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Antimicrobial Efficacy of Chitosan Nanoparticle Gel at the Implant Abutment Interface

Invitro Study

Type of manuscript : original research

Running Title: chitosan nanoparticle mediated gel to prevent bacterial accumulation in implant abutment interface - An Invitro Study

M. Sai Teja Reddy

Saveetha Dental College And Hospitals
Saveetha Institute Of Medical And Technical Sciences
Saveetha University
Chennai-600077,Tamilnadu,India
Email.id: msaitejareddy090@gmail.com

Subhabrata Maiti

Assistant professor
Department Of Prosthodontics And Implantology
Saveetha Dental College And Hospitals
Saveetha Institute Of Medical And Technical Sciences
Saveetha University
Chennai-600077,Tamilnadu,India
Email.id: drsubhoprostho@gmail.com

S Rajesh Kumar

Associate Professor
Nano Biomedicine Lab
Department of Pharmacology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Saveetha University
Chennai- 600077
Tamil Nadu, India

Dhanraj Ganapathy

Professor and Head of Department,
Department of Prosthodontics,
Saveetha Dental college and hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai-600077,TamilNadu ,India.
Contact:9841504523
Email id- dhanraj@saveetha.com

Corresponding Address:

Subhabrata Maiti

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Assistant professor
Department Of Prosthodontics And Implantology
Saveetha Dental College And Hospitals
Saveetha Institute Of Medical And Technical Sciences
Saveetha University
Chennai-600077,Tamilnadu,India
Email.id: drsubhoprostho@gmail.com
Phone Number: 9007862704

ABSTRACT -

Purpose: To develop a chitosan based thermosensitive hydrogel for sealing and lubricating purposes in dental implant systems.

Materials and Methods: To check for the bacterial leakage at the IAI, a stained implant is viewed under stereoelectronic microscope. Chitosan nanoparticle gel is prepared and placed around the implant abutment interface.The implants were placed in the Agar medium inoculated with streptococcus mutans and pseudomonas, incubated in an incubator for 120 hours at 37°C. For Anti-microbial analysis.

Results - There was no significant differences between two implant designs ($P > 0.05$), but showing activity of 100% better than the control group (control showed no zone of inhibitions against bacteria).

Conclusions: Based on our findings, it could be concluded that the thermosensitive and antimicrobial hydrogel with sealing and lubricating ability was successfully prepared and it has shown anti-microbial efficiency against both Streptococcus mutans and Pseudomonas.

KEYWORDS

abutment, implant, sealant, thermosensitive gel, chitosan gel

INTRODUCTION -

Tooth loss in adults and elderly individuals continues to be an oral health hazard that has negative impacts on quality of life and interferes with work activities [1]. In the past, several studies reported a strong association with mortality and tooth loss in elderly population[2], [3,4] and it is the ultimate endpoint for both pulpal and periodontal diseases [5],[6],[7]. It leads to: loss of masticatory function[8],[9] restricting specific food intake such as vegetables and fibers[10]; significant phonetic changes; and loss of self esteem due to appearance impacts. Fusion of all these aspects collapses into a significant reduction in the quality of life of these subjects, as previously reported [11]. Missing teeth can interfere with chewing ability, diction, and esthetics. Low self-esteem related to tooth loss can hinder an individual's ability to socialize, hamper the performance of work and daily activities, and lead to absence from work [12]. Caries and periodontal disease are the main reasons for tooth loss in adults[13].

In the last decades, implantology has emerged as one of the most innovative enrichments in the field of dentistry. The use of dental implants to rehabilitate the loss of teeth has increased in the last 30 years[14]. Considerable increase is expected in the future. Before dental implants, dentures and bridges were used, Compared to earlier preprosthetic methods, endosseous implantology is a very popular solution due to its simple treatment, high success rate and predictability of the procedure, better stability, higher chewing efficiency and greater biocompatibility than traditional methods[15] as well as its relatively few complications [16],[17] and offers many advantages for the patients that the procedure usually is not very stressful and the physiological transfer of chewing forces into the bone, which under certain conditions even generates renewed bone growth.Long term success of implant-based treatment depends on the control of mechanical and biologic factors[18]. Mechanical factors include the static and dynamic occlusal load on the prosthetic crowns and implants. In turn, biological factors also play a very important role in the short and long term success of a dental implant [19]. It is known that peri-implant infections can produce discomfort to the patient and also accelerate bone loss[20].

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However, despite these long-term success rates, recent studies have reported that unfavorable occlusal factors and microorganisms in the oral cavity, especially those related to periodontal diseases, are the main causes of implant complications[21],[22]. Several studies have related the presence of suppurative periodontal pathogens in the peri-implant sulcus to deleterious effects on the adjacent hard and soft tissues, resulting in implant failure[23].Bacteria and their products may cause inflammatory reactions in the peri-implant soft tissue[24], [25], [26], [27], [28] which is why the important role of microorganisms in implant survival should be considered.

With the two-stage implant system, the abutment is retained in the implant by using a mechanical attachment method. This results in gaps and cavities between the implant and the abutment acting as a bacteriological reservoir [29]. There are many studies showing that the implant/ abutment interface cannot totally seal the passage of microorganisms, regardless of the implant/abutment connection type (internal, external or morse taper connections) [30], [31]. The implant and the abutment cannot be accurately matched because of the precision limit during production [32]. The IAI microgap, defined as the microscopic space between implant and corresponding abutment, exists [33]. Even well adapted interfaces, with less than 5µm microgap, were shown to be unable to prevent infiltration and the consequent bacterial colonization inside the implants [19]. Thus, the internal colonization of the implant surface by microorganisms is eventually inevitable. The interior of the implant becomes a reservoir of pathogenic microorganisms, promoting and maintaining a chronic inflammation in tissues around the implants [34]. This may cause an inflammatory process in peri-implant tissues associated with the connective gap located near the level of the alveolar bone crest for most implant systems. Hence, microbial colonization of the gap may result in bone resorption. This condition causes bone loss and may lead to failure of the implant treatment [30], [20]. Marginal bone loss around the dental implant is one the most common complications after implantation and exerts remarkable influence on the future success and long-term stability of the implant. Factors contributing to the loss of marginal bone include surgical trauma, peri-implantitis, occlusal overload, microleakage, biologic width, and implant anatomy on the crest area [35].Generally, when the implant is placed into the alveolar bone, the resorption of marginal bone usually begins from the bone cortex (Branemark et al., 1969). The phrase microleakage of the implant-abutment interface (IAI) was coined in the 1990s, and it describes a microbial leakage between the implant and the abutment, which is attributed to the microgap and micromotion of the IAI.

Micro-gaps between the components are inevitable [36], [37], [38] but their clinical significance has been underestimated by manufacturers and clinicians. Several antimicrobial agents are used at the IAI such as Chlorhexidine gels,tetracycline gel, and Proheal ointment. None have been proven to be able to completely prevent bacteria from entering the microgap, ineffective or unable to last for a long time. necessary to fabricate sealants with strong and long lasting antibacterial effects. For that reason chitosan nanoparticles mediated gel has been used promisingly in antibacterial therapies because of their enhanced and unique physicochemical properties- ultra-small sizes, large surface area to mass ratio and increased chemical reactivity.

Nanoparticles are ultrafine particles with nano range dimensions in diameter (1–100 nm), and are made from basically any type of biocompatible substance.Nanoparticles are made of natural or artificial polymers ranging in size from 10–1000 nm[39]. Chitosan is a collective name for a group of polysaccharide bio-polymers obtained by deacetylation of chitin to various degrees[40]. Major sources of chitin include exoskeletons of crustaceans (crab, shrimp, lobster, crawfish, etc.) and cell walls of fungi and insects [41]. Chitosan and its derivatives have attracted considerable interest due to their antifungal activity and antimicrobial activity due to its promising in antibacterial therapies because of their enhanced and unique physicochemical properties, ultra-small sizes, large surface area to mass ratio and increased chemical reactivity[42], [43,44]. Chitosan exhibits higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Therefore, chitosan has a variety of current and potential applications in various fields, for example, biotechnology [45], pharmaceutics (Illum, 1998), wastewater treatment (Ramnani & Sabharwal, 2006), cosmetics[46], and food science. The antibacterial activity of chitosan has been widely explored [47]; Liu et al., 2006; [48]. A number of chitosan derivatives with different modifications have been prepared to improve its antibacterial activity [49], [50], [51]. Although the exact mode of action remains to be fully elucidated, the polycationic nature of chitosan, its chelating capacity and the binding/interaction with cell membrane structures are regarded as important factors resulting in a sequence of events that eventually lead to bacterial death[52].

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Our research and knowledge have resulted in high-quality publications from our team [53–63] Considering that bacterial infiltration is one of the main parameters in determining the quality of the connections, the objective of this current study is to evaluate different types of implant-abutment connection to assess bacterial infiltration through interfaces between the abutments and implants and the impact of sealing the dental implant-abutment interface with chitosan nanoparticle gel on the presence of the main oral cavity pathogens.

MATERIALS AND METHODOLOGY

CHECKING FOR BACTERIAL SEEPAGE AT THE IAI -

To check for the bacterial leakage at the IAI. A Nobel biocare implant was used in this study. The titanium abutment screws were tightened to 25 Ncm using a manual torque meter and the implant was placed in the solution containing artificial saliva (90%) and commercially available red wine (10%) for staining of the implant when viewed under stereoelectronic microscope. The implant is left in that for 48 hours and later the cover screw is removed and checked for staining in the inner hollow surface of the implant under an electronic microscope.

PREPARATION OF ANTI-MICROBIAL PASTE -

Raw materials for the chitosan preparation were chitosan nanoparticles, distilled water and glacial acetic acid. 500mg of chitosan nanoparticles dissolved in 49.5ml of distilled water. 0.5ml of glacial acetic acid was added to this solution and kept in a magnetic stirrer for 24 hrs for gel formation.

MICROBIOLOGICAL ASSESSMENT - All experiments were carried out by a single investigator in a sterile environment. The experiment, performed using 16 implants (NOBEL BIOCARE Dental Implant Systems Ltd) to check the efficacy of chitosan gel as a sealing agent at the IAI. 7 conical and 7 tri-channel connection implants were used. Three groups were established: One control group, which receives 1 conical and 1 tri-channel implant. Two experimental groups, one experimental group is the Streptococcus mutans group and other is the Pseudomonas group each of the experimental group received 3 conical and 3 tri-channel implants in which prepared chitosan gel is placed inside the hollow connection of an implant and closed with healing cap with a torque of 25 Ncm. The implants were placed in the Agar medium inoculated with streptococcus mutans and pseudomonas, incubated in an incubator for 120 hours at 37°C.

RESULTS -

After 24 hours of incubation the petri dishes are separated and see for changes

Bacterial Group	Study Group	Mean	t	df	SE	Sig (2-tailed)
Pseudomonas	conical trichannel	9.67 ± 1.62 12 ± 2.00	1.606	4	1.453	0.184
Streptococcus mutans	conical trichannel	8 ± 1.00 11 ± 1.73	2.958	4	1.15	0.06

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Table 1 : Pairwise comparison of the colony-forming units per milliliter for the 2 groups namely Streptococcus mutans and Pseudomonas in conical and tri-channel implant systems according to independent t-test.

Both the design of the Nobel implant system was showing zones of inhibitions against streptococcus and pseudomonas indicating the efficacy of prepared chitosan nano gel as a sealing antimicrobial agent at the junction of the implant abutment interface. There was no significant differences between two implant designs ($P > 0.05$), but showing activity of 100% better than the control group (control showed no zone of inhibitions against bacteria).

DISCUSSION -

This study shows that a complete seal is not found at the IAI, and the presence of sealing agents helps reduce microleakage. The bacteria that grow at the IAI will colonize and percolate through the microgap into the space within the implant that will act as a reservoir. Thus, this study clearly highlights that there is a leakage at the IAI and a complete hermetic seal is not possible [37], [34], [64] and it also shows the antimicrobial action in different culture media of which the zone of inhibition in conical and tri channel implant systems. The zone of inhibition in different groups is as follows, In Pseudomonas group, the zone of inhibition is more in tri-channel implant system is 12 ± 2.00 and in conical implant system is 9.67 ± 1.62 and the mean difference among groups was statistically non-significant ($P > 0.05$). In the Streptococcus mutans group, the zone of inhibition is more in the tri-channel implant system is 11 ± 1.73 and in the conical implant system is 8 ± 1.00 and the mean difference among groups was statistically non-significant ($P > 0.05$).

The microscopic space between implant and abutment (microgap) facilitates the infiltration of fluids and macromolecules from tissue fluids and saliva, facilitating bacterial invasion and proliferation [65], [66], even in patients with good oral hygiene [34], [30], [29], [67], [68] The presence of microorganisms inside implants can be a reservoir of peri-implant pathogens that may contact bone around the implant via the implant-abutment interface.The presence of microorganisms in the implant/abutment microgap may not cause significant bone loss that would greatly compromise the rehabilitation in a short term [69]. However, it will be significant in an aesthetic location, as even minimal bone loss will affect the quantity and quality of bone surrounding implants and consequently, compromise the shape and contour of the overlying soft tissues [70] .

Depending on the different implant systems, a microgap in the range of about 1 to 49 μm can be seen at the IAI. When the implant is subjected to masticatory forces the gap at IAI is further widened [71]. Pita MS et al., in their study tested both conventional flat-head and conical-head abutment screws, in External Hexagon (EH) and Trichannel Internal platform (TI) implants, under unloaded conditions with 38 microbial species. In both the EH and TI connections a large number of microbial species penetrated IAI. Implants attached with conical head abutment screws showed fewer microorganisms in comparison to conventional flat-head screws.[72]

Jansen et al. reported microbial leakage of 13 different implant abutment combinations using E. coli as the indicator bacteria. Callan et al.[73] described moderate to high levels of eight different periodontal pathogenic microorganisms, including A. actinomycetemcomitans and P. gingivalis, colonizing the microgap using DNA probe analysis. Tesmer et al. [70] assessed the potential risk for invasion of oral microorganisms into the fixture abutment microgap of dental implants with internal Morse-taper connections and the tri-channel internal connection. A wide variety of microorganisms seem to be able to penetrate along the implant components, ranging from gram positive cocci to gram-negative rods. Some of the identified species (Bacteroides species, Fusobacterium species and Peptostreptococcus micros) have been associated with peri-implantitis [73], [74]. It can be mixed with silicone products and make long lasting sealing agents.

LIMITATION AND FUTURE SCOPE -

This particular study has a lack of oral environment and decreased sample size. Furthermore, confirmation with experiments conducted under in vivo conditions in a larger sample size.

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CONCLUSION-

Within the limitations of this study it can be concluded that bacterial species from human saliva will penetrate along the implant-abutment interface. Chitosan gel has shown a good anti-microbial efficiency against both Streptococcus mutans and Pseudomonas but further studies should be done with larger sample size and the longevity of anti-microbial efficiency of chitosan gel.

CONFLICT OF INTEREST STATEMENT -

We declare that we have no conflict of interest.

Figures :-

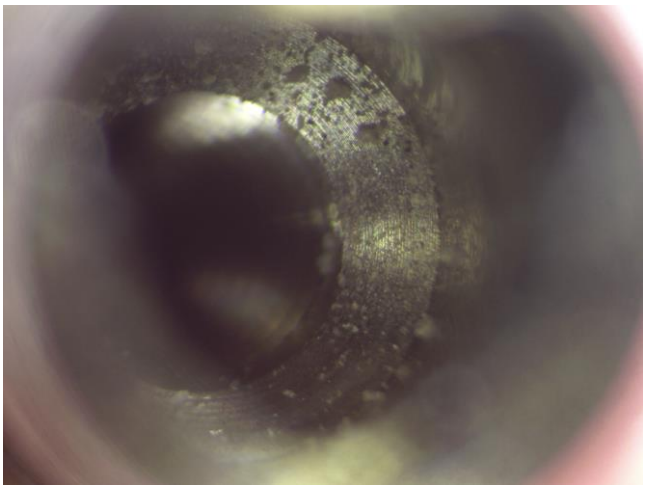

Figure 1- Stereoelectronic microscope showing internal connection of the implant

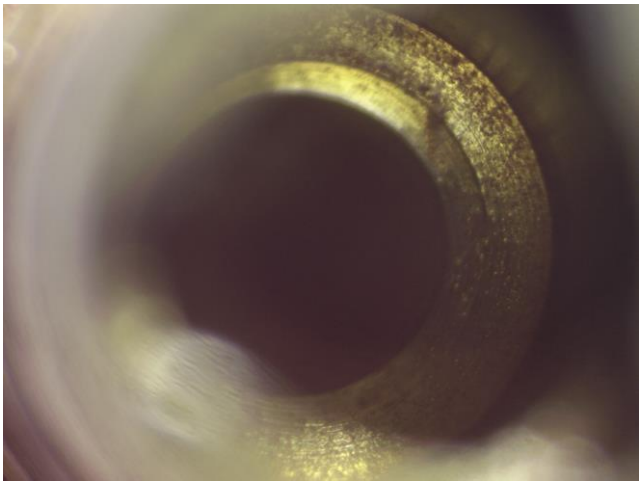

Figure 2- stereo electronic microscope of internal connection of implant after placing implant with healing cap in artificial saliva incorporated with red wine.

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Figure 3- healing cap showing stains of red wine after 72 hrs immersion in artificial saliva incorporated with red wine.

Figure 4 - chitosan prepared gel was incorporated into the implant abutment interface along with healing abutment and placed in prepared medium

Figure 5- zone of Inhibition around conical and tri-channel implant is seen in both S.mutans and Pseudomonas groups

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