Evaluation of hydrophilic gel made from Acemannan and Moringa oleifera in enhancing osseointegration of dental implants. A preliminary study in rabbits

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ABSTRACT

Background: Hydrophilic implant surface has gained increasing interest as a factor to stimulate osseointegration. **Purpose:** The study was done to formulate hydrophilic gel to be applied on to the dental implant surface, to enhance bone to implant contact (BIC).

Materials and methods: In first part of study, Acemannan and Moringa oleifera hydro gel 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 formulated from Acemannan and Moringa oleifera were coated on the study groups of implants. Histomorphometric analysis was carried out of the *en bloc* sections specimens. Student's unpaired *t*-test was used to compare mean values between the two groups.

Results: 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. Titanium disc coated with hydrogel of Acemannan and Moringa oleifera and seeded with Human MSC shows increased proliferation of osteoblast cells. Compare to study group implants, control group showed no new bone formation.

Conclusions: 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.

1. Introduction

Dental implants have become an integral part of dentistry and made it possible to rehabilitate edentulous arches with proper function and esthetics. Surface roughness properties is one of the factor in enhancing osseointegration.^{1–3} Researcher have been working on surface modifications since 3 decades to enhance osseointegration.^{4–7} Similarly, primary stability during implant placement, biological host bone and direct Bone-To-Implant contact (BIC) is very important for the survival and success of implants.^{8–10} BIC was studied extensively to find the amount of bone apposition adjacent to the implant surface through the histomorphometry at the light microscopic level. Studies had shown that BIC can be enhanced by implant surface characteristics such as chemistry, roughness, surface topography and energy.^{11–15}

It is well documented in literature that implants were coated with localized organic and inorganic osteogenic coatings on their surfaces to

improve implant surface activity and osteopromotive activity.¹⁶ Research had been done since long to improve the implant surface by making it hydrophilic, to further stimulate osseointegration.^{17–20} During initial stages of wound healing hydrophilic implant surface may facilitates bone integration and promote osseointegration.²¹ Positively charged implant surface makes the surface hydrophilic and thus enhances the adsorption of plasma proteins and initiates the osteogenic interactions.²² Hydro complex gels can be incorporated in the gel, which due to its hydrophilic nature provides a sustained release of bone proliferating medicine.²³

Since long, herbal extracts obtained from different plants have been used in bone healing process. Acemannan is an herbal extract obtained from Aloe vera gel. It is a biodegradable polysaccharide, consists of betaacetylated polymannose. Several researchers had showed that Acemannan can cause bone marrow-stromal cell proliferation and differentiation. They can also stimulate gingival fibroblast, dental pulp

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fibroblasts and cementoblasts.^{24–26} *Moringa oleifera* is the most widely cultivated species in the genus *Moringa*. Its seed is used as a natural coagulant. *Moringa oleifera* has anti-diabetic and anti-cancer properties.²⁷

The aim of the present study was to formulate a hydrophilic gel to be used along with the dental implant placement, so as to create a hydrophilic surface for the dental implant, to enhance implant to blood contact and also bone to implant contact.

2. Materials and methods

Institutional Animal Ethics Committee (IAEC) of the institution with reference no-RRDC&H/331/2014–2015 had approved the animal trial and was in compliance with the appropriate EQUATOR guidelines.²⁸ The study was conducted in two parts:

2.1. Part 1-basic study to find the biocompatibility of the material

Biocompatibility of the materials was evaluated by analyzing the viability of the cells and their differentiation to evaluate the long term effect of hydrophilic gel on osteoblast cells. Formulation of hydrogel was done in different proportions with close simulation of in vivo conditions.

2.2. Cytotoxicity of *Acemannan* and *Moringa oleifera* extracts against UMR106 cells

2.2.1. Cell culture

Evaluation of cytotoxicity with *Acemannan* and *Moringa oleifera* extracts had been done using UMR106 cells, as these cells were suitable for study related to osteoblast physiology and bone formation. Hydrogel was prepared according to the method given by Mizielińska et al.²⁹ The *Moringa oleifera* seeds and *Acemannan* leaves were dried and grounded in to powder separately. The powders were extracted with water and kept separately from the shaker for 2 h at 70 °C. The Aqueous extracts of *Acemannan* and *Moringa oleifera*, were filtered through a 0.2 µm filter and was stored at 2–8 °C for use. In this study, synthetic hydrogel was prepared using polyethylene glycol (PEG), a synthetic material that is nontoxic and inert and suitable for use in medical purposes. First, aqueous PEG solution was prepared, and then aqueous plant extract was dissolved in it and mixed well to obtain homogeneous solution. The hydrophilic gel was made thixotropic in nature so that it remains stable when applied on the specimen. Thixotropic behaviour of the gel was evaluated with the vial inversion method. The hydrogel placed in the vial was shaken and mechanically collapsed using a vortex genie (Scientific Industries, Inc) for several seconds. The gel converted to sol was again allowed to set at room temperature for its recovery back to gel state, which was determined by inversion of the vial by visual observation.

Grade 4 (ASTM F67) commercially pure titanium disks of diameter 5 mm and thickness 2 mm were used (Fig. 1) in the study. Disks were subjected to acid treatment for the removal of the oxide layers. Hydrophilic gels made of *Acemannan* and *Moringa oleifera* in three different ratio (1:1, 1:2; 2:1) were coated on the titanium disk using a spreader which was attached to the syringe containing hydrogel and this helps in even spread of the hydrogel on the complete implant surface, this was followed by immersion of disks in gel for around 45–60 s, for uniform coating and controlled release of gel. After 30 min coated disks were placed in each well of 96 well plates. Each well were seeded with 50,000 cells/well, 70–80% trypsinize confluent UMR106 rat osteosarcoma-derived cells and incubated at 37 °C in 5% CO₂ incubator for 24 h. Fresh media was placed and the incubation was repeated for next 24 h for cells to proliferate. Media was removed after 24 h from the well plates and 100 µl/well MTT (1 mg/2 ml of MTT in 1X PBS) working solution was added and incubated for 4 h. Later the media was removed from the wells, and 100 µl dimethyl sulfoxide

Fig. 1. Titanium disks 5 mm in diameter and 2 mm in width used in the study.

(DMSO) was added to rapidly solubilize the formazan. The absorbance was measured at 590 nm.

$$\% \text{ of Inhibition} = 100 - (\text{Sample/Control}) \times 100$$

2.2.2. Cell viability

Gel applied and non gel applied disks were seeded in 12 well plates, with 10,000 cells/cm² and *Kaighn's modification of Ham's F-12 medium* (F12K), with 20% fetal calf serum (FCS) and 100 µg/g streptomycin. Every week the cell medium was changed, and at different time interval 9 well/specimen was tested. MTT reagent was added to the cell medium after 1 day, 1 week, 2 weeks, and 3 weeks. Cells were incubated in dark for 4 h at 37 °C and cell medium was discarded followed by the lyses of cells in 0.004 N HCl in isopropanol. Cell lysates were centrifuged and supernatants obtained were transferred as triplets to a 96-well plate. The adsorption analysis was done at 570 nm and 630 nm with Synergy HT micro plate reader (BioTek, Bad Friedrichshall, Germany). MTT assay was also performed for titanium disks not seeded with cell culture, to rule out the material's effect on the test and to analyze the cells reactivity during the assay. The morphology of the cell was studied by a light microscope (Leica Microsystems GmbH, Wetzlar, Germany, Type 090–135.002) equipped with Ds-Fi1 digital camera (Nikon, Duesseldorf, Germany).³⁰

2.2.3. Alkaline phosphatase (ALP) content

Changes in the differentiation behavior of the bone-forming cells was indicated by Senso Lyte pNPP alkaline phosphatase assay (Ana Spec, Fremont, CA). Osteogenic differentiation was induced by cell culture in Dulbecco's modified Eagle's medium (DMEM), 10% FCS, low glucose with l-glutamine, 100 µg/g streptomycin, 100 U/ml penicillin, 0.005 µM ascorbic acid, 10 mM β-glycerolphosphate and 0.1 µM dexamethazone. After 1 day, 1 week, 2 weeks, and 3 weeks of culture Senso Lyte pNPP alkaline phosphatase assay was applied. 9 wells/specimen in total at different time interval was tested. Cell medium was changed every week during the experiment.

The cells were washed and frozen at –80 °C. PicoGreen dsDNA quantitation assay (In vitrogen, Eugene, OR) was done to measure the number of thawed frozen cells. The lyses of cells were done with 1% Triton X-100 in phosphate-buffered saline and cell lysates were centrifuged. The supernatants were mixed with the PicoGreen® working solution in 96-well plate. The sample was excited at 485 nm, and at 528 nm the measurement of fluorescence emission intensity was done. Buffer dilution of the supernatants was done in specific assay and was coated with ALP. Absorbance was measured at 405 nm. The correlation of absolute amounts of ALP was done with the number of cell obtained

from the PicoGreen assay. ALP and PicoGreen assays were also done for the disks not seeded with the cell culture, so as to exclude materials effect on the test and to find the cells reactivity behaviour during the assay.³¹

2.2.4. Transmission electron microscopy (TEM)

Titanium disks were incubated for 1 week and 3 weeks with Human mesenchymal stem cells (Human MSC) seeded in chamber slides (Nalge Nunc International, Rochester, NY). 2% glutaraldehyde, 0.02% picric acid and 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2–7.4) was used to fix the cells for 30 min, followed by 20 min fixation with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2–7.4). Samples were dehydrated and embedded in Epon. 80–100 nm ultrathin sections were done with collodion-coated copper grids. Leo 912 TEM (Carl Zeiss AG, Oberkochen, Germany) equipped with a TRS Sharpeye slow scan dual speed CCD camera (Albert Troendle Prototypentwicklung, Moorenweis, Germany) was used at accelerating voltage of 80 kV for analyses.

2.3. Part 2-in vivo animal study

2.3.1. Animals and anesthesia

Eight healthy lop-eared male New Zealand rabbits weighing 3–5 kg were used in the study. Rabbits were fed with standard pelleted food and water, and kept in animal facility of the institution. All the rabbits were kept under the required humidity and temperature and were monitored in a standard laboratory environment with a 12 h light and dark cycle. In-house veterinary surgeon regularly recorded and maintains the vital reading. Intramuscular injection of 0.15 ml/kg Xylazine hydrochloride (Xylaxin, Indian Immunologicals, Hyderabad, India) and 0.35 ml/kg Ketamine hydrochloride (Ketalar, Pfizer, Mumbai, India) was given as general anesthesia.

2.3.2. Surgical procedure

Hind legs were shaved and disinfected with 70% ethanol and 70% chlorhexidine before surgery. Careful opening of periosteal flap was done and standardized drilling protocol was followed to prepare the implant sites. Four implants were placed per rabbit using a randomization program for their distribution (www.randomization.com) with a 16 implants used as control and the other 16 as test implants. The surgical assignment was done by a person not involved in the study using sealed envelopes. The assignment was informed to the surgeon immediately before implant placement. The histological analysis was done by a different person who was not involved in any other section of the study. The sample size of the present study was calculated based on the results of a similar study and also based on statistical sample strength.

The study groups of implants were coated with hydrogel in the same manner as explained for the coating of titanium disks (Fig. 2A). The control groups of implants were not dipped in the gel. Pure machined surfaced prototype titanium implants (3 mm in diameter, 8 mm in length) were placed one each in tibia and femur of each rabbit (Fig. 2B). Flaps were closed with resorbable sutures (Petricryl 910, Dolphin sutures, MT Promed Consulting GmbH, Germany). Injection buprenorphine hydrochloride (Buprinor, Astra Zeneca Pharma, Bengaluru, India) 0.03 mg/kg was given subcutaneously as analgesic and doxycycline (Vibramycin, Pfizer, Mumbai, India) 3.2 mg/kg was given orally as antibiotic for 3 days. An overdose of sodium pentobarbitone (60 mg/ml) was given after 4 weeks to sacrifice the rabbits and required tissues were retrieved *enbloc* for further investigation.

2.3.3. Histomorphometric analyses

10% formalin was used for 10 days to fix the *enbloc* implants and the surrounding bone tissues. Dehydration of the specimens were done for 4 days in a graded series with ethanol in 70%, 80%, 90% and 100% and were embedded in methyl methacrylate (MMA) resin (Lucitone,

Fig. 2. A) Prototype implant coated with hydrophilic gel. B) Prototype implants placed in tibia and femur bone of rabbits.

Dentsply, West Philadelphia Street York, Pennsylvania). 0.14 mm sections of resin specimens were cut with the saw microtome (Accutome100, Struers, Denmark) at a speed of 0.1 mm/s with 1000 rpm. Cut sections were mounted on slide and grinding was done to a thickness of 0.08 mm. Cut sections were stained with Van Gieson's picrofuchsin and hot Stevenel's Blue and (Leica autostainer XL, ST5010, Nussloch, GmbH, Germany). The specimens were evaluated in a trinocular transmitted light microscope (Nikon E600, Germany) and photomicrographs were captured to the camera (Nikon DS Ri1) adhered to the microscope.

Bone implants contact (BIC) was measured along the total length of the implant, starting from first coronal microthread up to the apex of the implant. Bone volume (BV) was analyzed in a rectangular region of interest at the flat part of the implant.

2.3.4. Statistical analysis

SPSS (statistical package for social sciences, V.22, IBM, Corp.) software was used for statistical analysis. Mean and standard deviation (SD) were done to find the frequency distribution for continuous variables. Student unpaired *t*-test was used to compare mean values between the two groups. $P < .05$ was set as the level of significance.

3. Results

3.1. Cytotoxicity test

Cytotoxicity of aqueous extracts of plants of *Acemannan* and *Moringa oleifera* done in UMR106 cell lines were presented in Table 1. *Acemannan* showed less than 10% inhibition at 10 µg/ml, so used in formulation up to 10 µg/ml in the present study. Tested aqueous extracts of *Moringa oleifera* did not elicit toxicity up to 80 µg/ml and exhibited significant stimulatory activity up to 40 µg/ml.

3.2. MTT assay

The viability of the cells were tested with MTT assay and it was found that maximum cell inhibition of 20.08% was found for

Table 1
Cytotoxicity of Acemannan and Moringa oleifera using UMR 106 cells.

Plant Name	Concentration (µg/ml)	Absorbance 590 nm	% Inhibition	IC ₅₀
Control	0.0	0.347	0.00	NA
Acemannan (Aloe vera)	1.5	0.341	1.64	
	3.1	0.334	3.66	
	6.3	0.329	5.11	
	12.5	0.312	10.01	
	25.0	0.295	14.91	
	50.0	0.243	29.91	
Control	0.0	0.628	0.00	NA
Moringa oleifera	10.0	0.712	0.00	
	20.0	0.666	0.00	
	40.0	0.664	0.00	
	80.0	0.648	0.00	
	160.0	0.626	0.43	
	320.0	0.610	2.93	

IC₅₀:Half maximal inhibitory concentration.

Table 2
Formulations of Acemannan with Moringa oleifera evaluated for percentage cell inhibition by MTT assay and osteoblastic differentiation by ALP assay.

Formulations	Conc. 100 µg/ml	OD at 540 nm	% Inhibition	IC ₅₀
Acemannan: Moleifera oleifera	Control	0.344	0.00	NA
	1:1	0.275	20.08	NA
	2:1	0.328	4.45	
	1:2	0.297	13.53	

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ALP: Alkaline phosphatase; OD: Osteoblastic differentiation; IC₅₀:Half maximal inhibitory concentration.

Acemannan and Moringa oleifera in a ratio of 1:1 and at least cell inhibition of 4.45% was found for Acemannan and Moringa oleifera in a ratio of 2:1 (Table 2).

3.3. Alkaline phosphatase assay

The ALP assay showed the OD as 0.275 at 540 nm at which maximum cell inhibition of 20.08% was found for Acemannan and Moringa oleifera in a ratio of 1:1. The alkaline phosphatase assay showed the OD as 0.328 at 540 nm (Table 2) at which least cell inhibition of 4.45% was

found for Acemannan and Moringa oleifera in a ratio of 2:1 and the same formulation was used in the present study.

3.4. Transmission electron microscopy

Under TEM increased proliferation of osteoblast cells were seen on titanium disc coated with hydrogel of Acemannan and Moringa oleifera and seeded with Human MSC.

3.5. Histomorphometric analyses

Study group prototype implant showed new bone formation at the hydrophilic implant surface with increased BIC (Fig. 3). BIC and BV were measured by image J analysis software. Histomorphometric analyses showed absence of degenerative changes, necrotic changes, fibrosis, and inflammation at the new BIC (Fig. 4A and B). New woven bone formation arising from the periosteum was observed around the implant and filling the thread gap. Osteoblast proliferation was noticed at the edges of new bone. Compare to study group implants, control group implants showed no new bone formation (Fig. 4C). The statistical significant values found in the new bone formation of the tibia, indicate that the content of hydrogel helped in newer bone formation by increasing osteoblastic activity (Fig. 5) (Table 3). There was no statistical significant difference between study and control group implant for BIC and total bone volume (TBV) in tibia and femur sites (Table 4, Table 5). The mean value for BIC and TBV showed better values for gel coated implants place in tibia of rabbits.

4. Discussion

Osseointegration is defined as a time dependent healing process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading.³¹ Surface features of implants, such as topography, coatings and wettability mediate the host osteoblasts and contribute in bone formation during osseointegration. 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.^{10,15,21,32,33} Surface wettability or hydrophilicity of implants is one of the important aspect of osseointegration and is expressed by the water contact angle ranging from 0° for a hydrophilic surfaces to more than 90° on hydrophobic surfaces.³⁴ Hydrophilic surface initiates

Fig. 3. Graph presenting mean, standard deviation and P value for bone to implant contact of implants placed with gel and without gel applications.

Fig. 4. A) Histological analyses of study group showing new bone formation. New woven bone arising from the periosteum was observed around the implant and filling the thread gap. B) Histological analyses of study group showing absence of degenerative changes, necrotic changes, fibrosis, and inflammation at the new bone to implant contact. C) Micrograph of control group at 10× magnification showing with no relevant new bone formation.

the initial events of osseointegration by promoting the adhesion of monocytes, platelet activation, and blood clot formation.^{35,36} Hydrophilic surfaces exhibit anti-inflammatory properties and plays a key role in the osteogenic differentiation of mesenchymal stem cells.^{37,38}

Long-term effect of hydrophilic gel on different cell lines was evaluated in this study and in vivo conditions were simulated as closely as possible. Cell viability and differentiation were studied to evaluate the biocompatibility of the tested samples. Acemannan controls inflammation from bacteria contamination around dental implants.³⁹ Boonyagul et al.,²⁵ in their study found that Acemannan polysaccharide have negligible cytotoxicity against bone marrow stromal cells (BMSCs)

Fig. 5. Marking of bone to implant contact using image J software.

Table 3

Comparison of mean values of new bone volume in tibia and femur bone site between the two implant groups using student unpaired *t*-test.

Group	Sample	Mean	Standard deviation	<i>P</i> value
Tibia	With gel	33.36	4.88	0.05*
	Without gel	27.29	5.56	
Femur	With gel	26.5	5.86	0.3
	Without gel	30.37	7.47	

*Statistically significant ($P < .05$).

Table 4

Comparison of mean values of bone to implant contact (BIC) in tibia and femur bone site between the two implant groups using student unpaired *t*-test.

Group	Sample	Mean	Standard deviation	<i>P</i> value
Tibia	With gel	39.86	6.07	0.2
	Without gel	35.17	6.78	
Femur	With gel	46.31	14.57	0.14
	Without gel	35.97	8.86	

Table 5

Comparison of mean values of total bone volume (TBV) in tibia and femur bone site between the two implant groups using student unpaired *t*-test.

Group	Sample	Mean	Standard deviation	<i>P</i> value
Tibia	With gel	39.26	5.94	0.11
	Without gel	33.2	7.1	
Femur	With gel	32.89	7.22	0.42
	Without gel	35.95	6.37	

proliferation and found sound cytocompatibility. Acemannan treated groups in their study had higher bone mineral density and faster bone healing. Acemannan acts as a bioactive molecule and induces bone formation by stimulating BMSCs proliferation, osteoblasts differentiation, and synthesis of extracellular matrix.

The histomorphometric analysis in the present study was done to evaluate BV and BIC and showed that implants with gel showed favorable results, though there was no statistical significance difference for BIC, but the mean values of implant with gel was higher and similar to animal study by Vasak et al.,⁴⁰ where in hydrophilic implants caused tendency towards greater BIC. Buser et al.,²¹ also in their mini pig model showed that hydrophilic implants increased BIC after 2–4 weeks of healing when compared with non hydrophilic implants. Additionally

study in dog by Schwarz et al.,⁴¹ had reported with advantageous results for hydrophilic surface.

The significant values seen in the newer bone formation of the tibia indicate that the content of hydrogel helped in newer bone formation by increasing osteoblastic activity, as mentioned in a study Das et al.,⁴² that *Moringa oleifera* play a vital role in stimulating osteoblastic cells, similarly Patel et al.,⁴³ also showed osteoblastic stimulatory potential properties from *Moringa oleifera*. *Moringa oleifera* contains the large amount of flavonoids (kaempferol and quercetin). Flavonoids inhibits cyclooxygenase enzyme and also inhibit the release of histamine and thus possess anti-inflammatory property. Quercetin inhibits differentiation and activation as well induces apoptosis of osteoclast cells and can reduce the number of osteoclasts and increase the number of osteoblast cells.^{44–46}

Thus our study data demonstrates that hydrophilic implant surface not only increase BIC but also stimulate new bone formation. The clinical relevance of the present study findings must be interpreted with care, histomorphometric analysis provide surrogate parameter that cannot be directly translated in clinical survival rates, although hydrophilic surface has always shown tendency towards better implant stability. Addition of bone proliferating contents to these hydrogels would further shorten the time for osseointegration and also improve the quality of bone. Future studies can be carried out to find the effect of hydrogel coated implants in compromised patient's such as diabetes Mellitus, post radiation therapy, osteoporosis etc and also other clinical conditions with implant placement with guided bone regeneration and immediate implant cases.

5. Conclusion

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. Although addition of bone proliferating contents to these hydrogels would shorten the time for osseointegration and also improve the quality of bone, but the clinical relevance of the present study findings must be interpreted with care till further more new research can confirm it.

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Declaration of competing interest

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References

- Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. A long-term follow-up study of osseointegrated implants in treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants*. 2014;25:1378–1385.
- Asak C, Busenlechner D, Schwarze UY, et al. Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clin Oral Implants Res*. 2014;25:1378–1385.
- Wennerberg A, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. *Int J Oral Maxillofac Implants*. 2010;74:63–74.
- Chowdhary R, Jimbo R, Thomsen CS, Carlsson L, Wennerberg A. The osseointegration stimulatory effect of macrogeometry-modified implants: a study in the rabbit. *Clin Oral Implants Res*. 2014;25:1051–1055.
- Schulz MC, Korn P, Stadlinger B, et al. Coating with artificial matrices from collagen and sulfated hyaluronan influences the osseointegration of dental implants. *J Mater Sci Mater Med*. 2014;25:247–258.
- Chowdhary R, Halldin A, Jimbo R, Wennerberg A. Influence of micro threads alteration on osseointegration and primary stability of implants: an FEA and in vivo analysis in rabbits. *Clin Implant Dent Relat Res*. 2015;17:562–569.
- Kumararama SS, Mishra S, Chowdhary R. Evaluation of bone stimulation by different designs of microthreaded implants in enhancing osseointegration: an in vivo animal study supported by a numerical analysis. *Clin Implant Dent Relat Res*. 2018;20:215–221.
- Javed F, Romanos GE. The role of primary stability for successful immediate loading of dental implants. A literature review. *J Dent*. 2010;38:612–620.
- Javed F, Almas K, Crespi R, Romanos GE. Implant surface morphology and primary stability: is there a connection? *Implant Dent*. 2011;20:40–46.
- Gaviria L, Salcido JP, Guda T, Ong JL. Current trends in dental implants. *J Korean Assoc Oral Maxillofac Surg*. 2014;40:50–60.
- Trisi P, Lazzara R, Rao W, Rebaudi A. Bone-implant contact and bone quality: evaluation of expected and actual bone contact on machined and osseotite implant surfaces. *Int J Periodontics Restor Dent*. 2002;22:535–545.
- Novaes Jr AB, Souza SL, de Oliveira PT, Souza AM. Histomorphometric analysis of the bone-implant contact obtained with 4 different implant surface treatments placed side by side in the dog mandible. *Int J Oral Maxillofac Implants*. 2002;17:377–383.
- Froum SJ, Simon H, Cho SC, Elian N, Rohrer MD, Tarnow DP. Histologic evaluation of bone-implant contact of immediately loaded transitional implants after 6 to 27 months. *Int J Oral Maxillofac Implants*. 2005;20:54–60.
- Degidi M, Perrotti V, Piattelli A, Iezzi G. Mineralized bone-implant contact and implant stability quotient in 16 human implants retrieved after early healing periods: a histologic and histomorphometric evaluation. *Int J Oral Maxillofac Implants*. 2010;25:45–48.
- Novaes Jr AB, Souza SL, Barros RR, Pereira KK, Iezzi G, Piattelli A. Influence of implant surfaces on osseointegration. *Braz Dent J*. 2010;21:471–481.
- de Jonge L, Leeuwenburgh SC, Wolke JG, Jansen JA. Organic-inorganic surface modifications for titanium implant surfaces. *Pharm Res (N Y)*. 2008;25:2357–2369.
- Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res*. 2011;22:349–356.
- Kopf BS, Ruch S, Berner S, Spencer ND, Maniura-Weber K. The role of nanostructures and hydrophilicity in osseointegration: in-vitro protein-adsorption and blood-interaction studies. *J Biomed Res*. 2015;103:2661–2672.
- Sartoretto SC, Alves ATN, Resende RFB, Calasans-Maia J, Granjeiro JM, Calasans-Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. *J Appl Oral Sci*. 2015;23:279–287.
- Rupp F, Scheideler L, Eichler M, Geis-Gerstorf J. Wetting behavior of dental implants. *Int J Oral Maxillofac Implants*. 2011;26:1256–1266.
- Buser D, Brogini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res*. 2004;83:529–533.
- Jimbo R, Ivarsson M, Koskela A, Sul YT, Johansson CA. Protein adsorption to surface chemistry and crystal structure modification of titanium surfaces. *J Oral Maxillofac Res*. 2010;1:e3.
- Novaes Jr AB, Souza SL, Barros RR, Pereira KK, Iezzi G, Piattelli A. Influence of implant surfaces on osseointegration. *Braz Dent J*. 2010;21:471–481.
- Jittapiromsak N, Sahawat D, Banlunara W, Sangvanich P, Thunyakitpisal P. Acemannan, an extracted product from Aloe vera, stimulates dental pulp cell proliferation, differentiation, mineralization, and dentin formation. *Tissue Eng*. 2010;16:1997–2006.
- Boonyagul S, Banlunara W, Sangvanich P. Effect of acemannan an extracted polysaccharide from Aloe vera on BMSCs proliferation, differentiation, extracellular matrix synthesis, mineralization, and bone formation in a tooth extraction model. *Odontology*. 2014;102:310–317.
- Chantarawaratit P, Sangvanich P, Banlunara W, Soontornvipart K, Thunyakitpisal P. Acemannan sponges stimulate alveolar bone, cementum and periodontal ligament regeneration in a canine class II furcation defect model. *J Periodontol Res*. 2014;49:164–178.
- Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: a review on nutritive importance and its medicinal application. *Food Sci Hum Wellness*. 2016;5:49–56.
- EQUATOR network identification of reporting guidelines (Search strategy for MEDLINE database). www.equator-network.org/resource-centre/.
- Mizielinska M, Salachna P, Ordon M, Łopusiewicz L. Antimicrobial activity of water and acetone extracts of some Eucomis taxa. *Asian Pac J Trop Med*. 2017;10:892–895.
- Charyeva O, Dakischew O, Sommer U, Heiss C, Schnettler R, Lips KS. Biocompatibility of magnesium implants in primary human reaming debris-derived cells stem cells in vitro. *J Orthop Traumatol*. 2016;17:63–73.
- Zarb GA, Albrektsson T. Osseointegration: a requiem for periodontal ligament? *Int J Periodontal Restor Dent*. 1991;11:88–91.
- Junker R, Dimakis A, Thoneick M, Jansen JA. Effects of implant surface coatings and composition on bone integration: a systematic review. *Clin Oral Implants Res*. 2009;20(suppl 4):185–206.
- Dohan Ehrenfest DM, Coelho PG, Kang BS, Sul YT, Albrektsson T. Classification of osseointegrated implant surfaces: materials, chemistry and topography. *Trends Biotechnol*. 2010;28:198–206.
- Terheyden H, Lang NP, Bierbaum S, Stadlinger B. Osseointegration-communication of cells. *Clin Oral Implants Res*. 2012;23:1127–1135.
- Hong J, Kurt S, Thor A. A hydrophilic dental implant surface exhibit thrombogenic properties in vitro. *Clin Implant Dent Relat Res*. 2013;15:105–112.
- Milleret V, Tugulu S, Schlottig F, Hall H. Alkali treatment of microrough titanium surfaces affects macrophage/monocyte adhesion, platelet activation and architecture

- of blood clot formation. *Eur Cell Mater.* 2011;21:430–444.
37. Wall I, Donos N, Carlqvist K, Jones F, Brett P. Modified titanium surfaces promote accelerated osteogenic differentiation of mesenchymal stromal cells in vitro. *Bone.* 2009;45:17–26.
 38. Hamlet S, Alfarsi M, George R, Ivanovski S. The effect of hydrophilic titanium surface modification on macrophage inflammatory cytokine gene expression. *Clin Oral Implants Res.* 2012;23:584–590.
 39. Mangaiyarkarasi SP, Manigandan T, Elumalai M, Cholan PK, Kaur RP. Benefits of Aloe vera in dentistry. *J Pharm BioAllied Sci.* 2015;7(Suppl 1):S255–S259.
 40. Vasak C, Busenlechner D, Schwarze UY, et al. Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clin Oral Implants Res.* 2014;25:1378–1385.
 41. Schwarz F, Herten M, Sager M, Wieland M, Dard M, Becker J. Histological and immunohistochemical analysis of initial and early osseous integration at chemically modified and conventional SLA® titanium implants: preliminary results of a pilot study in dogs. *Clin Oral Implants Res.* 2007;18:481–488.
 42. Das S, Kanodia L. Effect of ethanolic extract of leaves of *Moringa oleifera* lam. On acetic acid induced colitis in albino rats. *Asian J Pharmaceut Clin Res.* 2012;5:110–114.
 43. Patel C, Rangrez A, Parikh P. The anti osteoporotic effect of *Moringa oleifera* on osteoblastic cells: SaOS2. *IOSR J Pharm Biol Sci.* 2013;5:10–17.
 44. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. *Food Chem Toxicol.* 2011;49:1270–1275.
 45. Wattel A, Kamel S, Mentaverri R, et al. Potent inhibitory effect of naturally occurring flavonoids quercetin and kaempferol on in vitro osteoclastic bone resorption. *Biochem Pharmacol.* 2003;65:35–42.
 46. Woo JT, Nakagawa H, Notoya M, et al. Quercetin suppresses bone resorption by inhibiting the differentiation and activation of osteoclast. *Biol Pharm Bull.* 2004;27:504–509.
- Praneeth Raj Pachimalla** had worked for the concept of the work and acquisition of the data required. He had drafted the paper and responsible for the final approval of the version of the article to be published. He agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any parts of the work are appropriately investigated and resolved.
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