500mg/ml, was administered into the ear vein till effect.

Removal Torque Analysis

At 6 weeks and 12 weeks, 6 rabbits respectively were sacrificed and immediately their tibia and femur were positioned and fixed in a splint to prevent any movement. Six animals were subjected to removal torque tests using a specially designed, calibrated electronic device. (Fig 10, Annexure IV) A fixed rotation rate was applied until failure of the bone-implant interface occurred. The peak force was registered and RTQ was calculated for statistical analyses. (Fig 28)

Histological Procedures

6 of the animals were sacrificed 12 weeks by an intravenous injection of Pentobarbital 100 mg/ml. Femur and tibial head tissue blocks containing the implant were removed en bloc. (Fig 29) the osseous specimens were fixed in N/10 formalin for 24 hours. Further the samples were subjected to decalcification in 10% formic and nitric acid combination solution for 6 days. Once the osseous specimens were soft enough, implants were carefully retrieved. Then the samples were taken for water wash for upto 1 and half hour. Later samples were processed in ascending grades of

alcohol (60%, 90% and 100%) and acetone to achieve dehydration followed by clearning through xylene and chloroform for one hour each at room temperature. Samples were immersed in melted paraffin wax for 24 hours. Then samples were embedded into wax and blocks were made. (Fig 30) The blocks were cut into 4µm thick sections using a soft tissue microtome, and the sections were taken on the glass slide and subjected to H&E stain and a special masson trichrome stain. All the slides were blinded and were observed under a light microscope by 3 experienced pathologists.

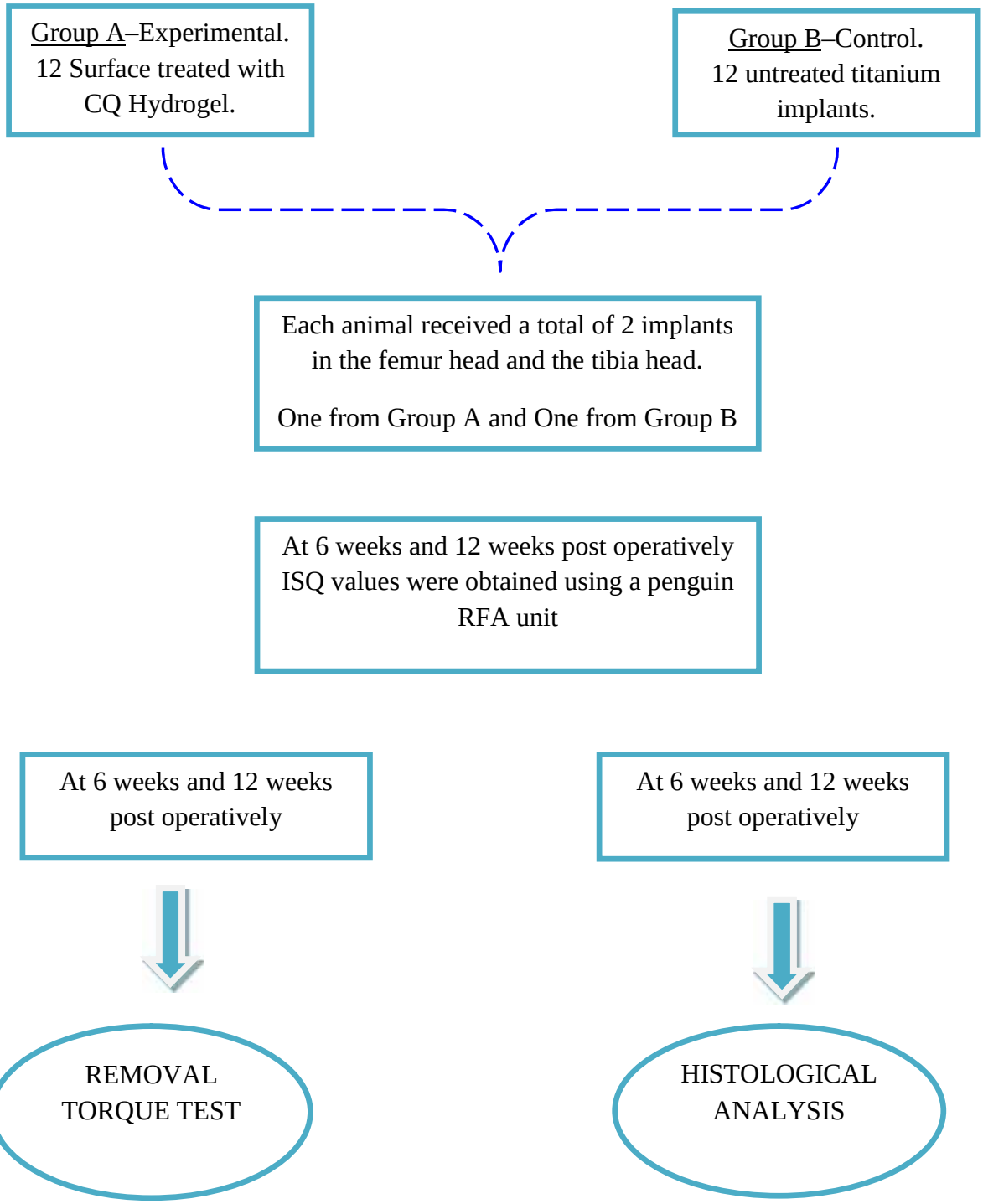

Group A–Experimental.
12 Surface treated with CQ Hydrogel.
Group B–Control.
12 untreated titanium implants.
Each animal received a total of 2 implants in the femur head and the tibia head.
One from Group A and One from Group B
At 6 weeks and 12 weeks post operatively ISQ values were obtained using a penguin RFA unit
At 6 weeks and 12 weeks post operatively
At 6 weeks and 12 weeks post operatively
REMOVAL TORQUE TEST
HISTOLOGICAL ANALYSIS

FIGURE 1: CISSUS QUADRANGULARIS DRY EXTRACT.

FIGURE 2: CHITOSAN POWDER.

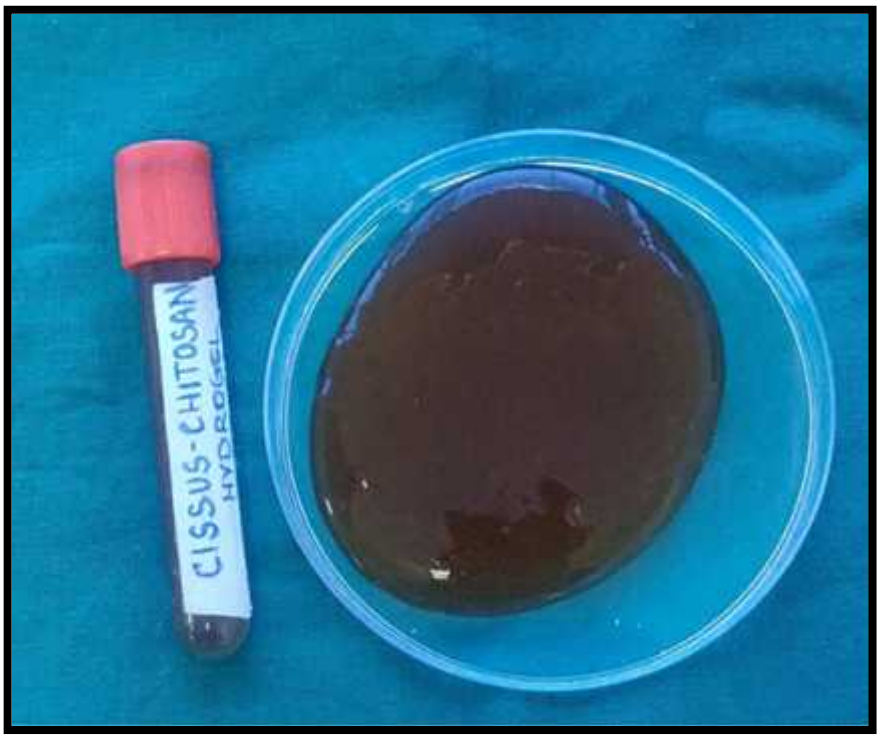


FIGURE 3: CISSUS QUADRANGULARIS HYDROGEL.

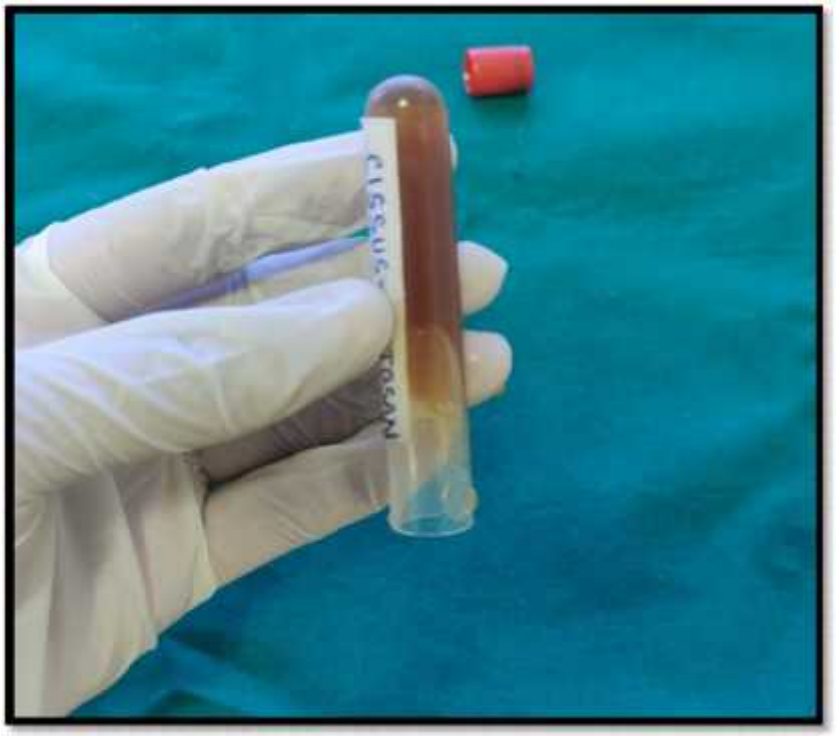


FIGURE 4: EVALUATION OF THIXOTROPIC BEHAVIOUR OF THE PREPARED HYDROGEL BY VIAL INVERSION METHOD.

FIGURE 5: TITANIUM DENTAL IMPLANTS.

FIGURE 6: PRE AND POST SURGICAL MEDICATIONS.

FIGURE 7A: IMPLANT SURGICAL KIT.

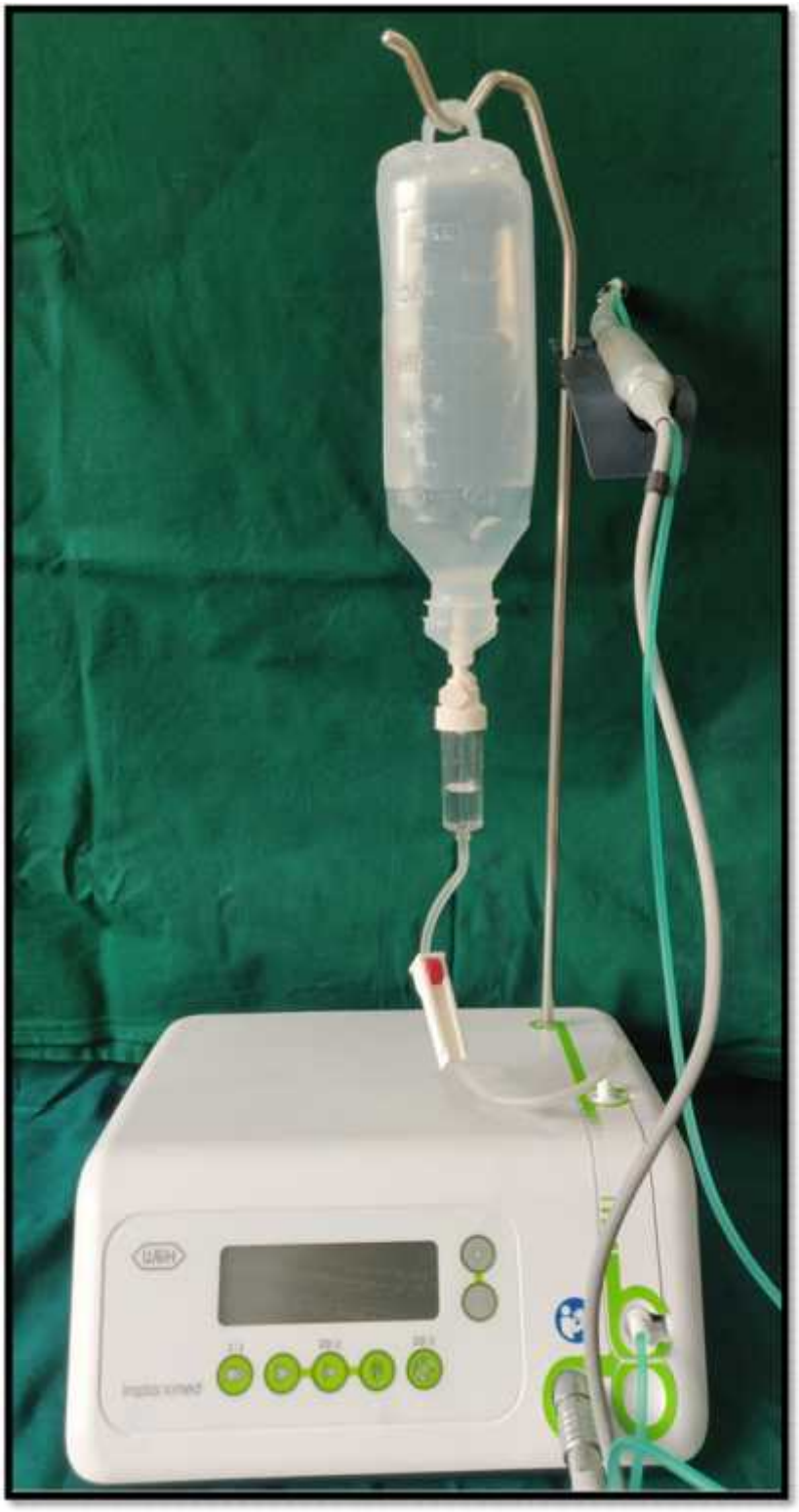


FIGURE 7 B: PHYSIO DISPENSER.

FIGURE 8: SURGICAL INSTRUMENTS.

FIGURE 9: RESONANCE FREQUENCY ANALYSER.

FIGURE 10:CUSTOMIZED CALIBERATED ELECTRONIC REMOVAL TORQUE WRENCH.

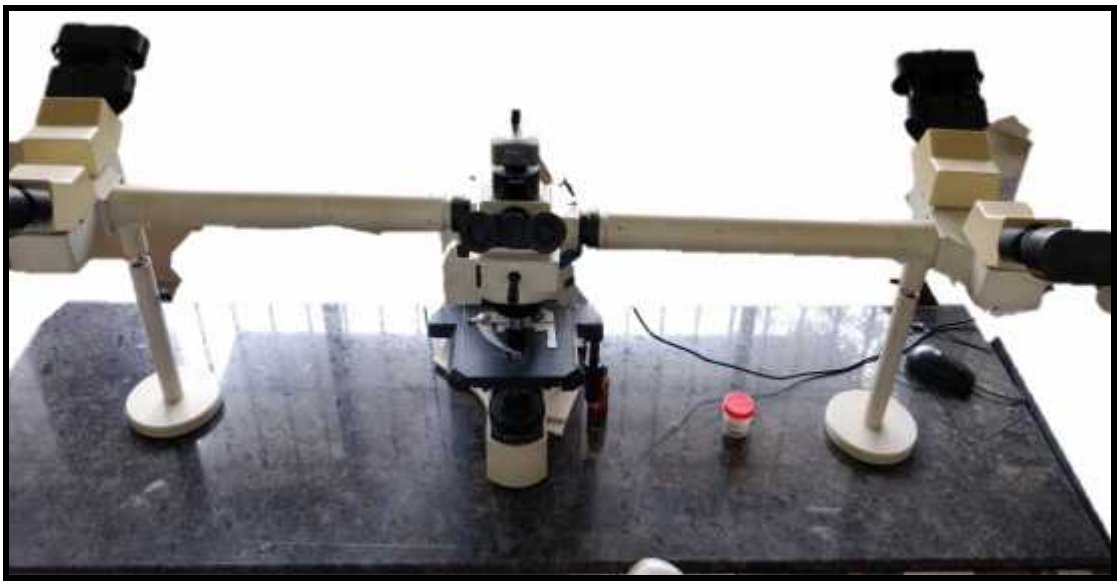


FIGURE 11: PENTAHEAD OPTICAL MICROSCOPE.

FIGURE 12: ANIMAL HOUSE HOUSING AND MAINTENANCE

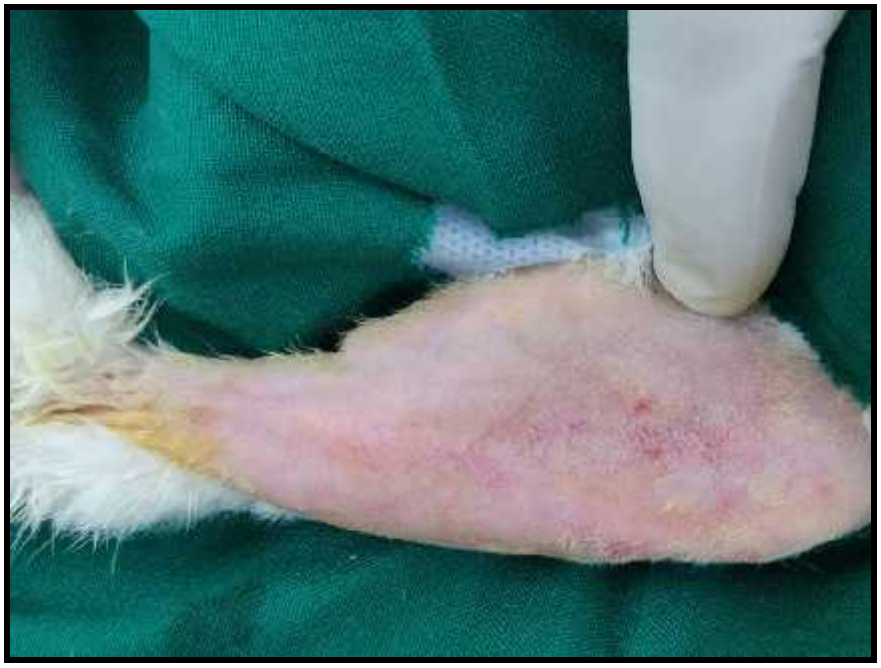


FIGURE 13: SHAVED AND TRIMMED RABBIT LIMB.

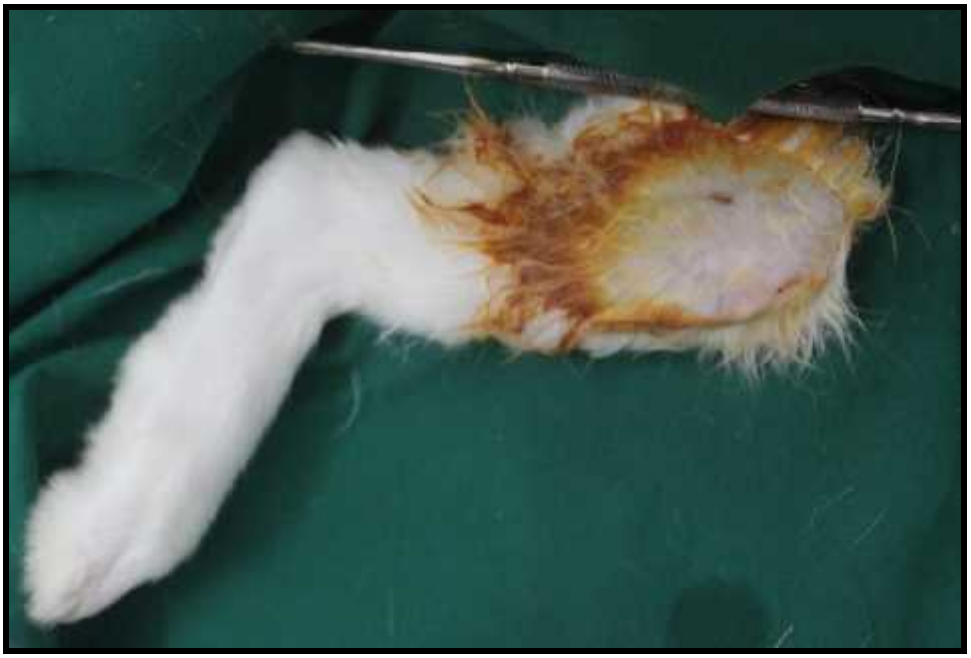


FIGURE 14: DRAPED AND CLAMPEDRABBIT LEG.

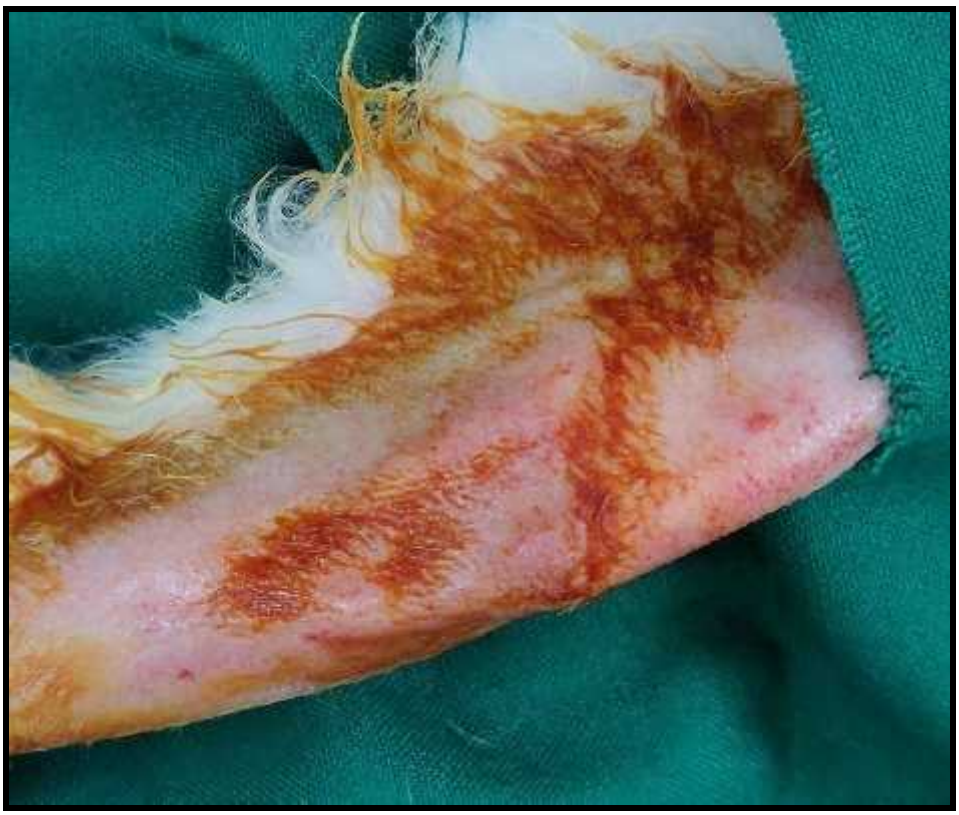


FIGURE 15: PRE-SURGICAL PREPARATION OF LIMB.

FIGURE 16: INCISION AT SURGICAL SITE.

FIGURE 17: REFLECTION OF FULL THICKNESS FLAP.

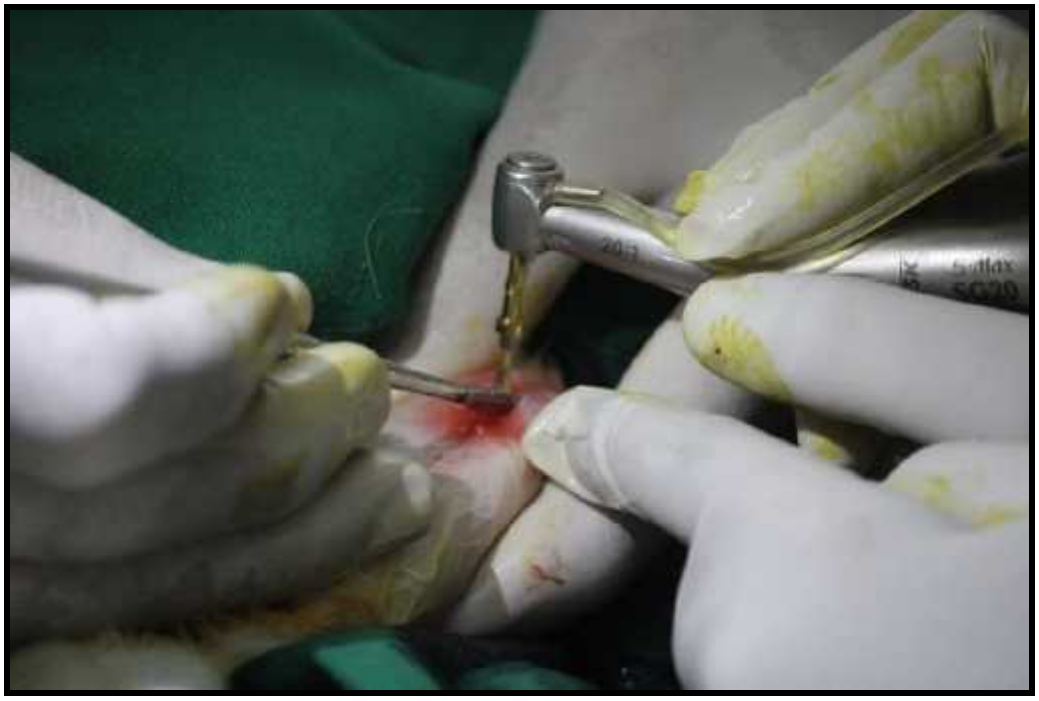


FIGURE 18: OSTEOTOMY SITE PREPARATION.

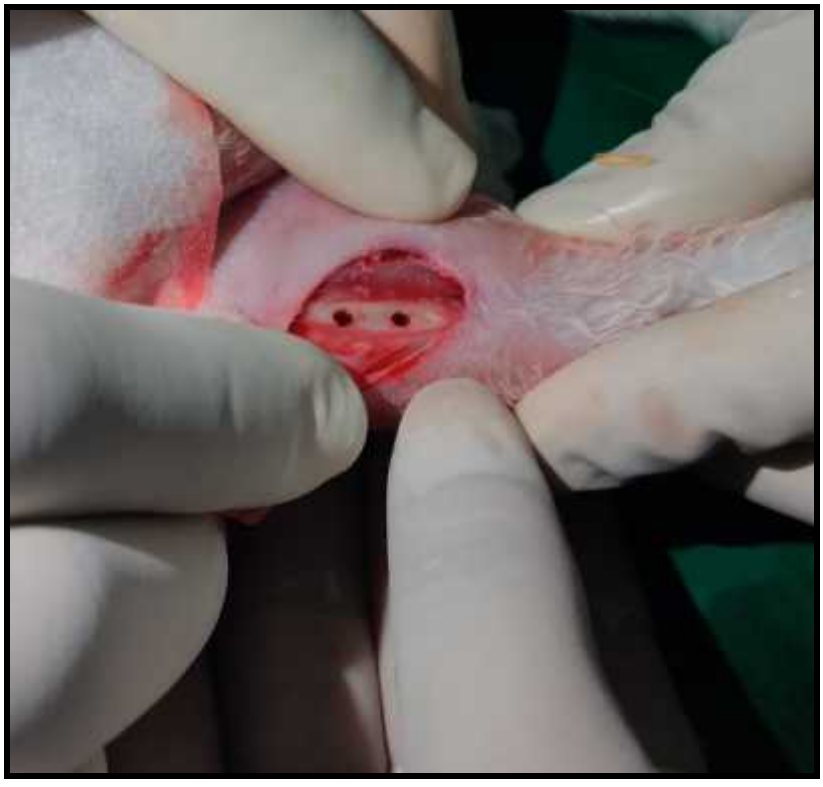


FIGURE 19: PREPARED OSTEOTOMY SITES.

FIGURE 20: SURFACE TREATMENT OF IMPLANT BY DIP COATING IN NOVEL HYDROGEL.

FIGURE 21: PLACEMENT OF IMPLANT.

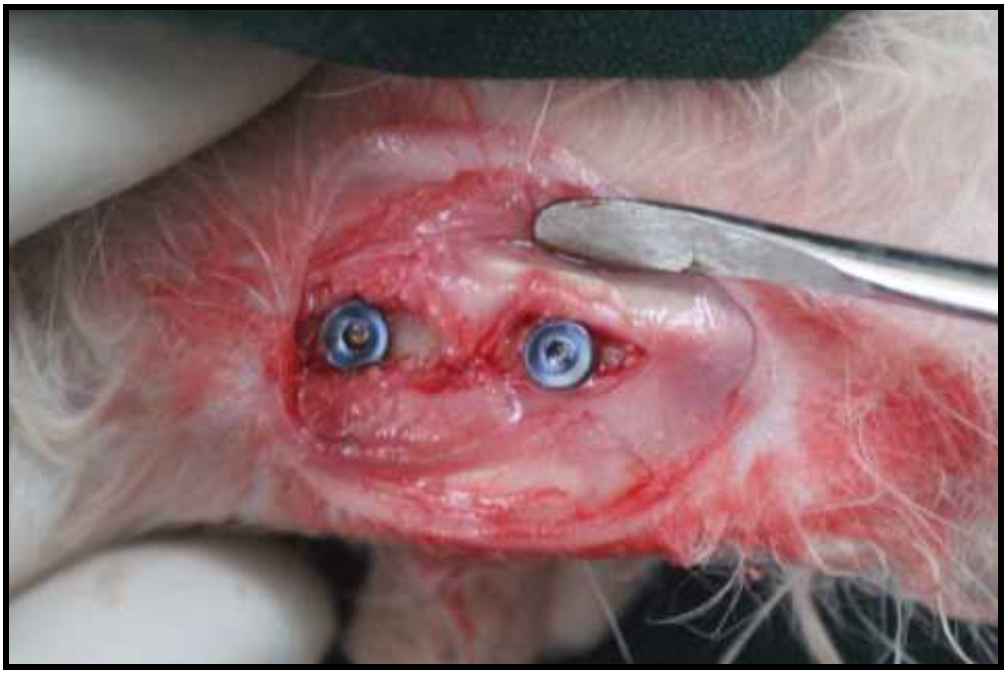


FIGURE 22: IMPLANT AT PREPARED OSTEOTOMY SITE.

FIGURE 23: FASCIA CLOSURE USING CATGUT SUTURES.

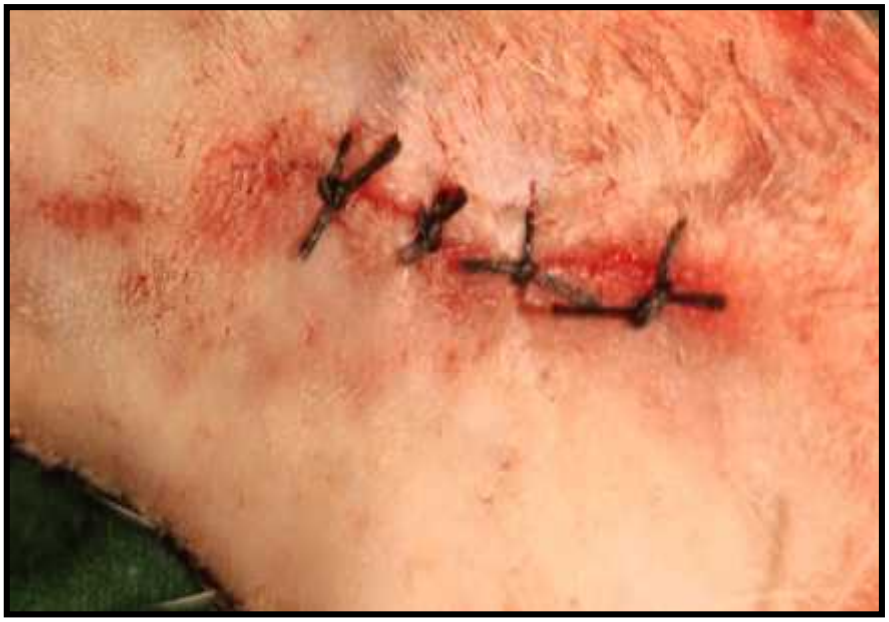


FIGURE 24: SKIN SUTURING WITH SILK SUTURES.

FIGURE 25: RABBIT WITH LAMP.

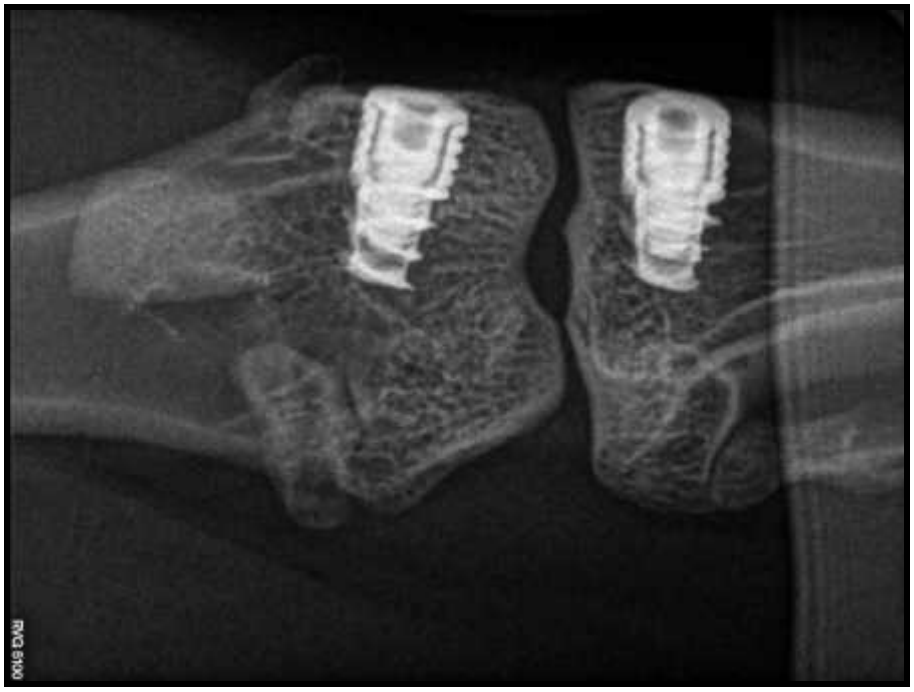


FIGURE 26: RADIOVISIOGRAPHY OF DENTAL IMPLANTS PLACED IN FEMUR AND TIBIA.

FIGURE 27: MEASUREMENT OF IMPLANT STABILITY QUOTIENT USING RESONANCE FREQUENCY ANALYSER (RFA).

FIGURE 28: MEASUREMENT OF REMOVAL TORQUE QUOTIENT USING A CUSTOMIZED CALIBRATED, ELECTRONIC REMOVAL TORQUE WRENCH.

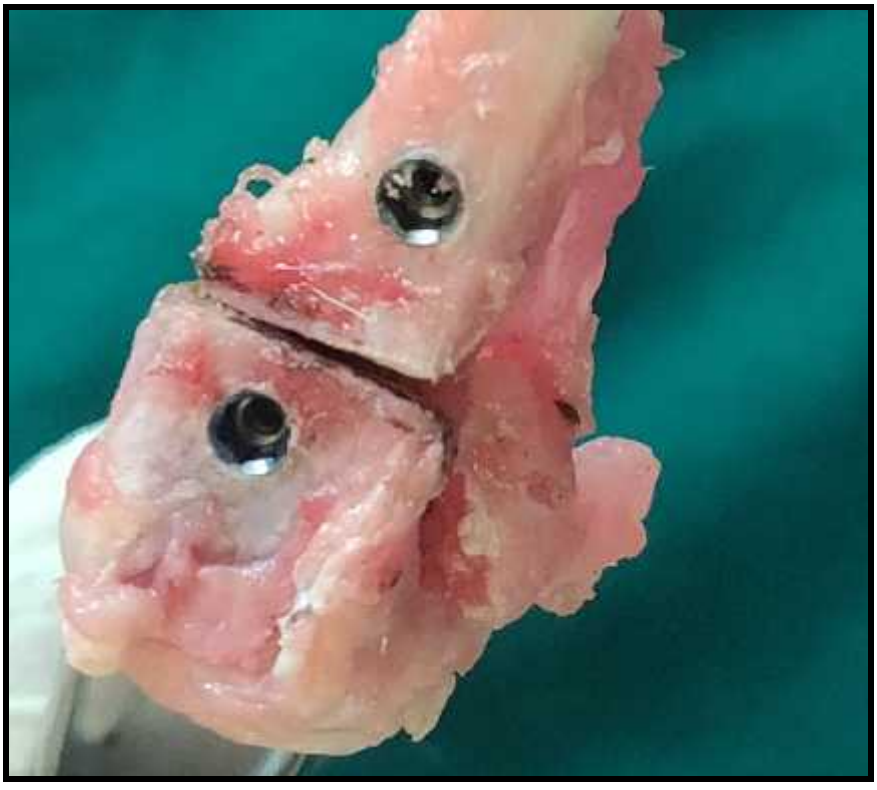


FIGURE 29: TRANSVERSE ENBLOC SECTION OF TIBIA AND FEMUR HEAD.

FIGURE 30: DECALCIFIED SPECIMENS EMBEDDED IN PARAFFIN WAX

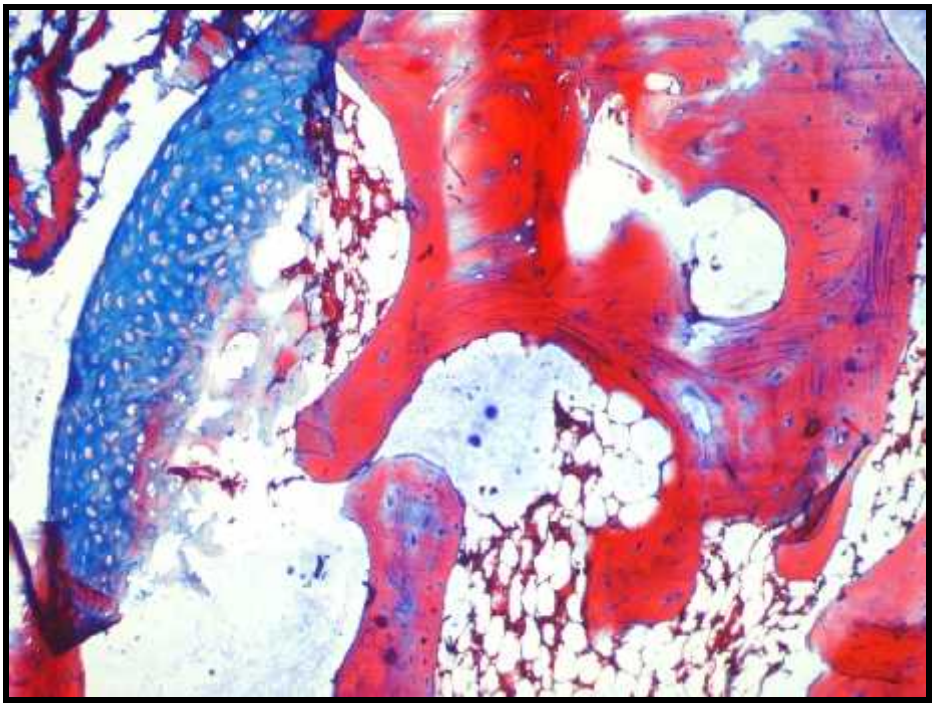


FIGURE 31: MASSON TRICHROME STAIN CLEARLY DIFFERENTIATING MATURE BONE (BLUE) FROM NEWLY FORMED IMMATURE BONE (RED)

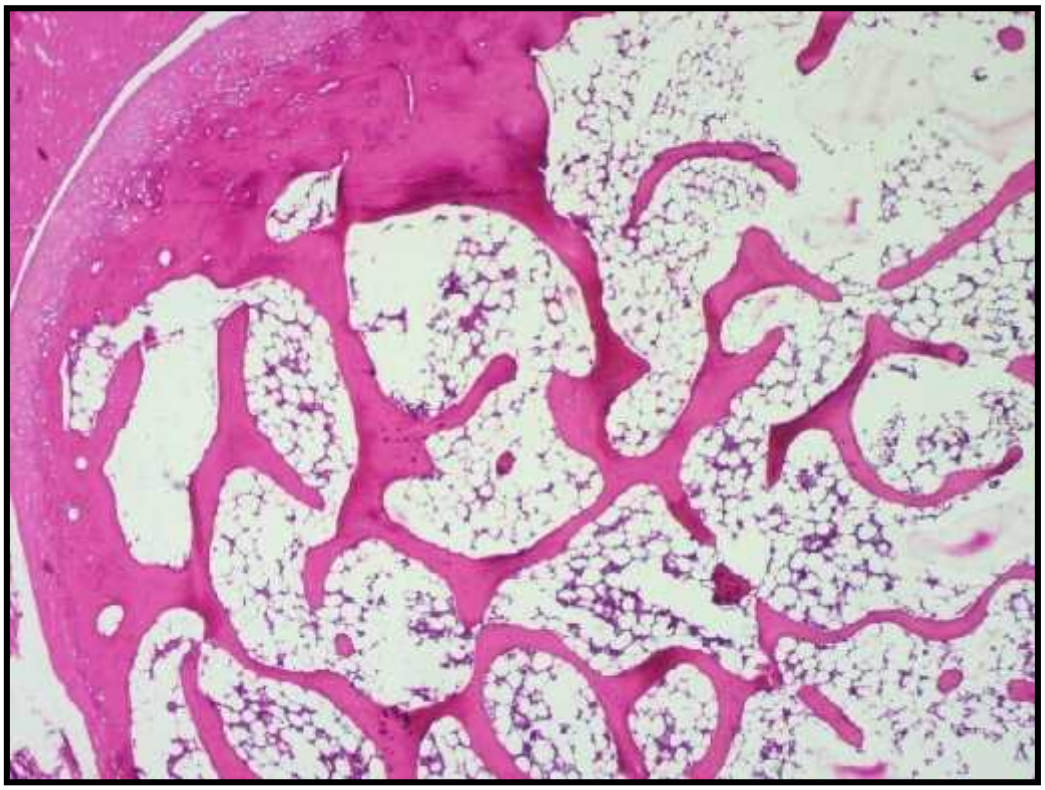


FIGURE 32: PHOTOMICROGRAPH SHOWING NEWLY FORMED SINGLE BONY TRABECULAE (H & E 4X)

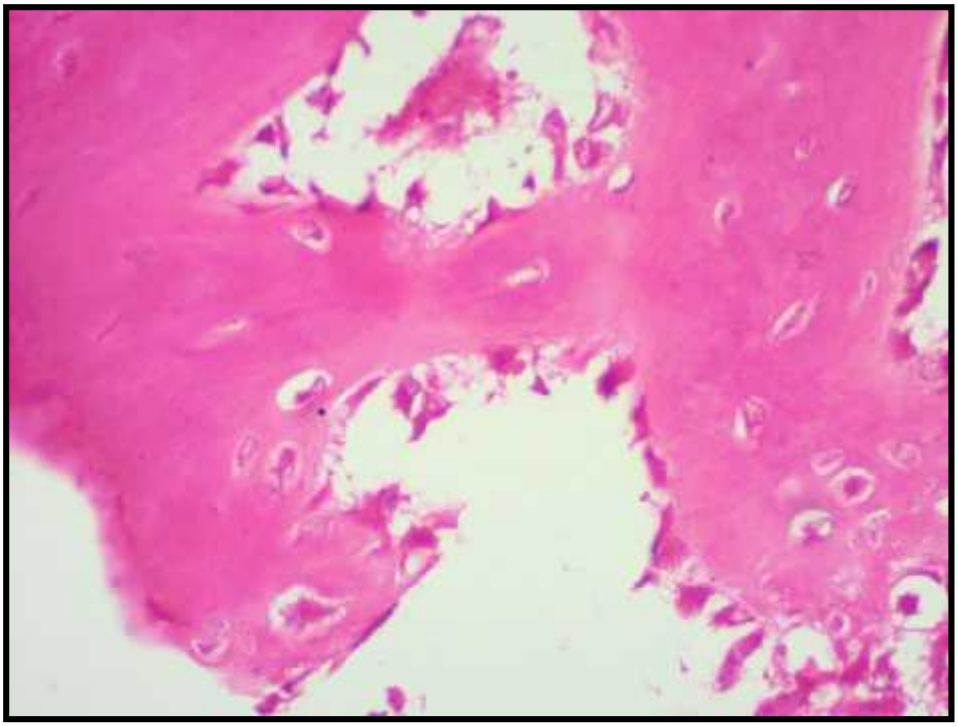


FIGURE 33: PHOTOMICROGRAPH SHOWING LINEAR CONCENTRATIONS OF OSTEOBLASTS AT THE PERIPHERY OF NEWLY FORMED BONE (H& E 40X)

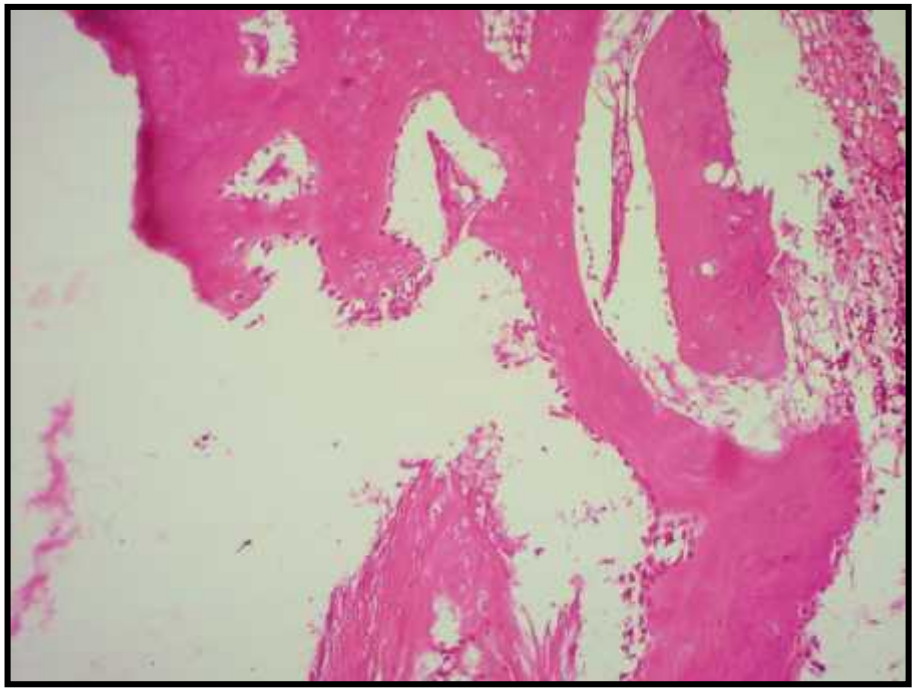


FIGURE 34: PHOTOMICROGRAPH SHOWING LINEAR CONCENTRATIONS OF OSTEOBLASTS AT THE PERIPHERY OF NEWLY FORMED BONE (H & E 10X)

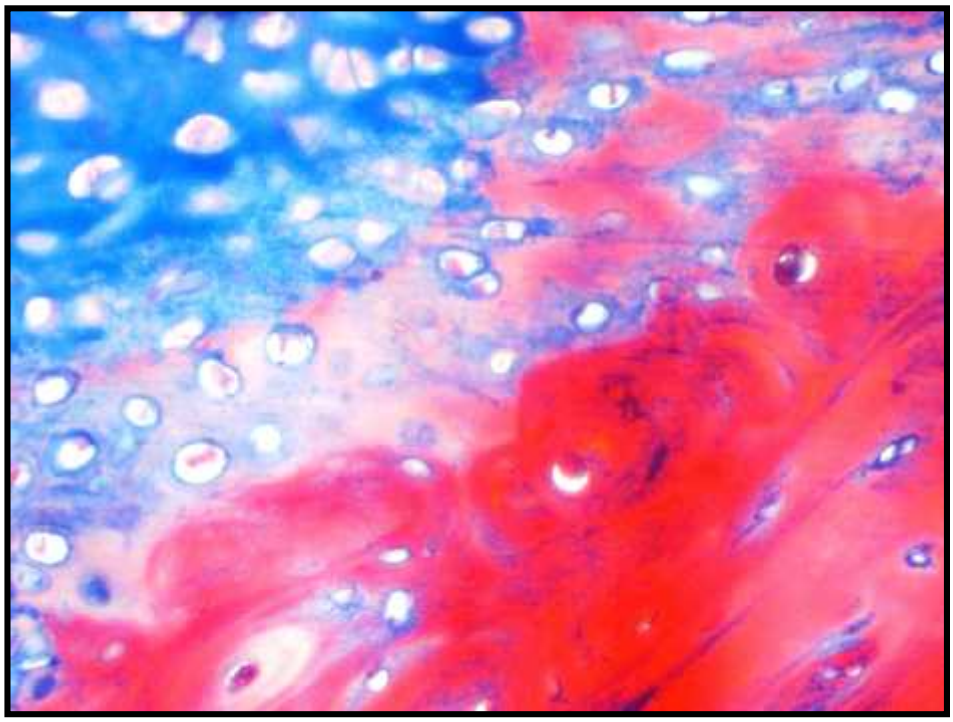


FIGURE 35: PHOTOMICROGRAPH SHOWING HIGH CONTRAST IMAGE WITH BONE IN RED AND OSTEIOD IN BLUE(MT 40X)

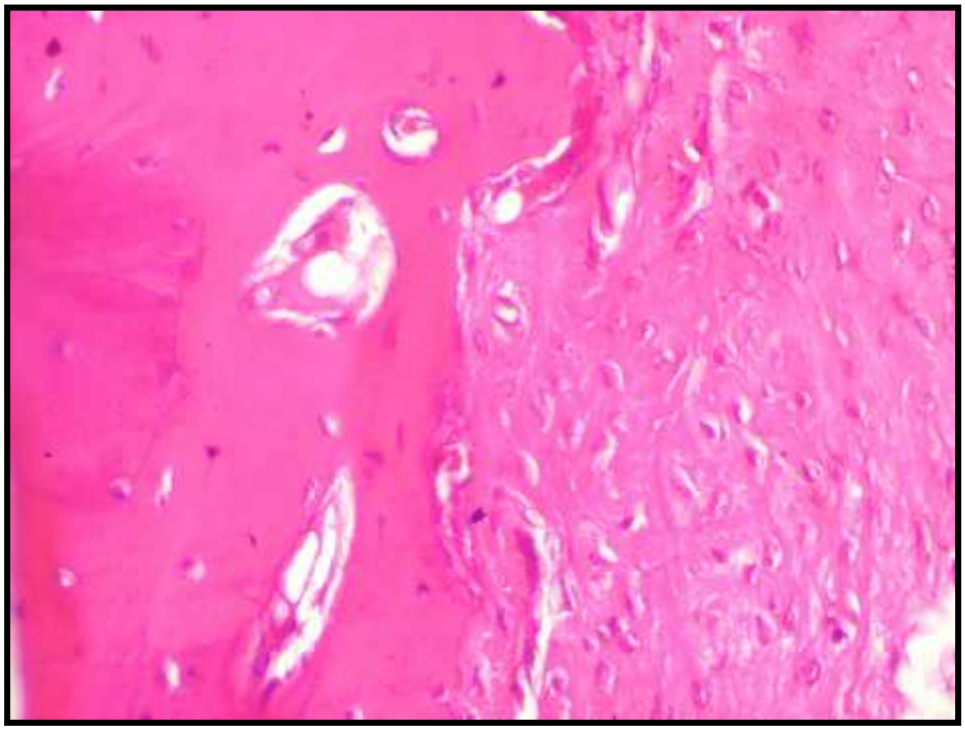


FIGURE 36: PHOTOMICROGRAPH SHOWING NON MINERALIZED OSTEOID MATRIX (H & E 40 X)

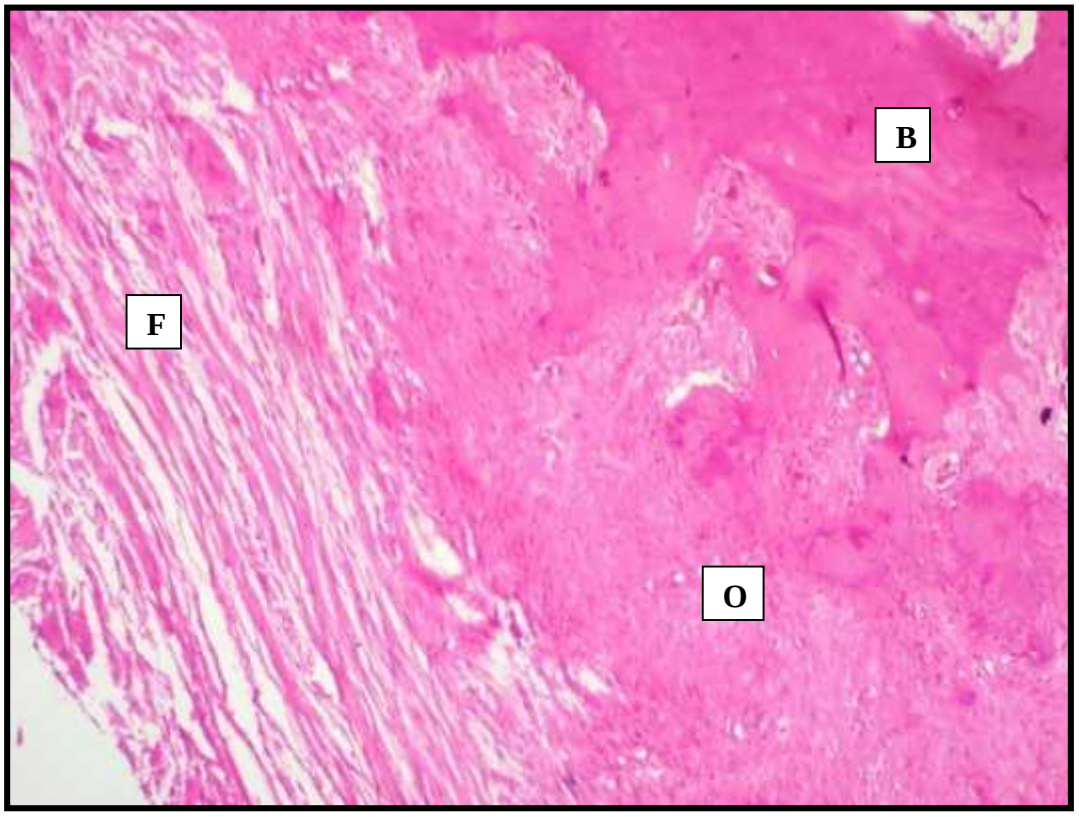


FIGURE 37: PHOTOMICROGRAPH SHOWING B BONE, O OSTEOID, F FIBROUS CALLUS (H& E 10 X)

RESULTS

The present study evaluated and compared osseointegration and osseoblast proliferation of surface coated dental implants and commercially pure titanium implants using resonance frequency analysis, removal torque quotient and radiovisiography analysis.

Statistical Analysis

Data gained from the study was uploaded in Microsoft excel sheet and SPSS version 20 software, was used to perform the statistical analysis.

TABLE 3: MTT ASSAY ON MG-63 OSTEOBLAST LIKE CELLS

CqChH 1%	**CONCENTRATION**	**% SURVIVAL OF CELLS**
		81
		79
		74
CqChH 3%	**CONCENTRATION**	**% SURVIVAL OF CELLS**
		97.32
		96.10
		95.53

Table 4: **Normality of scores of resonance frequency analysis, removal torque analysis and radiovisiography scores at different time points in two groups was assessed by Kolmogorov Smirnov test. The resonance frequency analysis, removal torque analysis and radiovisiography scores at different time points in two groups followed normal distribution. Therefore, the parametric tests were applied.**

		Control group		Test group	
		Z-value	p-value	Z-value	p-value
Resonance Frequency Analysis	6 weeks	0.7710	0.5920	0.7820	0.5740
	12 weeks	0.7250	0.6700	0.4800	0.9750
	6W-12W	0.7530	0.6220	0.8200	0.5130
Removal Torque Analysis	6 weeks	0.6230	0.8320	0.3810	0.9990
	12 weeks	0.4310	0.9920	0.4530	0.9870
	6W-12W	0.4690	0.9800	0.5490	0.9240
Radio Visio Graphy	Baseline	0.9130	0.3750	0.4950	0.9670
	6 weeks	1.0490	0.2210	0.5310	0.9400
	12 weeks	1.0010	0.2690	0.6420	0.8050
	BL-6W	0.6190	0.8390	0.9200	0.3660
	BL-12W	0.8580	0.4530	0.7650	0.6030
	6W-12W	0.7990	0.5460	0.4920	0.9690

Resonance frequency analysis

The mean ISQ of the commercially pure titanium implants group at 6 week, 12 weeks and between 6 and 12 weeks was found to be 59.73, 66.36 and 6.64 respectively. The mean ISQ for surface coated dental implants at 6 , 12 weeks and 6-12 week was found to be 60.27, 73.91 and 13.64 respectively.

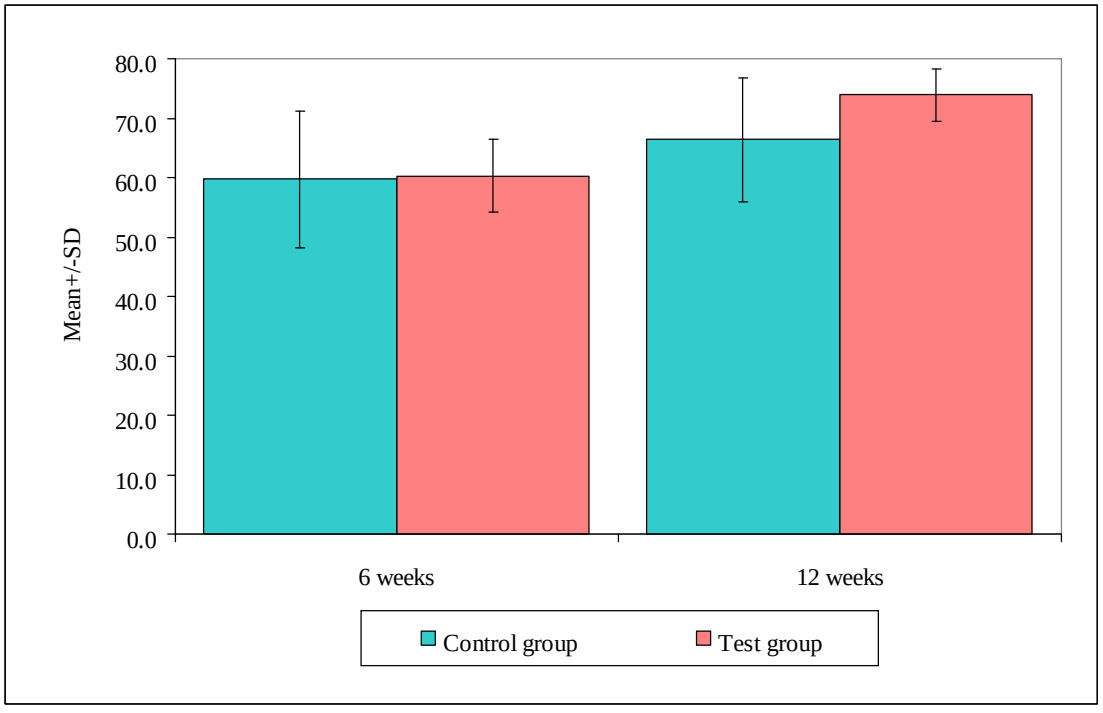
The standard deviation of ISQ for commercially pure titanium implants group at 6 week, 12 week and 6-12 week was found to be 11.51, 10.44 and 2.16 respectively. The standard deviation of ISQ for surface coated dental implants at 6 week, 12 week and 6-12 week was found to be 6.12, 4.39 and 3.23 respectively.

Pairwise comparison of ISQ values between commercially pure titanium implants and surface coated titanium implants at 6 weeks was found to be not significant. (p=0.8910) Pairwise comparison of ISQ values between commercially pure titanium implants and surface coated titanium implants at 12 weeks was found to be significant (p=0.0390*). Pairwise comparison of ISQ values between commercially pure titanium implants and surface coated titanium implants at 6-12 weeks was found to be significant (p=0.0001*). (Table 5)

Table 5: Comparison of control and test groups with resonance frequency analysis scores at 6 weeks and 12 weeks time points by independent t test

Time points	Groups	Mean	SD	SE	t-value	p-value
6 weeks	Control group	59.73	11.51	3.47	-0.1388	0.8910
	Test group	60.27	6.12	1.84		
12 weeks	Control group	66.36	10.44	3.15	-2.2090	0.0390*
	Test group	73.91	4.39	1.32		
6W-12W	Control group	6.64	2.16	0.65	-5.9728	0.0001*
	Test group	13.64	3.23	0.97		

Graph 1: Comparison of control and test groups with resonance frequency analysis scores at 6 weeks and 12 weeks time points

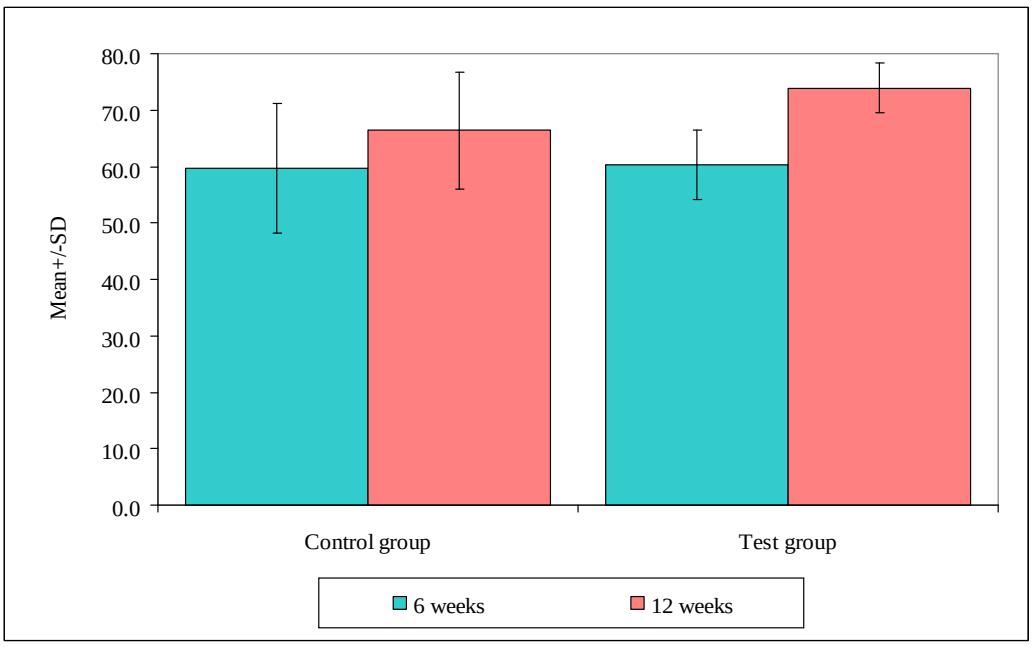


Pairwise comparison of ISQ values of commercially pure titanium implants between 6 week and 12 week was found to be significant. (p=0.0001) Pairwise comparison of ISQ values of surface coated titanium implants at 6 and 12 weeks was found to be significant (p=0.0001*). (Table 6)

Table 6: Comparison of 6weeks and 12 weeks time points with resonance frequency analysis scores in control and test groups by dependent t test

Groups	Time points	Mean	SD	Diff. Mean	Diff. SD	% of change	Paired t	P-value
Control group	6 weeks	59.73	11.51					
	12 weeks	66.36	10.44	-6.64	2.16	-11.11	-10.2021	0.0001*
Test group	6 weeks	60.27	6.12					
	12 weeks	73.91	4.39	-13.64	3.23	-22.62	-13.9876	0.0001*

Graph 2: Comparison of 6weeks and 12 weeks time points with resonance frequency analysis scores in control and test groups



Removal torque analysis

The mean RTQ of the commercially pure titanium implants group at 6 week, 12 weeks and 6-12 week was found to be 41.89, 45.80 and 3.91 respectively. The mean RTQ for surface coated dental implants at 6 week, 12 week and 6-12 week was found to be 49.85, 75.96 and 26.11 respectively.

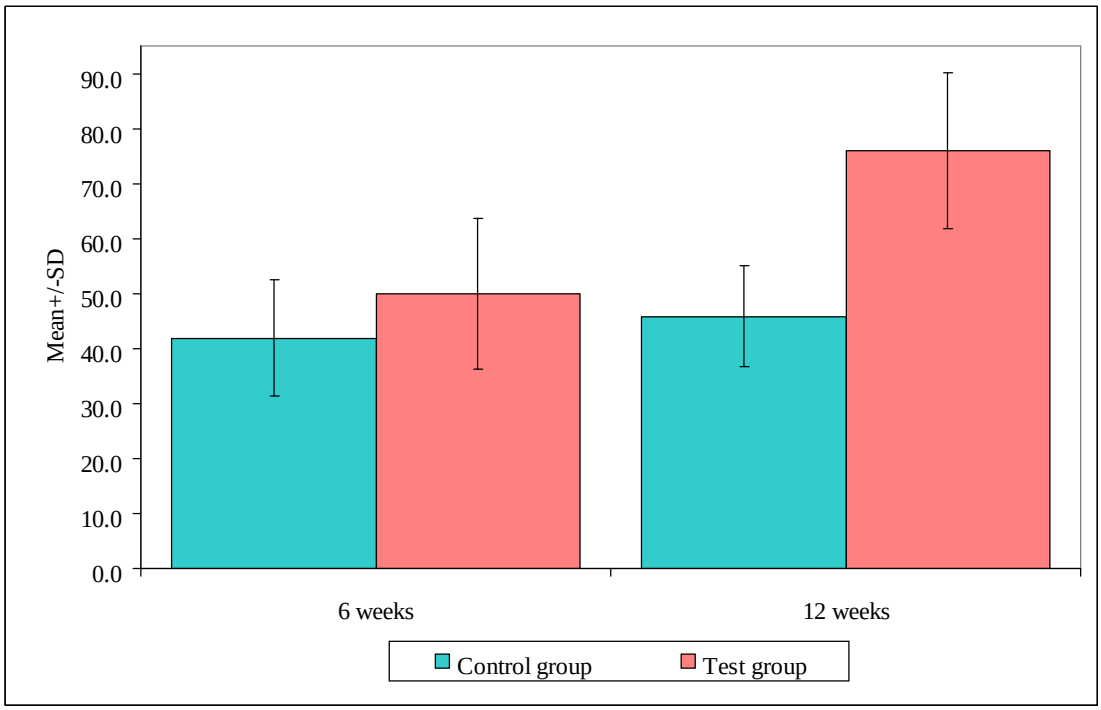
The standard deviation of RTQ for commercially pure titanium implants group at 6 week, 12 week and 6-12 week was found to be 10.64, 9.16 and 4.07 respectively. The standard deviation of RTQ for surface coated dental implants at 6 week, 12 week and 6-12 week was found to be 13.71, 14.10 and 18.97 respectively.

Pairwise comparison of RTQ values between commercially pure titanium implants and surface coated titanium implants at 6 weeks was found to be not significant. (p=0.3350) Pairwise comparison of RTQ values between commercially pure titanium implants and surface coated titanium implants at 12 weeks was found to be significant (P=0.004). Pairwise comparison of RTQ values between commercially pure titanium implants and surface coated titanium implants at 6-12 weeks was found to be significant. (0.0340). (Table 7)

Table 7: Comparison of control and test groups with removal torque analysis scores at 6 weeks and 12 weeks time points by independent t test

Time points	Groups	Mean	SD	SE	t-value	p-value
6 weeks	Control group	41.89	10.64	4.76		
	Test group	49.85	13.71	6.13	-1.0250	0.3350
12 weeks	Control group	45.80	9.16	4.09		
	Test group	75.96	14.10	6.31	-4.0110	0.0040*
6W-12W	Control group	3.91	4.07	1.82		
	Test group	26.11	18.97	8.48	-2.5590	0.0340*

Graph 3: Comparison of control and test groups with removal torque analysis scores at 6 weeks and 12 weeks time points

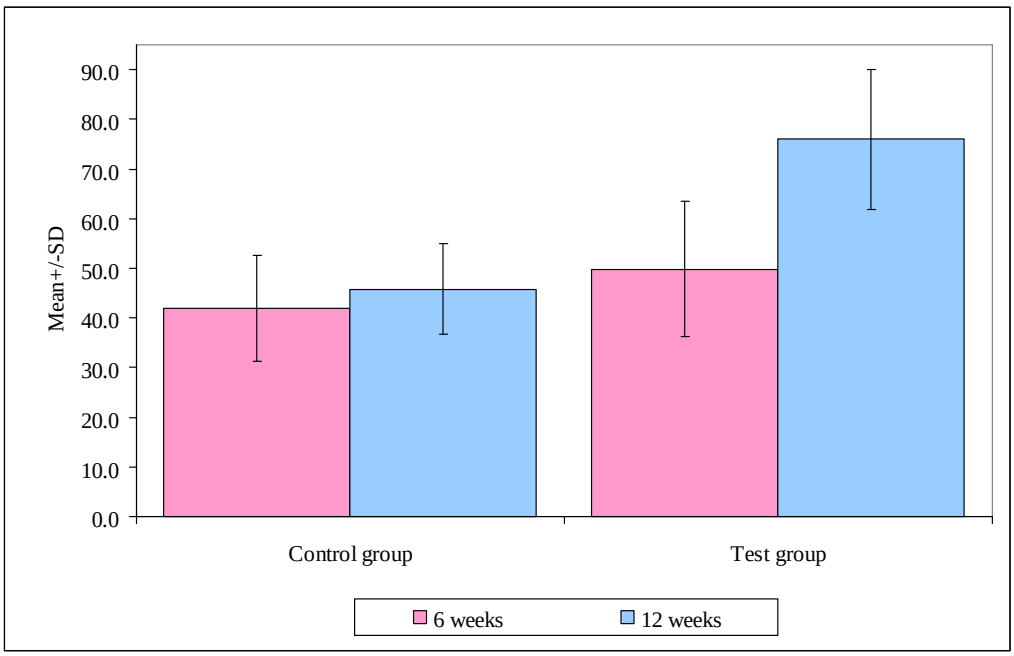


Pairwise comparison of RTQ values of commercially pure titanium implants between 6 week and 12 week was found to be not significant. (p=0.0990) Pairwise comparison of ISQ values of surface coated titanium implants at 6 and 12 weeks was found to be significant (p=0.0370*). (Table 8)

Table 8: Comparison of 6weeks and 12 weeks time points with removal torque analysis scores in control and test groups by dependent t test

Groups	Time points	Mean	SD	Diff. Mean	Diff. SD	% of change	Paired t	P-value
Control group	6 weeks	41.89	10.64					
	12 weeks	45.80	9.16	-3.91	4.07	-9.32	-2.1440	0.0990
Test group	6 weeks	49.85	13.71					
	12 weeks	75.96	14.10	-26.11	18.97	-52.37	-3.0780	0.0370*

Graph 4: Comparison of 6weeks and 12 weeks time points with removal torque analysis scores in control and test groups



Radiovisiography Scores:

The mean RVG values of the commercially pure titanium implants group at baseline, 6 weeks and 12 weeks was found to be 84.80, 92.20and 102.80respectively.The mean RVG values of the commercially pure titanium implants group atBL-6W, BL-12W and 6W-12W was found to be 7.40, 18.00 and 10.60 respectively. The mean RVG for surface coated dental implants at baseline, 6 and 12 weeks was found to be 61.10, 96.30 and 113.80 respectively. The mean RVG for surface coated dental implants at BL-6W, BL-12W and 6W-12W was found to be 42.30, 52.70 and 17.50respectively.The standard deviation of RVG for commercially pure titanium implants group at baseline, 6 and 12 weeks was found to be 42.27, 40.19 and 43.28respectively. The standard deviation of RVG for commercially pure titanium implants group atBL-6W, BL-12W and 6W-12W was found to be 4.12, 5.56 and 5.04respectively. The standard deviation of RVG for surface coated dental implants at baseline, 6 and 12 weeks was found to be 16.99, 15.76 and 16.09respectively.The standard deviation of RVG for surface coated dental implants at BL-6W, BL-12W and 6W-12Wwas found to be 40.76, 26.71 and 3.75respectively.

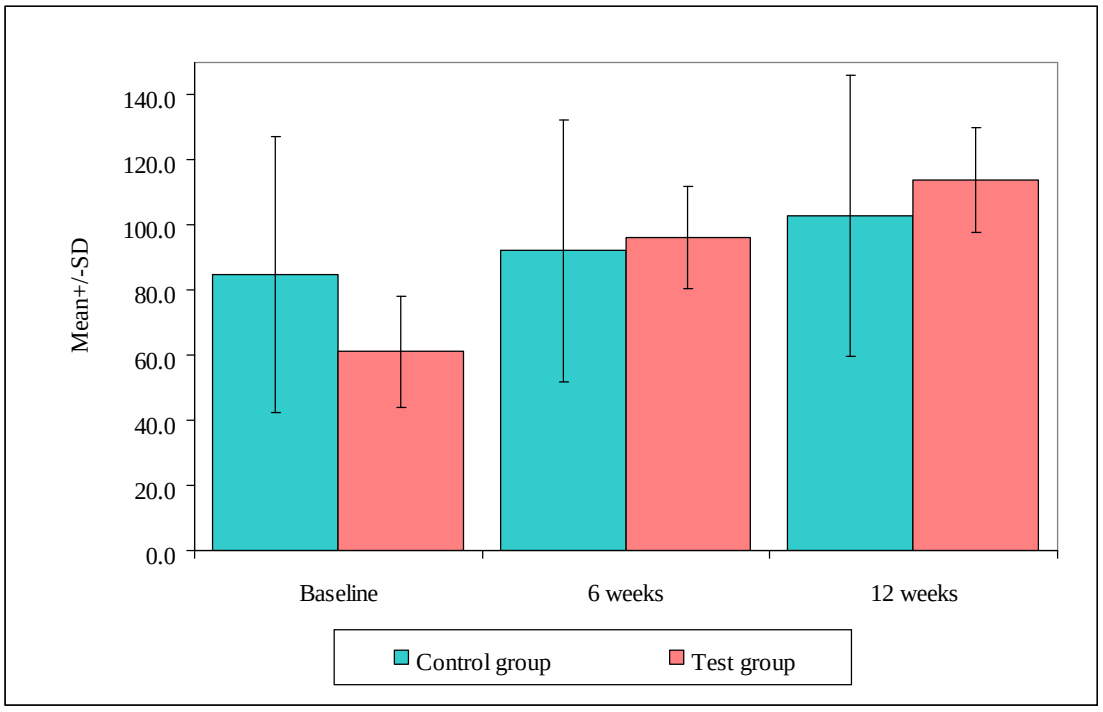
Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at baseline was found to be not significant. (p=0.1173) Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at 6 weeks was found to be not significant (P=0.7674) Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at 12 weeks was found to be not significant (p=0.4610).

Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at BL-6Wwas found to be significant. (p=0.0148) Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at BL-12Wwas found to be significant (P=0.0008) Pairwise comparison of RVG values between commercially pure titanium implants and surface coated titanium implants at 6W-12Wwas found to be significant (p=0.0.0027) (Table 9)

Table 9: Comparison of control and test groups with radio visiography scores at baseline, 6 weeks and 12 weeks time points by independent t test

Time points	Groups	Mean	SD	SE	t-value	p-value
Baseline	Controlgroup	84.80	42.27	13.37	1.6452	0.1173
	Testgroup	61.10	16.99	5.37		
6 weeks	Controlgroup	92.20	40.19	12.71	-0.3003	0.7674
	Testgroup	96.30	15.76	4.98		
12 weeks	Controlgroup	102.80	43.28	13.69	-0.7534	0.4610
	Testgroup	113.80	16.09	5.09		
BL-6W	Controlgroup	7.40	4.12	1.30	-2.6940	0.0148*
	Testgroup	42.30	40.76	12.89		
BL-12W	Controlgroup	18.00	5.56	1.76	-4.0223	0.0008*
	Testgroup	52.70	26.71	8.45		
6W-12W	Controlgroup	10.60	5.04	1.59	-3.4747	0.0027*
	Testgroup	17.50	3.75	1.19		

Graph 5: Comparison of control and test groups with radio visio-graphy scores at baseline, 6 weeks and 12 weeks time points



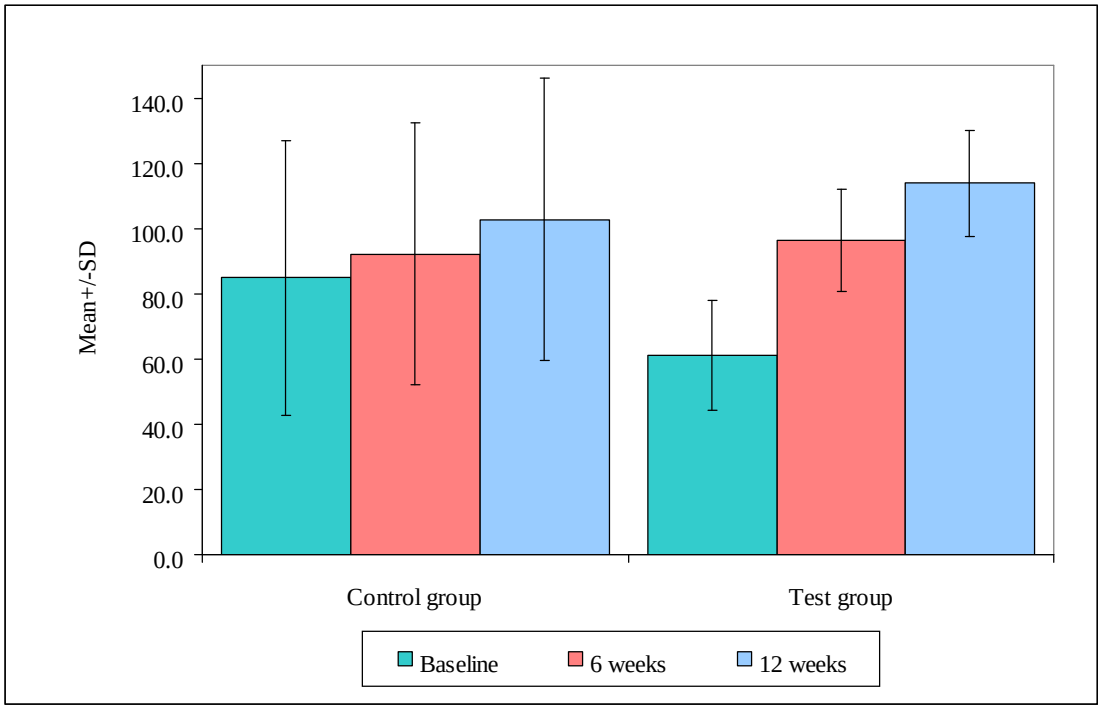
Pairwise comparison of RVG values of commercially pure titanium implants between Baseline and 6 week was found to be significant. (p=0.0003). Pairwise comparison of RVG values of commercially pure titanium implants between baseline and 12 week was found to be significant. (p=0.0001). Pairwise comparison of RVG values of commercially pure titanium implants between 6 week and 12 week was found to be significant. (p=0.0001)

Pairwise comparison of RVG values of surface coated titanium implants at Baseline and 6 week was found to be significant (p=0.0019). Pairwise comparison of RVG values of surface coated titanium implants at baseline and 12 weeks was found to be significant (p=0.0.0002*). Pairwise comparison of RVG values of surface coated titanium implants at 6 and 12 weeks was found to be significant (p=0.0001). (Table 10)

Table 10: Comparison of baseline 6weeks and 12 weeks time points with radio visio-graphy scores in control and test groups by dependent t test

Groups	Time points	Mean	SD	Diff. Mean	Diff.SD	% of change	Paired t	P-value
Control group	Baseline	84.80	42.27					
	6 weeks	92.20	40.19	-7.40	4.12	-8.73	-5.6867	0.0003*
	Baseline	84.80	42.27					
	12 weeks	102.80	43.28	-18.00	5.56	-21.23	-10.2417	0.0001*
	6 weeks	92.20	40.19					
	12 weeks	102.80	43.28	-10.60	5.04	-11.50	-6.6539	0.0001*
Test group	Baseline	61.10	16.99					
	6 weeks	96.30	15.76	-35.20	25.60	-57.61	-4.3476	0.0019*
	Baseline	61.10	16.99					
	12 weeks	113.80	16.09	-52.70	26.71	-86.25	-6.2397	0.0002*
	6 weeks	96.30	15.76					
	12 weeks	113.80	16.09	-17.50	3.75	-18.17	-14.7609	0.0001*

Graph 6: Comparison of baseline 6weeks and 12 weeks time points with radio visio-graphy scores in control and test groups



DISCUSSION

Tooth extraction is an undesirable and uncontrollable event that can affect oral function as well as esthetics of patients. The absence of the extracted tooth, becomes a void that needs to be tended to almost immediately.[83] To keep up with the ever increasing demands of patients, dental implant placement is slowly yet steadily becoming the go-to treatment to replace missing teeth, all over the world. Dental implant placement is also followed by the wait for optimum osseointegration to occur, which ranges from 3-6 months depending on the amount of available bone. Surface chemistry plays a major role in the osseointegration of implants as it determines adsorption of proteins from body fluids.[84] In vitro and animal studies have already demonstrated that alterations to the implant surface, improves the interaction between the titanium implant and the human body.[85, 86] Surface topography is very crucial for adhesion and differentiation of osteoblasts to implant surface during the initial phase of osseointegration and also in long-term bone remodeling.[87, 88, 89] Surface wettability or hydrophilicity of implants is one of the important aspects of osseointegration.[90] In this study, a novel hydrophilic hydrogel coated on the titanium implant surface resulted in higher values of bone volume and enhanced osteoblast proliferation. Scwartz et al. showed that hydrophilic surfaces have a higher affinity to form an initial bone contact, with improved angiogenesis, and a greater bone density after a 14-day period of bone healing.[91] An animal study by Vasak et al., reported hydrophilic implants caused tendency towards greater BIC.[92]Buser et al., also reported in the mini pig model that hydrophilic implants increased BIC after 2–4 weeks of healing when compared with non hydrophilic implants.[93] Additionally, a study in the dog model by Schwarz et al[91] had reported advantageous results for hydrophilic surface similar to this study where significant values showed new bone

formation of the test group implants both in the tibia and femur which indicated that the content of the novel hydrophilic hydrogel used in this study helped in newer bone formation by increasing osteoblastic activity. The effect of the novel Cissus hydrogel used in this study was tested in vitro on osteoblast like cell lines and its effect was evaluated in varying concentrations of 1% and 3%. Since cell viability and differentiation was similar in both concentrations the higher concentration was recommended in this in-vivo study. Hydrogel-based treatment for bone tissue engineering seems rather promising.[94] Creating hydrogels with positively charged domains have improved the hydrogel's ability to encourage cellular adhesion within in vitro models.[45]Chitosan is a positively charged linear polysaccharide.[95] This polymer is hydrophilic in nature with the ability to degrade via human enzymes which results in excellent biocompatibility and biodegradability properties. Li et al.[96] in 2017 conducted in vitro and in vivo experiments which demonstrated that chitosan-based biocomposite scaffolds are not toxic, and have very good properties of biocompatibility. Vandevord et al., 2002 confirmed that implantable chitosan scaffolds are safe and free from immunogenic response when used in animals. These scaffolds are histocompatible and support cell attachment and regeneration.[97] Chitosans efficient antimicrobial properties together with its biodegradability and histocompatibility made it our distinguished choice as scaffold material for the novel hydrogel used in this study. Chitosan is low in cost and can be easily converted into various forms such as film, scaffold and hydrogels with a required tensile strength. [98]Cissus quadrangularis, a vining plant, native to India has several studies reporting its bone fracture healing and anti-osteoporotic activity.[99] Results of our study are in line with several studies that have used compounds extracted from Cq and showed bone protective and bone strengthening properties. A series of studies by Potu et al. was

conducted where a petroleum ether extract of Cissus was used, and reported evidence of the ability of Cissus extracts to stimulate bone growth and healing.[100, 101, 37, 102, 103]The extract resulted in increased serum levels of alkaline phosphatase and tartrate-resist acid phosphatase activities, and hydroxyproline content, all indicative of osteoproliferative activity.[103] Though the exact mechanism of action has not been identified, Mishra in 2010 conducted a radioactive study with calcium (Ca45) indicated that CQ stimulated cells of mesenchymal origin—the fibroblasts, the chondroblasts and osteoblasts at early stage and hastened the healing at the fracture site by about 10–14 days in the treated group.[104] Other studies have also found that a phytogenic steroid isolated from CQ stimulates osteoblasts and leads to early fracture healing.[105, 51, 50]

In this study bone response was evaluated using resonance frequency analysis and removal torque measurement and confirmed with histopathologic analysis. The resonance frequency analysis measures the ISQ non-invasively at any point in the study. It has been reported to be a reliable and accurate method for the early assessment of implant stability that is directly related to the bone implant interface.[106],[107] The Resonance Frequency Analysis was performed after 6 weeks of healing and 12 weeks of healing to evaluate the strength of the osseointegration. In the current study, the ISQ values for both the groups after the healing period increased through osseointegration; significant differences were observed among the groups. Meredith in 1997 demonstrated that implants with higher ISQ can reach peak of stability in shorter healing periods and concluded that RFA is a useful research technique and can be valuable in studying the stability of implants in the bone and can subsequently be used clinically to hasten loading protocols.[108] In the present study, all implants surface coated with Cissus hydrogel had significantly higher ISQ values at 6

weeks and 12 weeks when compared with commercially pure titanium implants. (Table 5 Graph 1) Pairwise comparison of ISQ between commercially pure titanium implants and surface coated implants at 12 weeks was found to be significant with significance set at p =0.0390. (Table 5) This result agreed with that of previous studies reporting that the ISQ values of modified implants were higher than those of topographically modified implants.[109,110,111] (Graph 1) Predictably, between 6 weeks and 12 weeks statistically significant differences were observed which agrees with studies by Parisuthiman et al., 2011[38] which demonstrated that the ethanolic extract of Cissus may regulate osteoblastic activity by enhancing alkaline phosphatase activity, which is associated with osteoblast differentiation, and the mineralization process. Similarly, Singh et al. (2011) conducted a human clinical evaluation in which they compared the ability of a Cissus extract, a Moringa olifera extract and a product containing a 4:1:2 ratio of Cissus, Asparagus and Moringa extracts (Osteoseal™) to hasten mandibular fracture bone healing and confirmed that the Cissus extract alone shortened the duration of bone healing by about two to three weeks, demonstrating clinical efficacy in decreasing fixation time.[112]Parusithiman in 2008 conducted an in vitro study, which reported for the first time, that CqE may regulate the osteoblastic activity by enhancing the ALP activity. [38] Several studies reported that Cq contains anabolic and phytogenic steroids like Ketosteroids, silosterol, alpha amayrin, alpha ampyrone and tetracyclic treterpenoids which plays a significant role in bone healing.[113, 114, 115] Siddaram et al. also supported the inference that these anabolic and steroidal component showed a marked influence on fracture healing.[116] Ketosteroid acts as antagonists to the glucocorticoid receptor and promotes good bone health. It mobilizes fibroblast and chondroblasts to an injured tissue and enhances regeneration of osteoblasts.[117]Ambarish et al confirmed that the plant contains a high percentage of

calcium probably due to its thick cell wall, which makes it suitable for growth of mineral crystals. The presence of phosphorous in the plant can also be exploited for synthesizing hydroxyapatite, thus utilizing the traditional knowledge of bone-fracture healing in advanced technique of new material synthesis adopted in this study.[51]The removal torque test is common among the invasive mechanical tests used to evaluate the strength of the interaction between the bone and the implant surface. High resistance to implant removal encountered during these tests indicates good integration between the bone and the implant surface. It is well known that surface modification can enhance bone integration of titanium implants, which, in animal studies, can be observed as higher bone-contact ratios and greater resistance to RTQ than non-modified implants. Removal torque scores calculated in this study also supports enhanced bone formation in surface coated implants. Removal torque measurements were calculated for 5 rabbits after 6 weeks and 5 rabbits after 12 weeks with a specially designed customized electronic torque wrench. RTQ has been used as a biomechanical measure for anchorage of the implants in the bone wherein greater forces required to remove the implants are interpreted as an increase in the osseointegration strength. Although removable torque is an invasive biomechanical test that provides information on the rigidity of dental implants in the bone, Ivanoff in 1996 reported that removal torque was closely related to bone to implants contact and the amount of bone within the threads. Removal torque analysis conducted in this study revealed a significant increase of bone formation via increased removal torque scores. Although removable torque scores at 6 weeks were not statistically significant ($p=0.3350$) (Table 7), pairwise comparison of removable torque quotient at 12 weeks was found to be significant ($p=0.0040$) when level of significance was set at $p < 0.05$. (Table 7, Graph 3) On comparison of removable torque scores at 6 weeks and 12

weeks it was observed that an increase in the removable torque quotient was achieved with surface coated implants with p=0.0370. (Table 8, Graph 4) Studies have confirmed that Cq reduces the usual fracture healing time from 14–16 to 8–10 weeks, indicating faster, better bone formation, in the same way our removal torque values revealed. It was also noted that there was increased serum calcium and serum phosphorus levels because of administration of Cq group.[112, 119, 120] Potu in 2009, clearly reported that the CQ plant extract enhances the proliferation and differentiation ability of MSCs into osteoblasts. ALP activity, the most widely recognized biomarker for osteoblast activity, was enhanced by a short treatment with Cq.[101]Our findings are in line with several previous in vivo experiments that have demonstrated that Cq promotes ALP activity and enhances collagen synthesis thereby hastening bone formation.[104] The histopathologic analysis, in the present study, revealed that new bone formation was significantly higher around test group implants when compared with the control group. Osteoblast proliferation was evaluated which showed that implants coated with the novel gel showed enhanced osteoblast differentiation and proliferation. Similar to what the histopathological staining with convention H&E stain and MT special stain revealed in our study, an increase in osteoblastic activity was also confirmed by Potu et al. Our study revealed apparent osteoid formation adjacent to the surface coated implant site. (Fig 35) There were plump osteoblast rimming seen all along the bone surrounding the surface treated implants new bony trabeculae lined by active osteoblasts were observed surrounding the test group implants.(Fig 33, Fig 34) Additionally, connective tissue surrounding the new bone trabeculae was more vascular in the Cissus treated bone blocks compared to the control group blocks. Active osteoblasts synthesizing new bone matrix were numerous at new bone sites, lining the edge of the trabeculae.(Fig 36)

Kumar et al. (2010) used primary cultures of osteoblasts, and showed that various constituents of Cissus quadrangularis increased osteoblast differentiation and mineralization in rat osteoblasts.[121]Muthusami et al. (2011) [122, 123]demonstrated that an ethanolic extract of Cissus positively influenced proliferation, differentiation and mineralization of human osteoblast like cells without exhibiting toxicity to the cells, similar to our histopathologic analysis.

In a study by Brahmakshatriya, radiographs of the Cissus group shows the early callus formation and complete new bone formation at 7–8 weeks.[36]Similarly, radiovisiography analysis in this study revealed marginally higher isodensity values of the peri implant bone in the test group when compared to the control group. Although removable torque scores at baseline, 6 weeks and 12 weeks were not statistically significant (p=0.1173, p=0.7674 and p=0.4610) (Table 9), pairwise comparison of radiovisiography scores at BL-6W, BL-12W and 6W-12W was found to be significant (p=0.0148, 0.0008 and 0.0027) when level of significance was set at $p < 0.05$. (Table 9, Graph 5) Heterogeneity of data can be caused due to variation in the animal itself as well as variation in positioning of the implant. Clinical observation indicated that the final healing time for each rabbit differed owing to iatrogenic errors and operation conditions.

Our study demonstrated that hydrogel coated implant surfaces not only increased removal torque values but also significantly increased osteoblast proliferation. Further research can be conducted to evaluate similar hydrogels in cases of systemic conditions that affect the density of bone. We concluded that surface coated dental implant with Cissus hydrogel showed faster osseointegration and bone healing than commercially pure titanium and should be considered as an excellent economic and efficient surface modification of implants.

SCOPE OF THE STUDY

The present study evaluated and compared the osseointegration and osteoblast proliferation on titanium implants coated with a novel Cissus quadrangularis hydrogel. The implant stability quotient was conducted using the resonance frequency analyser.

Further research is suggested to assess the differentiation of cells quantitatively using histomorphometry.

This study should be expanded by the use of bigger animals for closer resemblance to human bone formation and maturation, before conducting human clinical trials.

Future studies to determine efficacy of higher concentrations of Cissus quandrangularis extract could be conducted.

Since the hydrogel proved excellent properties, further research to determine the effect of Cissus hydrogel coated implants in compromised patients such as diabetes mellitus, post radiation therapy, osteoporosis etc can be tested.

Hydrogel can be electrospun into nanofibres to ensure better adaptation on the implant surface.

LIMITATIONS OF THE STUDY

1. Osteoblast like cells MG-63 were used in the study to which some researchers claim inconsistencies in its cell differentiaition abilities.
2. Variables such as bone quality and quantity, local and systemic diseases and use of medications may affect the outcome of the study.
3. Histologic evaluation was only performed qualitatively. Quantitative analysis should be done and correlated with the qualitative data.

CLINICAL IMPLICATIONS

One of the most significant problems in the treatment planning for implant surgery is bone response, which refers to rate, the quantity and quality, which is directly related to implant surface properties. Surface characterization in dental implants with hydrogels will lead to improved stability and shorter healing periods. Use of hydrogels for coating implants could yield successful results in enhancing osteointegration due to its hemostatic properties, angiogenic ability, faster healing, and bone proliferative nature, as well as osteoconductive property. The use of bone tissue engineering with hydrogels in bone defects is one of the alternative methods for grafts. With the objective of improving the clinical performance in aspects showcasing poor quantity or quality of bone, of expediting the bone healing and thereby enabling immediate or early loading protocols along with stimulating bone growth to permit implant placement in sites that lack sufficient residual alveolar ridge. With these benefits, surface coated implants with the novel Cissus hydrogel can be a brilliant approach concidering its excellent regenerative potential in atrophic jaws and compromised cases. It is a common dental practice to allow a minimum of 4-6 months for optimum osseointegration following dental implant placement. The limited available bone at the time of placement further delays time taken for osseointegration. The availability of novel biopolymers and biomimetic biomaterials, in the form of hydrophilic hydrogels, similar to what is used in this study, has set the optimal stage for early loading protocols even in compromised cases.

CONCLUSION

In conclusion, within its limitations, this study suggests that in a rabbit tibia model, at 6 weeks and 12 weeks follow up, implants coated with the novel Cissus hydrogel showed a significant difference in bone formation when compared to commercially pure titanium implants. Removal torque and ISQ values were found to be statistically significant in implants surface modified with coatings of Cissus hydrogels, thus proving our research hypothesis. Histologic evaluation was found to be in favour of surface coated implants. No necrotic, degenerative tissue was observed along with absence of fibrosis and inflammation surrounding the surface treated implants. Since the reduced density of alveolar bone is a common problem encountered during treatment planning of implants, the use of this novel hydrophilic hydrogel appears to be a favourable adjunct to implant placement. Implants surface coated with Cq hydrogels shortens time taken for osseointegration thereby having a positive effect on healing. This opens the door for further researches in this regard. Also in a broader sense these natural biopolymer coatings have a significant role to play in increasing regenerative potential of bone in compromised situations.

SUMMARY

The present study was conducted with the aim to evaluate and compare the osseointegration and osteogenesis of titanium dental implants coated with novel hydrogels. The Cell proliferation was studied by MTT Assay as Optical densities of different concentrations of the novel hydrogels which were then used to measure surviving cells mentioned as percentage. In the present study, a total of 24 commercially pure titanium implants, measuring 3.5mm in diameter and 6mm in length were placed in 12 male, New Zealand white rabbits, included in the study. These implants were divided into a test group i.e. Group A implants, coated with the novel hydrogel and 12 control Group B commercially pure titanium implants. The study was done with null hypothesis that there is no significant difference in bone regeneration surrounding implants coated with Cissus quandrangularis hydrogel, (CqH) and a research hypothesis that there is significant difference bone regeneration in response to Cissus quandrangularis hydrogel. (CqH). In the present study, ISQ of all the implants was performed using a Resonance Frequency Analyser at 6 weeks and 12 weeks respectively. At similar time intervals, animals were sacrificed and removal torque quotient was calculated. Histologic analysis of the retrieved en bloc samples was conducted to evaluate the osteoblast proliferation. Statistical Analysis was done by using following tests: independent t-test - to compare pair wise comparison of test and control groups at 6 and 12 weeks. Dependent t test – to compare pair wise comparison of control group at 6 and 12 weeks and test group at 6 and 12 weeks respectively. When intragroup and intergroup comparison was performed statistically significant result was observed with $p < 0.05$.

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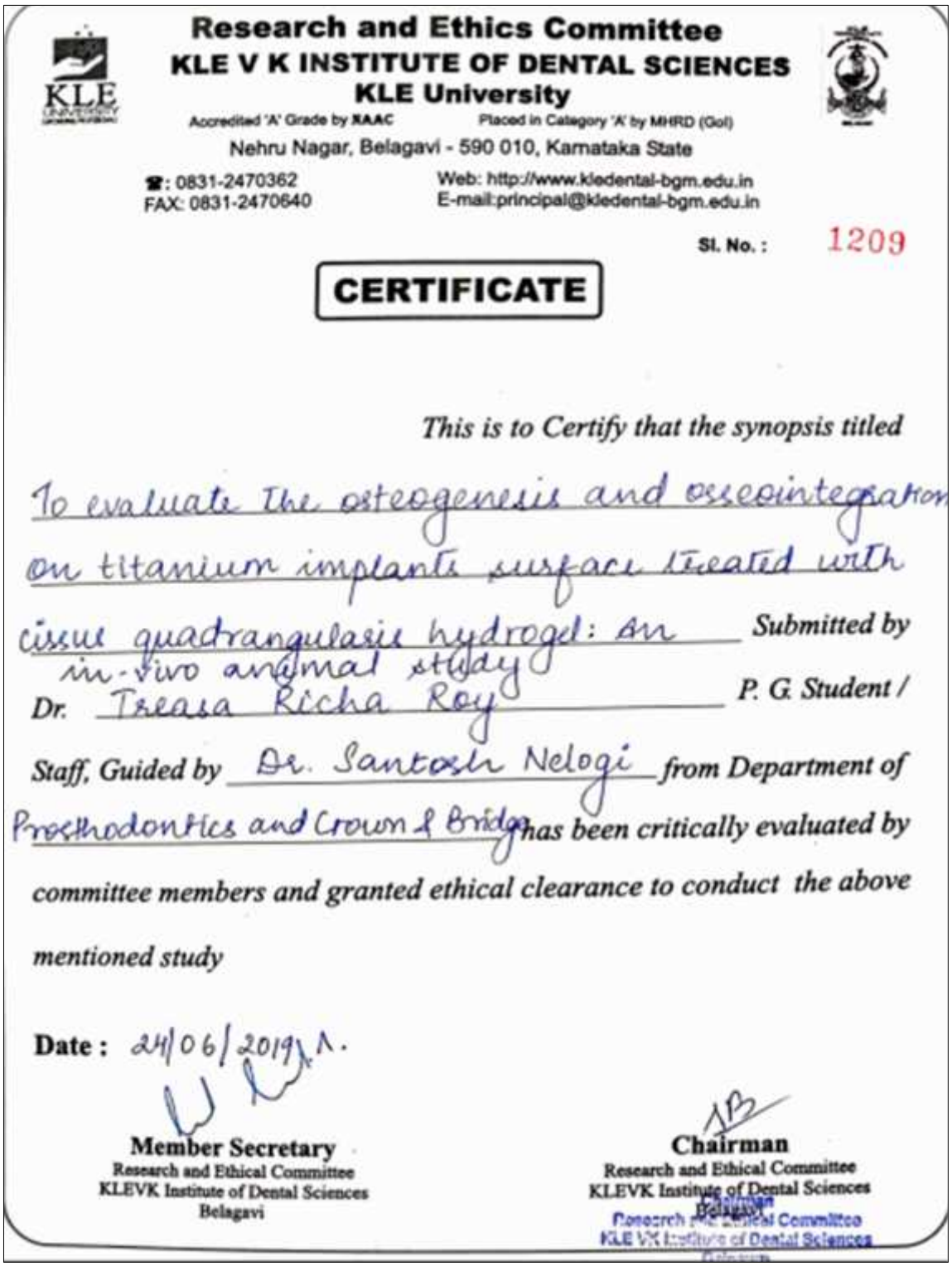
ANNEXURE – I – ETHICAL CLEARANCE LETTER



Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
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Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1209

CERTIFICATE

This is to Certify that the synopsis titled

To evaluate the osteogenesis and osseointegration on titanium implants surface treated with tissue quadrangulasis hydrogel: An in-vivo animal study

Submitted by

Dr. Treasa Richa Roy *P. G. Student /*

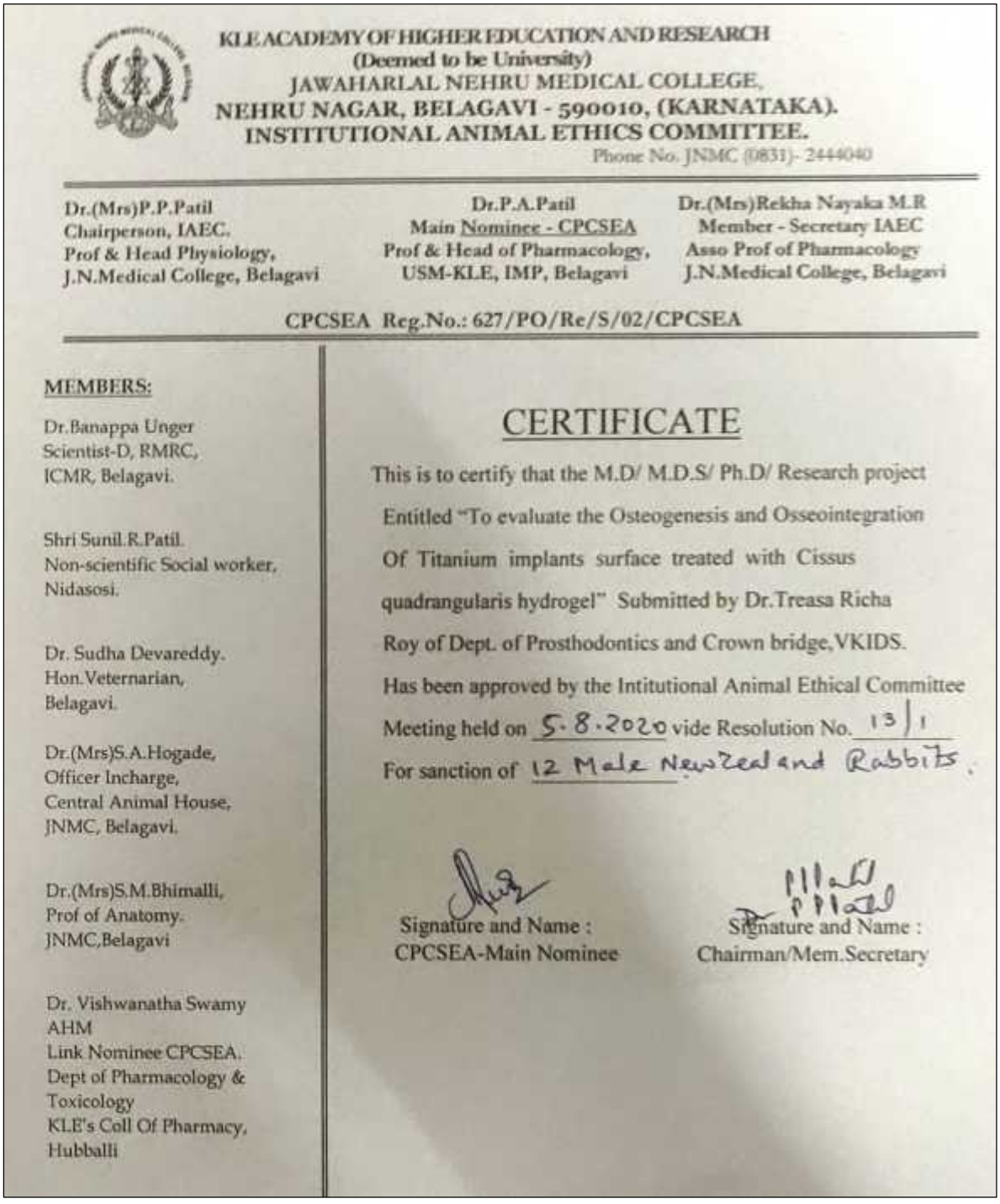
Staff, Guided by Dr. Santosh Nelogi *from Department of* Prosthodontics and Crown & Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE – II –ETHICAL CLEARANCE LETTER FROM INSTITUITIONAL ANIMAL ETHICS COMMITTEE



KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI - 590010, (KARNATAKA).
INSTITUTIONAL ANIMAL ETHICS COMMITTEE.
Phone No. JNMC (0831)- 2444040

Dr.(Mrs)P.P.Patil
Chairperson, IAEC.
Prof & Head Physiology,
J.N.Medical College, Belagavi

Dr.P.A.Patil
Main Nominee - CPCSEA
Prof & Head of Pharmacology,
USM-KLE, IMP, Belagavi

Dr.(Mrs)Rekha Nayaka M.R
Member - Secretary IAEC
Asso Prof of Pharmacology
J.N.Medical College, Belagavi

CPCSEA Reg.No.: 627/PO/Re/S/02/CPCSEA

MEMBERS:

Dr.Banappa Unger
Scientist-D, RMRC,
ICMR, Belagavi.

Shri Sunil.R.Patil.
Non-scientific Social worker,
Nidasosi.

Dr. Sudha Devareddy.
Hon.Veternarian,
Belagavi.

Dr.(Mrs)S.A.Hogade,
Officer Incharge,
Central Animal House,
JNMC, Belagavi.

Dr.(Mrs)S.M.Bhimalli,
Prof of Anatomy.
JNMC,Belagavi

Dr. Vishwanatha Swamy
AHM
Link Nominee CPCSEA.
Dept of Pharmacology &
Toxicology
KLE's Coll Of Pharmacy,
Hubballi

CERTIFICATE

This is to certify that the M.D/ M.D.S/ Ph.D/ Research project Entitled "To evaluate the Osteogenesis and Osseointegration Of Titanium implants surface treated with Cissus quadrangularis hydrogel" Submitted by Dr.Treasa Richa Roy of Dept. of Prosthodontics and Crown bridge,VKIDS. Has been approved by the Intitutional Animal Ethical Committee Meeting held on 5.8.2020 vide Resolution No. 13/1 For sanction of 12 Male New Zealand Rabbits.

Signature and Name :
CPCSEA-Main Nominee

Signature and Name :
Chairman/Mem.Secretary

ANNEXURE – III – CERTIFICATE OF ANALYSIS FOR CISSUS QUADRANGULARIS EXTRACT

KSHIPRA BIOTECH

KSHIPRA BIOTECH PVT. LTD.

(An ISO 9001:2015 | WHO – GMP | FSSAI | Organic Certified Company)

CIN. : U24100MP2015PTC033525 | GSTIN: 23AAFCK7461H1ZI | PAN: AAFCK7461H |

CERTIFICATE OF ANALYSIS

PRODUCT: CISSUS QUADRANGULARIS (CISSUS) D.E.
ACTIVES: TOTAL KETOSTERONES> 10%
PART USED: STEM
SOLVENTS USED: METHANOL, WATER

BATCH: KBAI-0919-009
DATE: SEPTEMBER' 2019
SHELF LIFE: 3 YRS
COUNTRY OF ORIGIN: INDIA

TEST	SPECIFICATIONS	RESULTS	PROTOCOL
PHYSICO-CHEMICAL			
DESCRIPTION	BROWINSH COLOUR POWDER WITH SLIGHTLY BITTER TASTE	COMPLIES WITH DESCRIPTION.	VISUAL/ ORGANOLEPTIC
IDENTIFICATION	BY TLC	POSITIVE	BY TLC
SOLUBILITY : WATER	MINIMUM 50%	COMPLIES	USP <561>
PH 1% WATER SOLUTION	5-7	7	USP <791>
BULK DENSITY(UNTAPPED) (TAPPED)	0.4-0.8 G/ML 0.5-0.9 G/ML	0.58 G/ML 0.74 G/ML	USP <616>
TOTAL ASH	MAXIMUM 20%	3.57%	USP <561>
LOSS ON DRYING	MAXIMUM 5%	3.93%	USP <731>
MESH SIZE	100% PASSES THROUGH #40	COMPLIES	USP <786>
ASSAY			
TOTAL KETOSTERONES	**MINIMUM 10%**	**11.98%**	**GRAVIMETRIC**
MICROBIAL PROFILE			
TOTAL PLATE COUNT	NMT 10000 CFU/G	COMPLIES	USP <61>
YEAST & MOULDS	NMT 100 CFU/G	COMPLIES	USP <61>
E-COLI	ABSENT	COMPLIES	USP <62>
SALMONELLA	ABSENT	COMPLIES	USP <62>
S.AUREUS	ABSENT	COMPLIES	USP <62>
PSEUDOMONAS	ABSENT	COMPLIES	USP <62>
HEAVY METALS PROFILE			
HEAVY METALS CONTENT	NOT MORE THAN 10 PPM	COMPLIES	BY ICPMS/ AAS

- STORAGE: STORE IN COOL, DRY PLACE IN CLOSED CONTAINERS, AWAY FROM DIRECT SUNLIGHT
- NO-IRRADIATED. NON-GMO

HERBAL EXTRACTS, BEING A NATURAL PRODUCT ARE SUBJECT TO MINOR VARIATIONS IN COLOUR, TEXTURE AND SMELL OVER A PERIOD OF TIME AND FROM BATCH TO BATCH.

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Mfg. Facility : Jamna Nagar, Maxi Road, Dewas 455001 (M.P) INDIA,
Email: info@kshiprabiotech.com, biotechkshipra@gmail.com , **Web :** www.kshiprabiotech.com

ANNEXURE – IV – CERTIFICATE FOR CALIBRATION OF CUSTOMISED REMOVAL TORQUE WRENCH

CRESCENT INDUSTRIES

TÜV SÜD

(An ISO 9001: 2008 Company)
Pioneer Vendor for Quality Assured Components and Measurements
108/2, SHINOLI (B.K.), TAL: CHANDGAD
DIST. KOLHAPUR – 416 507, INDIA
Tel: (Off): 0831- 2446598; Email: crescent.ind.bk@gmail.com

Report on Removal Torque procedure carried on 10 distinct Rabbits with a Calibrated Digital-Torque Wrench*(DM-3)*

❖ **Abstract –**

The aforementioned Study and procedure are hereby carried to explicitly understand the effect of applying the bio-medical coating on standard implants (Inserted into rabbits' leg (Femur and Tibia)), thereby to indirectly measure the extra-bone adhesion incurred by the application of the same which in turn affects the removal torque. So, by measuring the same we shall get the extent of adherence obtained. This is one of the invasive tangible method to accomplish the said study.

❖ **Procedure –**

A mini-customized digital torque wrench is used to perform the removal torque procedure, the specifications of the same are as follows.

Sl.no	Parameter	Specification	Range/Value
1.	Measuring Quantity	Force-applied	0.0009N – 9.80N
2.	Perpendicular length	From-axis of rotation	14.5 cm
3.	Display type	Digital, with stabilized Re-zeroing option	-
4.	Device material	-	Wrench body – PLA Wrench – SS
5.	Gross Weight	Device weight	Approx. 350 grams
6.	Input Voltage	Device I/P	3 Volts, DC
7.	O/P Tolerance	Force applied	±0.1 Grams.
8.	Angular variation	Gravity Effect	±2.0 Grams.
9.	Calibration Range	-	250-1000 Grams.
10.	Error	Related to load application point	(- ve) 50-Grams.

The Same as described with above specifications was used

** Note.*

- *Here the described range of calibration is performed using physical end to end calibration with standard deadweight testers.*
- *The Error range is measured for a 2 cm radius from point of load application to avoid any misinterpretation for unknown/unpredictable value addition while calculation.*
- *And the perpendicular length is measured by a calibrated vernier device – VERNIER "Aerospace CI/VC/6","01".*

For Crescent Industries

Proprietor

ANNEXURE – V

Resonance Frequency Analysis

Sl No	Group	ISQ		
		6 week	12 week	6-12 week
1	**Control Group**	60	67	7
2		65	69	4
3		89	93	4
4		64	73	9
5		54	63	9
6		44	53	9
7		55	60	5
8		60	64	4
9		61	67	6
10		50	57	7
11		55	64	9
12	**Test Group**	60	75	15
13		59	69	10
14		52	68	16
15		59	74	15
16		62	78	16
17		66	77	11
18		63	74	11
19		48	68	20
20		60	71	11
21		70	80	10
22		64	79	15

ANNEXURE – VI

Removal Torque Analysis

Sl No	Group	RTQ value		
		6 weeks	**12 weeks**	**6W-12W**
1	Control group	42.46	45.77	3.31
2		30.00	36.28	6.276
3		38.00	38.33	0.33
4		59.00	59.01	0.01
5		40.00	49.60	9.6
6	Test Group	44.60	55.83	11.23
7		30.05	77.26	47.21
8		49.60	95.01	45.41
9		65.00	72.25	7.25
10		60.00	79.44	19.44

ANNEXURE – VII

Radiovisiography Analysis

Sl No	Group	Isodensity value					
		Baseline	**6 weeks**	**12 weeks**	**BL-6W**	**BL-12W**	**6W-12W**
1	Control Group	157	160	172	3	15	12
2		46	56	66	10	20	10
3		56	65	75	9	19	10
4		68	84	89	16	21	5
5		80	88	101	8	21	13
6		166	170	188	4	22	18
7		54	58	58	4	4	0
8		61	71	82	10	21	11
9		74	81	96	7	22	15
10		86	89	101	3	15	12
11	Test Group	40	111	132	71	92	21
12		56	78	98	22	42	20
13		63	101	119	38	56	18
14		80	99	112	19	32	13
15		90	105	119	15	29	14
16		43	117	132	74	89	15
17		60	69	88	9	28	19
18		63	80	92	17	29	12
19		75	95	118	20	43	23
20		41	108	128	138	87	20