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Dynamic Assessment fo Biocidal Properties of Silicon-Chitosan -Containing Hydrogels

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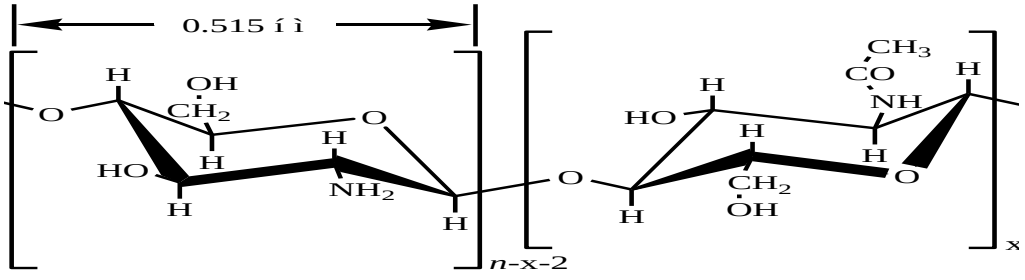
ABSTRACT: Our researches were focused on the study of the possibility to use the Silicon-chitosan-containing hydrogels as the more active antimicrobial substances needed for the manufacture of drugs for the treatment of many infectious diseases. Up until now, its antimicrobial activity is not well researched and its validity remains only vaguely defined.

KEYWORDS: Silicon-Chitosan , Chitosan , Antimicrobial.

I. INTRODUCTION

Chitin is a polysaccharide of animal origin found abundantly in nature and characterized by a fibrous structure. It forms the basis of the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs and lobster[1]. The chemical structure of chitin is similar to cellulose, having one hydroxyl group on each monomer substituted with an acetylamine group (Figure 1). The extraction of chitin involves an acid removal of calcium carbonate (demineralization), generally by hot reaction with HCl, HNO3 or HCl, etc., followed by a deproteinization (removal of proteins). This step usually performed by alkaline treatments (e.g. with NaOH)[2]. In its extracted crude form, chitin has a highly ordered crystalline structure, is translucent, resilient and quite tough. It has, however, poor solubility and low reactivity.
chitosan depends on its biological origin, molecular weight and degree of acetylation Since chitosan is soluble in diluted acid solutions, films can be readily prepared by casting or dipping, resulting in dense and porous structure[3].
Chitosan film is regarded as biofunctional material, well tolerated by living tissues, particularly applicable as edible coatings to prolong shelf-life and preserve quality of fresh foods In medical field, chitosan films have been tested as curative wound dressing and as scaffolds for tissue and bone engineeringAdditionally the reactive functional groups present in chitosan (amino group at the C2 position of each deacetylated unit and hydroxyl groups at the C6 and C3 positions) can be readily subjected to chemical derivatization allowing the manipulation of mechanical and solubility properties enlarging its biocompatibility [4]. (Fig. 1).



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Now our researches are focused on the study of the possibility to use the silicon-chitosan-containing gels (Si-Chit-Gels) as the more active antimicrobial substances needed for the manufacture of drugs for the treatment of many infectious diseases and in wound therapy. Up until now, its antimicrobial activity is not well studded and its validity remains only vaguely defined.

II. MATERIALS AND METHODS

Bacterial strains:

Three well-characterized standard laboratory strains were used in this study: gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* and *Bacillus cereus.*

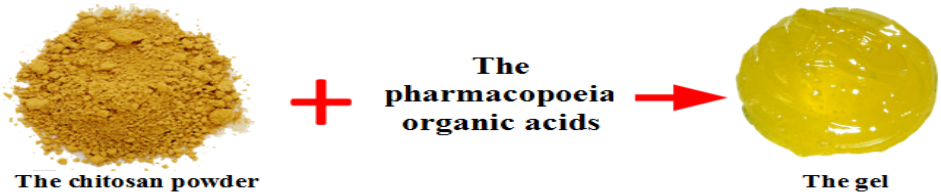
Bacterial killing assay:

Cultures of the indicator strains were incubated separately in the absence (control) and presence of Si-Chit-Gel for a period of 5 h at 37°C.

The delayed growth of the cell suspensions in broth with/without Si-Chit-Gel was monitored as a decrease in optical

Producing gel for Technology based on Chitosan and Ascorbic acid

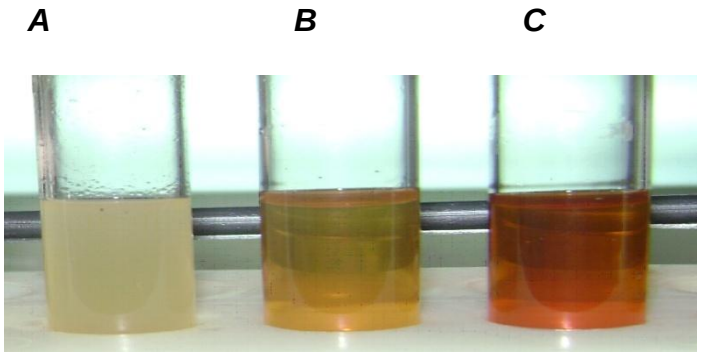
To obtained gel of chitosan based on the solubility chitosan powder with ascorbic acid



Properties of this Gel

antioxidative, anti-inflammatory , and wound healing immunostimulatory effect anti-inflammatory and wound healing immunostimulatory

Antimicrobial Activity of Gel



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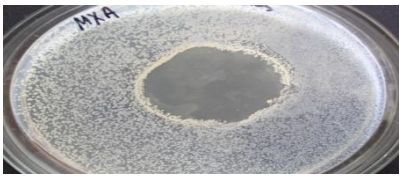
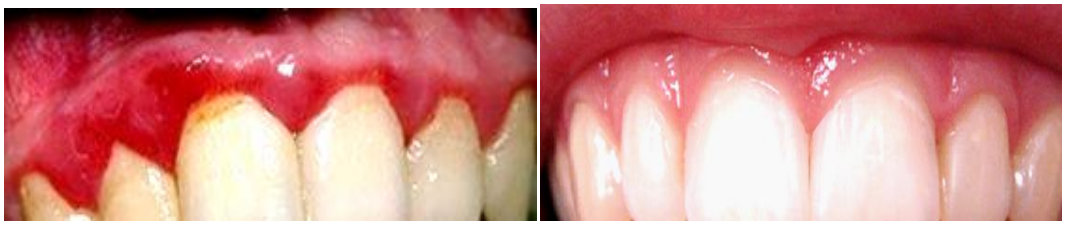
Growth of the culture Escherichia coli
in the Meat-Peptone Broth without (A) and with the addition of chitosan gel in ascorbic acid (B) and chitosan gel in ascorbic acid with Si (C)



No growth of the culture Bacillus cereus
in the area of application of chitosan gel on Mueller-Hinton agar
Application of Gel in Ascorbic Acid on the Gingival margin



(a) ***(b)***

The results of mild generalized periodontitis's treatment by application of gel:
(a) status of periodontal tissue before treatment; (b) after 5 days.

III. MANUFACTURE AND PROPERTIES OF CHITOSAN

Chitosan was discovered by Rouget in 1859. is a technologically important polysaccharide biopolymer. Chemically, it is a high-molecular-weight linear polycationic heteropolysaccharide consisting of two monosaccharides and D-glucosamine linked together by β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [5] [6]. . The production of chitosan from chitin primarily takes place through exhaustive alkaline deacetylation this involves boiling chitin in concentrated alkali for several hours (40–45% sodium hydroxide, 120°C, 1–3 h). Since this *N*-deacetylation is almost never complete, chitosan is considered as a partially *N*-deacetylated derivative of chitin. Consequently, a sharp distinction between chitin and chitosan on the basis of the degree of *N*-deacetylation cannot be drawn. Chitosan is also found in nature, such as in cell walls of fungi of the class *Zygomycetes*, in the green algae *Chlorella* sp., yeast and protozoa as well as in insect cuticles Advances in fermentation technology suggest that the cultivation of fungi (*Aspergillus niger*) can provide an alternative source of chitosan[7]. However, chitosan from both sources differs slightly: whereas the acetyl groups in chitosan produced from crustacean chitin are uniformly distributed along the polymer chain, a chitosan of similar degree of deacetylation(DD) isolated from fungal cell walls would possess acetyl residues that are grouped into clusters. In contrast to most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, which are neutral or acidic in nature, chitosan is an example of a highly basic polysaccharide, with a nitrogen content varying between 5% and 8%, depending on the extent of deacetylation[8].

The main difference between chitin and chitosan lies in their solubility; chitosan is therefore said to be chitin that has been *N*-deacetylated to such an extent that it
becomes soluble in dilute aqueous acids. Pure, native chitosan (pKa ~ 6.3) is insoluble in water, in alkaline medium and even in organic solvents. However, water soluble salts of chitosan may be formed by neutralization with organic acids (e.g. 1–10% aqueous acetic, formic, succinic, lactic, glutamic and malic acids) or inorganic acids such as hydrochloric acid [9].

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IV. SAFETY

The low toxicity profile of chitosan compared with other natural polysaccharides is another of its many attractive features. It has been reported that the purity of chitosan influences its toxicological profile, yet its safety in terms of inertness and low or no toxicity has been demonstrated by *in vivo* toxicity studies. Its oral LD50 (median lethal dose) in mice was found to be in excess of 16 g day-1 kg-1 body weight, which is higher than that of sucrose. [10]. Ylitalo and colleagues (2002) reported the absence of significant side-effects following chitosan ingestion in human studies (for up to 12 weeks). However. We were unable to identify any epidemiological studies or case reports investigating the association of chitosan exposure and cancer risk in humans, any carcinogenicity studies on chitosan in animals and any *in vitro* or *in vivo* studies evaluating chitosan for mutagenic effects in the available literature. [11].

V. SENSITIVITY OF MICROBIOLOGY STRAINS TO CHITSAN

Chitosan has several advantages over regular type of disinfectants owing to its broad spectrum of activity. Chitosan has been observed to act more quickly on fungi than on bacteria and activity against typhoid organisms are comparable to the standard antibiotics used in clinical practiceAs discussed this antimicrobial activity has a strong dependence on MW and DA characteristics and also varied according microorganism strains[12].

There are many studies about the minimum inhibitory concentration (MIC) for chitin, chitosan, their derivatives or combination, with different results for different microorganism. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. It is dependent of many factors and the non-standardized procedures make difficult to compare MIC results from author to author. MIC however is useful as a practical indicator of a primary activity against a selected pathogenic microorganism[13] .

VI. INTERACTION OF CHITOSAN WITH THE BACTERIAL CELL SURFACE

The mode of action of cationic antibacterial agents is widely believed to be the interaction with and disruption of the cell envelope. Electron microscopical examinations of various chitosan-treated microorganisms suggest that its site of action is indeed at the microbial cell surface [14].. nature of chitosan, conveyed by the positively It is generally assumed that the polycationic charged —NH3+ groups of glucosamine, might be a fundamental factor contributing to its interaction with negatively charged surface components of many fungi and bacteria, causing extensive cell surface alterations, leakage of intracellular substances, and ultimately resulting in impairment of vital bacterial activities[15].

VII. CLINICAL STUDY RESULTS

- Studies have shown that the gel can significantly increase the effectiveness of basic treatment for gingivitis and chronic periodontitis .
- The use of the gel was recommended for the treatment of periodontitis in the pre- and postoperative periods and also during maintenance therapy for the early elimination of inflammatory processes in periodontal tissue.

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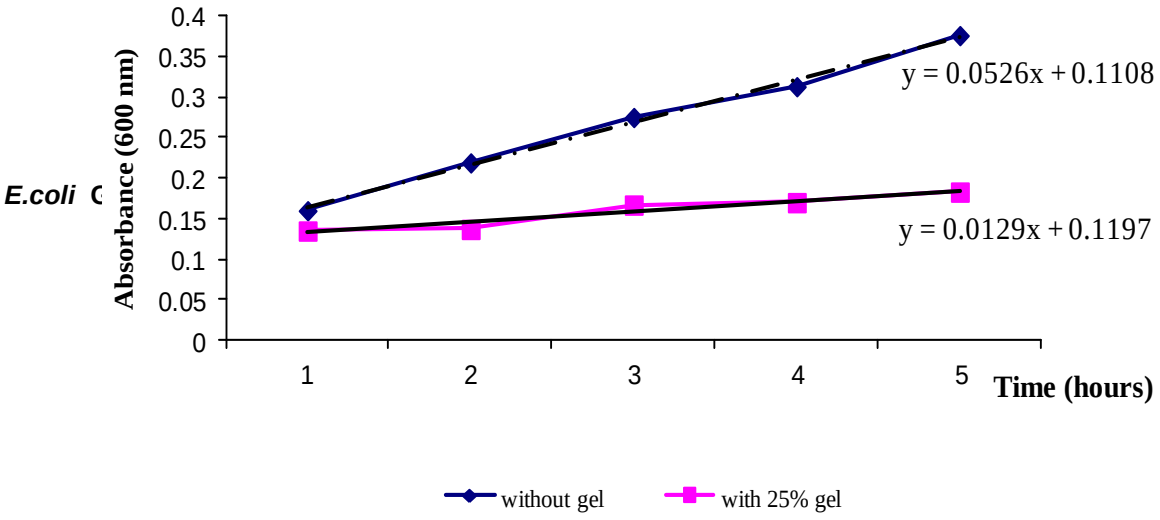
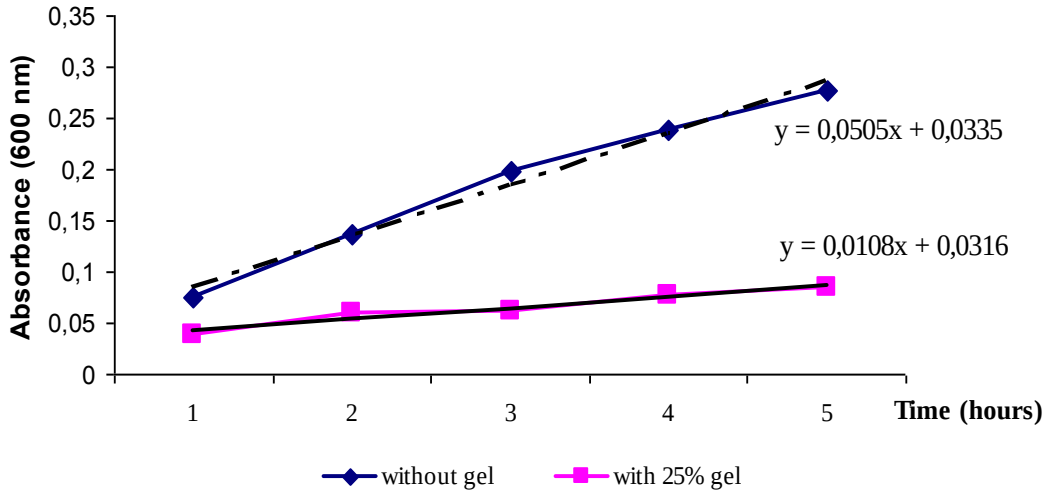
***E.COLI* GROWTH IN MEDIA WITH WITHOUT FRESHLY MADE Si-CHIT-GEL**



***E.COLI* GROWTH IN MEDIA WITH /WIYHOUT TWO-MONTHS Si-CHIT-GEL**



VIII. CONCLUSIONS

- The gram-negative culture of *E.coli* exhibited delayed growth in broth containing Si-Chit-Gel compared to growth of the microorganism in the absence of Si-Chit
- Similar effects of Si-Chit-Gel on bacterium growth were seen with the use of two additional gram-positive strains *S.aureus* and *B.cereus*.
- Si-Chit-Gel does not lose antimicrobial activity against gram-negative and gram-positive bacteria within two months of storage at $4^{o}C$.

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- Si-Chit-Gel can be recommended for outdoor use in the treatment of the infectious diseases.

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