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Evaluation of tetracycline containing chitosan hydrogels as potential dual action bio-active restorative materials capable of wound healing: *in vitro* approach

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ABSTRACT

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. Wound healing progresses through a series of interdependent and overlapping stages in which a variety of cellular and matrix components act together to reestablish the integrity of damaged tissue and replacement of lost tissue. The objectives of this study was to evaluate the novel chitosan based functional drug delivery systems which can be successfully incorporated into "dual action bioactive restorative materials" capable to induce an improved wound healing prototype *in-vitro.* The system contained a common antibiotic such as tetracycline, krill oil as an antioxidant, hydroxyapatite as a molecular bone scaffold, which is naturally present in bone and is reported to be successfully used in promoting bone integration when implanted as well as promoting healing. The hydrogels were prepared as previously reported. The bio-adhesive capacity of the materials in the 2 separate "*in vitro*" systems were tested and quantified. Additional action of chitosan:vitamin C pre-complex was investigated and it was found that favorable synergistic effect of free radical build-in defense mechanism of the new functional materials. Additional action of chitosan:vitamin C precomplex was investigated and it was found that favorable synergistic effect of free radical build-in defense mechanism of the new functional materials increased dentin bond strength and sustainable bio-adhesion and therefor act as a "proof of concept" for the functional multi-dimensional restorative materials with potential application in wound healing *in vitro*. A steady slow release of tetracycline, while maintaining antibiotic effects against the tested bacteria for at least 10 days, was shown from designer chitosan-therapeutic agent hydrogels. Based on our results, we can conclude that the chitosan-antioxidant containing hydrogels are a suitable carrier for tetracycline to be slow-released. Within the limitations of the study design chitosan based hydrogels are suitable materials for functional restorative and wound healing applications in vitro. However, future investigations are necessary to validate this hypothesis. The addition of antioxidants to the tetracycline containing prototype delivery system had a beneficial effect on the design of the hydrogel by slowing down the release of tetracycline and thereby enabling a sustainable antifungal activity over time.

Keywords: *chitosan, hydrogels, tetracycline, reactive oxygen species, antioxidants, functional biomaterials, microbiological activity, release %*

1. INTRODUCTION

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. Wound healing progresses through a series of interdependent and overlapping stages in which a variety of cellular and matrix components act together to reestablish the integrity of damaged tissue and replacement of lost tissue [1, 2]. The wound healing process has been reviewed and described as a process comprising five overlapping stages that involve complex biochemical and cellular processes [3]. These are described as haemostasis, inflammation, migration, proliferation and maturation phases. In fact, the understanding of wounds lies beyond the cellular level to a molecular context as well and therefore there is a need to approach wound healing at multiple levels (cellular and molecular) to help improve wound treatment and management.4 Wound healing formulations (dressings) and novel technologies developed to date focus on one or more of these aspects of the natural healing process [5]. Reactive oxygen species (ROS) are associated with all the stages of the healing process [6]. ROS are produced by the inflammatory cells and play an integral role during this process [7-12]. Antioxidants administration is beneficial for healing [13]. Bio-adhesive polymers appear to be particularly attractive for the development of alternative etch

free dentin bonding systems with an added advantage of additional therapeutic delivery systems to improve intra-dental administration of therapeutic and prophylactic agents if necessary [10-15]. Chitosan, which is a biologically safe biopolymer, has been proposed as a bio-adhesive polymer and are of continuous interest due its unique properties and flexibility in a broad range of oral applications reported by recently [16-20]. The additional benefits of the site specific "functional restorative material" for use of dressings to deliver antibiotics to wound sites can provide tissue compatibility, low occurrence of bacterial resistance and reduced interference with wound healing. The issue of lower antibiotic doses within the dressing also reduces the risk of systemic toxicity considerably. Also local drug deliveries from a dressing or functional restorative material can overcome the problem of ineffective systemic antibiotic therapy resulting from

the compromising blood circulation caused by other systemic conditions. The objectives of this study was to evaluate novel chitosan based functional drug delivery systems which can be successfully incorporated into "dual action bioactive restorative materials" capable to induce in-vitro improved wound healing prototype and containing common antibiotics such as tetracycline, krill oil as an antioxidant, hydroxyapatite as a molecular bone scaffold, which is naturally present in bone and is reported to be successfully used in promoting bone integration when implanted as well as promoting healing. The novel hydrogels were investigated with respect to the antioxidant capacity and drug release capacity of tetracycline from the designer drug delivery system, the use of SEM imaging for the characterization of the surfaces, bio-adhesive property, antioxidant capacity, free radical defense, antioxidant and active ingredient stability and reactive features of novel materials with antimicrobial potential.

2. EXPERIMENTAL SECTION

2.1. Materials.

Chitosan, Vitamin C (Aldrich, Australia), glycerol (Sigma, USA), glacial acetic acid (E. Merck, Germany) were used as received. The chitosan used in this study had a molecular weight of 2.5x103 KD and a 87% degree of de-acetylation, typical of commercial chitosan. The isoelectric point is 4.0–5.0. Krill oil (Aurora Pharmaceuticals, Australia), tetracycline were used as received. The physico-chemical features including surface morphology (SEM), release behaviors, stability of the therapeutic agent-antioxidant-chitosan and the effect of the hydrogels on the shear bond strength of dentin were measured and compared to the earlier reported chitosan-antioxidant containing hydrogels [22]. Structural investigations of the reactive surface of the hydrogel are reported. Release of tetracycline was investigated for all newly prepared hydrogels. Bio-adhesive studies were performed in order to assess the suitability of these designer materials. Free radical defense capacity of the biomaterials was evaluated.

2.2. Preparation of the various antibiotic containing hydrogels.

Chitosan hydrogels have been prepared using the methodology previously described [21-23]. Briefly, the corresponding antibiotic and antioxidant mixtures, were incorporated by dispersion of corresponding antioxidant powder 0.2 grams in glycerol (5% w/w) using a mortar and a pestle and 1 milliliter of glacial acetic acid (2% w/w). The corresponding antioxidant mixtures were incorporated into the mixture and a summary of the newly prepared materials is highlighted in Table 1. The pH of each gel was measured by accurately weighing one gram of the prepared gel and dispersing it in 10 ml of purified water. The pH of the dispersions was measured using pH meter (HANNA instruments, HI8417, Portugal) [21-23].

Table 1. Gel formulations prepared in the study

Gel formulation		Chitosan /Vitamin C (5:1) (w/w%)	Tetracycline (w/w%)	Krill Oil (w/w%)	Hydroxyapatite (w/w%)	pH
Ch/Vit C	Gel-1	5	0	0	0	5.46
Ch/Vit C/tetr	Gel-2	5	1	0	0	6.84
Ch/VitC/HA/T	Gel-3	5	1	0	1	6.74
Ch/VitC/T/Krill	Gel-4	5	1	1	0	6.94
Ch/VitC/T/Krill/HA	Gel-5	5	1	1	1	6.65

Where T is tetracycline, K is Krill oil, Vit C is Vitamin C, HA is hydroxyapatite.

2.3. Morphology of the gels.

The samples were prepared by freezing in liquid nitrogen for 10 min, and then were freeze-dried for 24 h. The prepared samples were fractured in liquid nitrogen using a razor blade. The fractured samples were dried under vacuum, attached to metal stubs, and sputter coated with gold under vacuum for the SEM study. The interior and the surface morphology were observed under scanning electron microscope (SEM, Hitachi S4800, Japan).

2.4. Gel stability.

Stability of the gel formulations was also investigated. The organoleptic properties (color, odor), pH, drug content, and release profiles of the gels stored at 20oC were examined on days 0, 15, 30 and 178 using previously tested established methodology [22].

2.5. Equilibrium swelling of the gels.

Infusion bags containing a known weight of dry gels were immersed in pH 4.0, pH 9.0 buffer solutions, respectively, and kept at 25oC for 48 h until equilibrium of swelling had been reached. The swollen gels were taken out and immediately weighed with microbalance after the excess of water lying on the surfaces was absorbed with a filter paper. The equilibrium swelling ratio (SR) was calculated using the following equation:

$$SR = (W_s - W_d)/W_d \times 100\%$$

where Ws and Wd are the weights of the gels at the equilibrium swelling state and at the dry state, respectively.[21-23] experiments were repeated in triplicate for each gel specimen and the mean value calculated.

2.6. Bio-adhesive investigation.

Bio-adhesion studies were done using a Chatillon apparatus for force measurement.[25] This method determines the maximum force and work needed to separate two surfaces in intimate contact.[25] The hydrogels (0.1g) were homogeneously spread on a 1cm2 glass disk and then the disks were fixed to the support of the tensile strength tester using double sided adhesive. The gel was brought into contact with the commercially available band aid, in order to simulate the skin attachment or the contact with a slice of dentin was established in order to imitate adhesion of the gel to the tooth structure. After a preset contact time of 1 min under a contact strength of 0.5N, the 2 surfaces were separated at a constant rate of displacement of 1mm/s. The strength was recorded as a function of the displacement, which allowed to determine the maximal detachment force, Fmax, and the work of adhesion, W, which was calculated from the area under the strength-displacement curve.

2.7. Shear bond strength testing.

Extracted non-carious, intact, human molars stored in water containing a few crystals of thymol at 4°C were used within two months using a protocol previously described [21-23]. 56 teeth samples prepared and divided into 7 groups of 8 each, A-F (Table 2), stored in a solution of artificial saliva were then treated as outlined in Table 2. After 24 hours a stud of each tooth was tested for shear bond strength. An Instron Universal Testing Machine at a crosshead speed of 0.5 mm/minute was used to test the de-bonding strength. All data tests were analyzed using the non-parametric ANOVA test.

Table 2. Groups tested (8 teeth per groups)

Group A	37% of phosphoric acid +primer+ Bonding immediately (negative control)
Group B	Self-etching primer + Bonding immediately (positive control)
Group C	Gel1+primer+ Bonding immediately
Group D	Gel2+primer+ Bonding immediately
Group E	Gel3+primer+ Bonding immediately
Group F	Gel4+primer+ Bonding immediately
Group J	Gel5+primer+ Bonding immediately

2.8. In vitro tetracycline release from the gels.

The release study was carried out with USP dissolution apparatus type 1, Copley U.K., slightly modified in order to overcome the small volume of the dissolution medium, by using 100 ml beakers instead of the jars. The basket of the dissolution apparatus (2.5 cm in diameter) was filled with 1 gm of tetracycline gel on a filter paper. The basket was immersed to about 1 cm of its surface in 50 ml of phosphate buffer pH 6.8, at 37ºC ± 0.5 and stirred at 100 rpm. 24 Samples (2ml) were collected at 0.2, 1, 2, 3, 4, 5, 6, 7, 8,10, 15 and 24 hours and were analyzed spectrophotometrically with a U.V. spectrophotometer (Cintra 5, GBC Scientific equipment, Australia). The UV-vis absorption spectrum of tetracycline hydrochloride in water is typical at 361 nm, using the calibration curves (A1% 1cm=337 for tetracycline hydrochloride both evaluated in PB, pH 6.8). Three replicate measurements were performed for each designed formulation [22]. Each sample was replaced by the same volume of phosphate buffer pH 6.8 to maintain its constant volume and sink condition [21-24].

2.8. Microbiological Investigations.

A type strain of Staphylococcus aureus (ATCC 12600), obtained from the American Type Culture Collection (Manassas, USA) was used as test bacterium for estimating the antibacterial activity of the hydrogels. The antibacterial activity of the prepared tetracycline/antioxidant chitosan hydrogels were tested using the standard Kirby-Bauer agar disc diffusion method (Bauer et al). Five to 6 mm deep Muller-Hinton agar (Oxoid, Basingstoke, UK) plates were inoculated by streaking a standardized inoculum suspension that match a 0.5 McFarland standard and containing 107- 108 colony forming units/ml with a throat cotton swab. For each test sample 500µg of hydrogel was applied to a 6 mm diameter paper disc. The paper discs were placed on the inoculated Muller-Hinton agar medium and incubated at 37ºC for 24 hours. The diameter of the zones of growth inhibition was measured with a caliper. Each measurement was done in triplicate and the testing of each sample was repeated 3 times. The antibacterial efficacy of the prepared gels were compared to antibiotic sensitivity discs (Mast Laboratories, Merseyside UL) containing 30 µg of tetracycline per disc.

3. RESULTS SECTION

3.1. Morphology of the gels.

The SEM images were obtained to characterize the microstructure of the freeze-dried gels and are presented in Figure 1. It could be seen that the gels displayed a homogeneously pore structure similar to a sponge. SEM analysis revealed interconnected pores of different size and flat, relatively smooth walls. The biomaterial remained intact after 24 days of immersion in artificial saliva as it was confirmed by SEM.

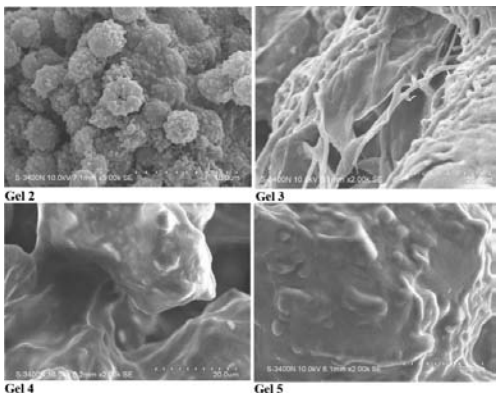

Figure 1. SEM photographs of interior morphology of the selected gels under investigation for (a) Gel-1, (b) Gel-2, (c) Gel-3, (d) Gel-4 and (e) Gel-5.

It was thought that the micro-porous structure of the gels could lead to high internal surface areas with low diffusional resistance in the gels. The surfaces of the gels were also presented (Figure 1). The collapse of the surface pores in the 'skin' of the gels that can be seen may be due to artifacts produced during the freeze-drying process.

3.2. Gel stability.

The results suggest there is no significant decomposition observed after 6 months storage at room temperature (24oC) as antioxidant capacity of the materials stored for 6 month have showed no diminished capacity compare to the freshly prepared hydrogels as indicated in Figures 2 and 3.

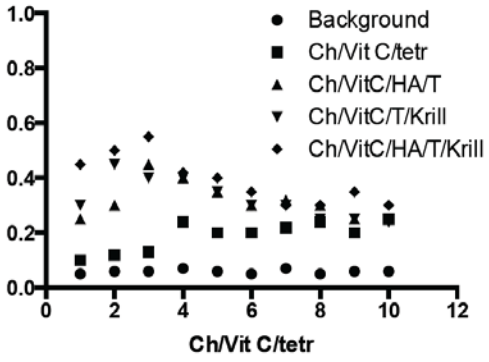

Figure 2. Antioxidant capacity measured at 450nm using the previously described spectrophotometric assay to asses the hydrogels and corresponding ingredients antioxidant capacity after 24 hours under storage under ambient temperature condition. Antioxidant capacity was measured during the first 2 hours of exposure.

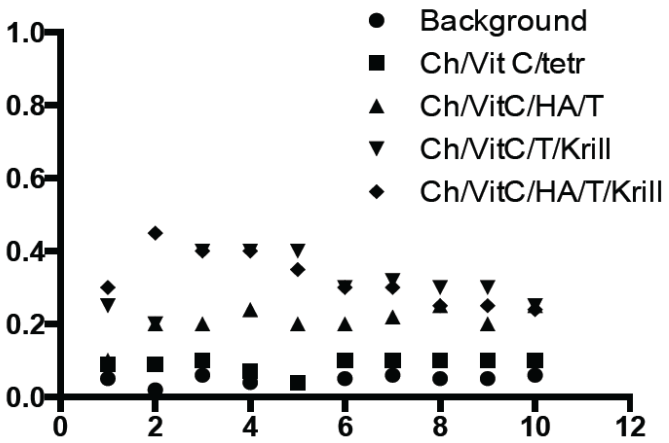

Figure 3. Antioxidant capacity measured at 450nm using the previously described spectrophotometric assay to asses the hydrogels and corresponding ingredients antioxidant capacity after 6 month under storage under ambient temperature condition. Antioxidant capacity was measured during the first 2 hours of exposure.

3.3. Studies of equilibrium swelling.

The hydrogels remain in the cylindrical form after swelling. Compared with dry state hydrogels, the swollen state hydrogel volume displays significant increases and are summarized in Figure 4.

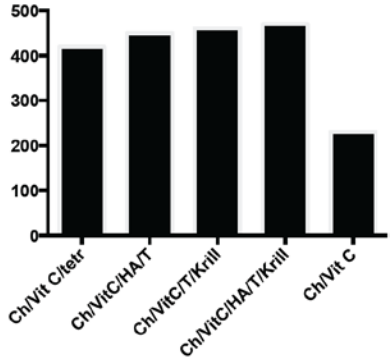

Figure 4. Water uptake degree of the gels Gel-1 – Gel-5 (n=3, $p<0.05$).

3.4. Bio-adhesion in vitro model.

Higher adhesiveness of the gels is desired to maintain an intimate contact with skin or tooth structure and results are summarized in Table 3. Chitosan hydrogels showed the highest adhesive force and the work of adhesion can be expected because of the well known intrinsic bioadhesive properties of chitosan [35]. The adequate water absorption capacity together with the cationic nature which promotes binding to the negative surface of skin or dentin structure can also interpret this results.

Table 3. Bioadhesion testing in vitro.

Hydrogel	Adhesive Force(N) ±SD (skin)	Adhesive Force (N) ± SD (Dentin)	Work of Adhesion (Ncm)±SD (Skin)	Work of Adhesion (Ncm) ±SD (Dentin)
Gel-1	1.20±0.30	1.21±0.35	3.35±0.48	2.92±0.34
Gel-2	1.12±0.27	1.37±0.44	3.19±0.52	3.49±0.42
Gel-3	1.01±0.30	1.12±0.60	2.85±0.41	2.94±0.29
Gel-4	1.15±0.40	1.27±0.24	3.31±0.31	3.38±0.31

The presented values are an average (n=5).

3.5. Shear bond strength.

Mean shear bond strength values and difference between the groups are summarized in Figure 5 for bonding to dentin after 24 hours.

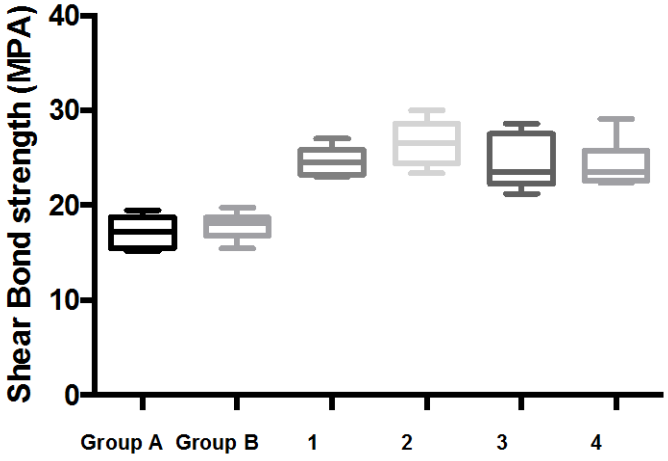

Figure 5. Shear bond strength of hydrogels after 24 hours of bonding to dentin.

In general there was an increase in bond strength of the dentin treated with the antioxidant (chitosan:Vit C complex) containing hydrogels compared to the bond strength of the conventionally bonded teeth. Interestingly the increase in bond strength was also observed in the groups of mhydrogen peroxide exposed samples suggesting that there are additional benefits associated with chitosan:therapeutic agent:antioxidant system that are in need of further investigations [21-23].

3.6. In vitro tetracycline release.

The release study was carried out with USP dissolution apparatus type 1, Copley U.K., slightly modified in order to overcome the small volume of the dissolution medium, by using 100 ml beakers instead of the jars. The basket of the dissolution apparatus (2.5 cm in diameter) was filled with 1 gm of tetracycline gel on a filter paper. The basket was immersed to about 1 cm of its surface in 50 ml of phosphate buffer pH 6.8, at 37ºC ± 0.5 and stirred at 100 rpm. 24 Samples (2ml) were collected at 0.2, 1, 2, 3, 4, 5, 6, 7, 8,10, 15 and 24 hours and were analyzed spectrophotometrically with a U.V. spectrophotometer (Cintra 5, GBC Scientific equipment, Australia). The UV-vis absorption spectrum of tetracycline hydrochloride in water is typical at 361 nm, using the calibration curves (A1% 1cm=337 for tetracycline hydrochloride both evaluated in PB, pH 6.8). Three replicate measurements were performed for each designed formulation [22]. Each sample was replaced by the same volume of phosphate buffer pH 6.8 to maintain its constant volume and sink condition [21-24].

3.7. In vitro microbial activity of tetracycline/antioxidant containing chitosan hydrogels.

Paper discs impregnated with the chitosan hydrogels without tetracycline gave no inhibition zones. However all the test samples containing tetracycline give inhibition zones larger than the clinical breakpoint inhibition zone diameters (European Committee on Antimicrobial Susceptibility Testing, Basel, Switzerland) for S. aureus sensitivity for tetracycline. Using the students T test, no statistically significant difference ($p<0.05$) between the averages of the inhibition zone diameters for all the samples were found (Table 4).

Table 4. (tetracycline) tested for antibacterial activity against Staphylococcus aureus NCTC 12600.

Tetracycline inhibition zone diameters						
Sample no. (n=10)	Tetracycline disc (30µg)	Ch/Vit C	Ch/VitC/tetr	Ch/VitC/HA/T	Ch/VitC/T/Krill	Ch/VitC/T/Krill/HA
Average ± standard deviation	26.44 ± 0.28	0	26.44 ± 0.32	22.80 ± 0.64	23.43 ± 1.34	23.20 ± 0.66

(Clinical breakpoints for tetracycline: 30µg/disc >22mm is sensitive and <19mm is resistant).

Chitosan, a linear abundant polysaccharide, is selected as the wall material of the delivery system.[25] Due to its biodegradable, biocompatible, muco-adhesive and non-toxic nature, it has been widely used in numerous drug delivery systems. Compared to other delivery systems, chitosan nanoparticles have a special feature. They can adhere to the mucosal surface and transiently open the tight junction between epithelial cells. Some reports have indicated that chitosan can increase membrane permeability, both in vitro [25,26] and in vivo [27]. Microencapsulation of antioxidants have been an important area of research for several years in order to preserve the beneficial effects of antioxidants[28]. In this work, the gelation method was used to microencapsulate biomaterials into the chitosan.

3.8. Stability of antioxidants in the chitosan hydrogels during storage.

Stability of various conventional antioxidants in the newly designed drug delivery system during storage is an important factor to determine whether chitosan-coated nano-size delivery vehicle can protect various conventional antioxidants. So the stability of the microencapsulated antioxidants has been measured by UV absorbance. The performance of the hydrogels was not affected by the storage conditions, suggesting remarkable stability of the novel biomaterials under investigation. This observation suggests that the antioxidant had been protected by the molecular carrier and can lead to a development of broad range to novel functional drug delivery systems and dual action restorative material.

3.9. Bond strength.

The results suggests that the optimum result for the strengthening of dentin can be achieved throughout the immediate treatment with antioxidant:chitosan:vitamin C "host:guest" complex with the increase of dentin bond strength. The additional advantage of the system may suggest that, antioxidant release from chitosan gel depends on the physical host:guest structure as well as pH properties and flexibilities of the material. The additional benefit of using chitosan:antioxidant system as a bonding/pre-bonding to enamel and dentin system lies in its ability to show favorable immediate results in terms of bonding effectiveness as well as the durability of resin-dentin bonds for a prolonged time (up to 6 months) [26,27]. It is well documented that the hydrostatic pulpal pressure, the dentinal fluid flow and the increased dentinal wetness in vital dentin can affect the intimate interaction of certain enamel and dentin adhesives with dentinal tissue [26,27]. Therefore the newly developed chitosan derivatised systems are supporting our earlier reported results that addresses the shortfalls affecting the long-term bonding performance of modern adhesives and addresses the current perspectives for improving bond durability of conventional adhesive systems [27].

3.10. In vitro release of antibiotics.

Drug release from topical formulations is affecting the efficiency of topical therapies to a great extent. Tetracycline, a model antimicrobial drug, which can be used in topical wound treatment [28], was incorporated into chitosan-based delivery systems and cumulative release was tested. After a 10 h time period, no significant difference in drug release could be seen between the three different formulations. Al-Khamis and group claimed that drug release from gel is controlled by two factors, namely the thermodynamic activity of the drug and the microviscosity of the gel [29]. Ji *et al*. confirmed these findings through the determination of protein release from hydroxypropyl methylcellulose gels [29]. Kristl *et al* [31] suggested that in the microenvironment of chitosan high molecular weight gels, the drug release is strongly affected by the degree of deacetylation of chitosan. Tetracycline release is expected to be fully diffusion controlled as the cumulative percentage of drug released is proportional to the square-root of time. The regression lines show a good fit with $R2 \geq 0.97$. Higuchi reported this kind of relation between release and square-root of time for suspensionointments originally [22]. Another indicator for the similar microviscosity of prepared hydrogels investigated in this study is the similarity in the values of the release constant k (5.91, 5.03, 4.54 and 5.216 respectively), which are reflecting structural and geometric characteristics of the hydrogel [22].

3.11. Free radical defense capability of the prepared hydrogels.

When wound occurs, it is generally accompanied by classical symptoms of inflammation, such as pain, redness and edema. The inflammation stage begins immediately after injury, first vasoconstriction, platelet aggregation at the injury site and then infiltration of leukocytes and the T lymphocytes to the wound area. The cicastration process proceeds naturally, since the damaged tissue attempt to re-establish hemostasis. In the inflammation stage, the main aim is the removal of debris, damage tissue, and bacteria by neutrophils and macrophages, which have a role in antimicrobial defense and debridement of devitalized tissue by production of proteolytic enzyme and reactive oxygen species [32]. The amount of uncontrolled ROS is the main cause of the inability of healing process to continue and therefor it would be ideal to utilize the antioxidant capacity of the "designer hydrogels " to detect and able to fight the free radical excess" have been assessed using previously described model using well-established that HO radical can be generated from a reaction known as the biologic Fenton reaction and this reaction requires the presence of H_2O_2 [34]. The generation of $HO^{\cdot}$ from the biologic Fenton reaction has been shown to be a critical factor in various ROS-induced oxidative stresses [21-23]. H_2O_2 and $HO^{\cdot}$ might be related to apoptosis in atherosclerosis [34]. Godley *et al*. also reported that blue light induces mitochondrial deoxyribonucleic acid damage and cellular aging [35]. We reported earlier that protein cross-linking as a model for detection of free radical activity and activation of "molecular defense forces". Bovine serum albumin

(BSA), a completely water-soluble protein, was polymerized by hydroxyl radicals generated by the Fenton reaction system of Fe^{2+}/EDTA/ H_2O_2/ascorbate [21-23]. As a result, the protein loses its water-solubility and the polymerized product precipitates. The decrease in the concentration of the water-soluble protein can easily be detected. We considered worthwhile to study the chitosan as a "build in defense mechanism" for the in-vitro generated free radical production and "site specific" in vitro model counter reaction of the hydrogel. Therefore we adopted the method for recording changes in water solubility of the model protein bovine serum albumin (BSA) exposed to free radicals generated by an inorganic chemical system. As clearly demonstrated by the Figure 5, upon exposure to standard H_2O_2 in the form of Fe^{2+}/EDTA/H_2O_2/ascorbate solution as a base line determinate free radical generation under "prototype in-vitro free radical damage", upon incorporation of the chitosan substituted hydrogels, the build in antioxidant capacity and therefor free radical defense of the in-vitro model has been activated and are of significant value to take notice. This model represents the practical approach of in-situ monitoring and test the amount of free radical production and synergistic antioxidant defense of the system. Further investigations and fine-tuning of the system are currently on the way in our laboratory.

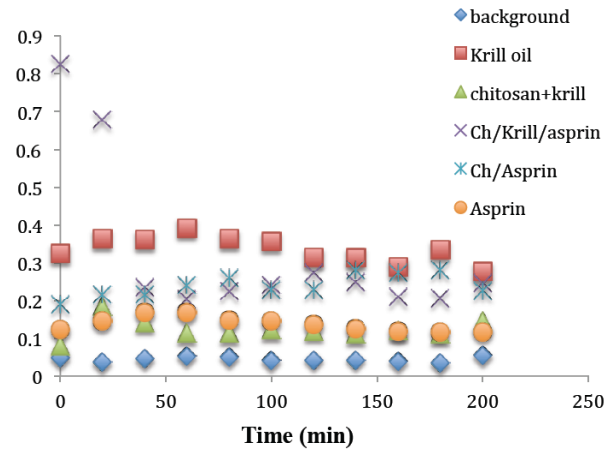

Figure 5. Antioxidant capacity measured at 450 nm using the previously described spectrophotometric assay to asses the hydrogels and corresponding ingredients antioxidant capacity after 6 month under storage under ambient temperature condition. Antioxidant capacity was measured during the first 2 hours of exposure.

3.12. Insight into microbiological investigations.

In the present study, tetracycline was selected because of its wide application, both locally and systemically, in wound healing. A commercially available standard bacterial strain was selected to investigate whether the preliminary goal of preserving the activity of antibiotics, after incorporation into preparations, can be obtained. Our results showed that the cross-linked chitosan sponges were able to deliver active antibiotic for 10 days. Chitosan hydrogen scaffolds were designed in this study as carriers for antibiotics and showed a steady release of the medication. Three-dimensional chitosan matrices have been shown to be excellent tissue engineering scaffolds for cell attachment and growth. Chitosan has a scalloped structure and has been used in tissue engineering to culture hepatocytes, fibroblasts and cartilage cells because of its ability to promote cell attachment and growth [32–38]. In our investigation, chitosan was selected as the carrier for tetracycline, mainly because it can both carry and deliver the medication, but also because it has other useful bioactivities such as antioxidant and anti-inflammatory properties [40].

3.13. Bioadhesion.

Chitosan hydrogels showed the highest adhesive force and the work of adhesion this can be expected because of the well known intrinsic bioadhesive properties of chitosan. The adequate water absorption capacity together with the cationic nature, which promotes binding to the negative surface of skin or dentin structure can also interpret this results. According to Caffaggi, hydration of the polymer causes mobilization of the polymer chains and hence influences polymeric adhesion [36]. Appropriate swelling is important to guarantee adhesivity; however, over hydration can form slippery non-adhesive hydrogels [37]. In addition the molecular arrangement of the polymeric chains, which are present in the new hydrogels, such as propolis, aspirin, ibuprofen and naproxen can further unable to interact further with the substrate The correlation between the force and work of adhesion is noticeable for all. Further experiments are to be conducted on the skin samples to evaluate the bio-adhesive capacity of the designer hydrogels.

4. CONCLUSIONS

The materials were tested using effective in-vitro free radical generation model as functional additive prototypes for further development of "dual function restorative wound healing materials". We quantified the effects of functional designer biomaterials on the dentin bond strength of a composite and evaluate the bio-adhesive capacity of the materials in the 2 separate "in vitro" systems. The added benefits of the chitosan:vitamin C host:guest complex treated hydrogels involved positive influence on the tetracycline release, increased dentin bond strength as well as demonstrated in vitro "build in" free radical defense mechanism and there for acting as a "proof of concept" for the functional multi-dimensional restorative wound healing materials with the build in free radical defense mechanism. A steady slow release of tetracycline, while maintaining antibiotic effects against the tested bacteria, for at least 10 days was shown from designer chitosan-therapeutic agent hydrogels. Based on our results, we can conclude that the chitosan-antioxidant containing hydrogels are a suitable carrier for tetracycline to be slow-released. Within the limitations of the study design chitosan based hydrogels are suitable materials for functional restorative and wound healing applications in vitro. However, future investigations are necessary to validate this hypothesis. The addition of antioxidants to the tetracycline containing prototype delivery system had a beneficial effect on the design of the hydrogel by slowing down the release of tetracycline and thereby enabling a sustainable antifungal activity over time.

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6. ACKNOWLEDGEMENTS

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