ORIGINAL ARTICLE

Insights into functional tea infused-chitosan hydrogels as potential bio-active restorative materials

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ABSTRACT

Introduction: We described novel chitosan hydrogels (chitosan-H) containing tea infusions (green, red and black) as functional additive prototypes with special focus on the design and functionality of dual action composite restorative materials. Their intended uses include remineralizing bases/liners, therapeutically active restorative materials and/or functional additives as well as functional prototype of the drug delivery system. **Materials and Methods:** The above mentioned hydrogels were prepared by dispersion of the corresponding component in glycerol and acetic acid with the addition of chitosan gelling agent. The surface morphology scanning electron microscope (SEM), release behavior (physiological pH as well as acidic conditions), stability of the hydrogels as well as antioxidant capacity of the tea infused hydrogels was evaluated. **Results:** It was found that all the anti-oxidant chitosan-H hydrogels treated dentine gave significantly ($P < 0.05$; Non-parametric ANOVA test) higher shear bond strength values than dentine treated or not treated with phosphoric acid. Overall, there was a small relapse in the shear bond strength after 6 months. The SEM is employed to observe the surface of the newly made functional restorative materials. The anti-oxidant capacity of various black, red and green tea infusions was investigated and demonstrated increased antioxidant stability of the newly prepared material stability. **Conclusion:** We have developed and evaluated several functional chitosan hydrogels with several targets as therapeutic restorative materials, the added benefits of their unique functionality involve increased dentin adhesive bond strengths (after 24 h and after 6 month), concept of using functional materials as carriers for pro-drugs as well as display certain degree of defense mechanism for a free radical damage.

Key words
Antioxidant-chitosan hydrogels, bond strength, functional restorative materials, scanning electron microscope, tea antioxidants

INTRODUCTION

Bioadhesive polymers appear to be particularly attractive for the development of alternative etches free dentin bonding system with an added advantage of additional therapeutic delivery systems to improve intradental administration of therapeutic and prophylactic agents if necessary.[1-5] Chitosan, which is a biologically safe biopolymer, has been proposed as a bioadhesive polymer and are of continuous interest to us due to their unique properties and flexibility in a broad range of oral applications reported by others and us recently.[6-10]

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The objectives of the following study were to evaluate the effect of chitosan-green tea; chitosan-black tea and chitosan-red tea extract hydrogels on the bond strength of a composite resin to dentine in the etch-free protocol, secondly to use scanning electron microscope (SEM) imaging for the characterization of the surfaces of the novel materials.

MATERIALS AND METHODS

Three types of tea were studied (Green, Red and Black). Two commercially available tea products were studied for each tea type. (1) Green (Tetley Green Tea, Lipton Green Tea) (2) Red (Vital Organic Rooibos Tea, Just Rooibos African Tea) (3) black (Tetley Black Tea, Lipton Black Tea). All teas were purchased from a commercial supplier (Safeway, Priceline and Wholesale Chemist, QLD, Australia). While Chitosan (Aldrich, Australia), glycerol (Sigma, USA), glacial acetic acid (E. Merck, Germany) were used as received. The degree of de-acetylation of typical commercial chitosan used in this study is 87%. Chitosan with a molecular weight

2.5 × 10^3 KD was used in the study. The isoelectric point (PI) is 4.0-5.0.

Infusion preparation

Each tea mixture (1.5 g) was mixed with 100 ml of boiling water for 5 min, with constant shaking and the samples were then filtered through Whatman No. 1 filter paper (Sigma-Aldrich, Australia). Every experiment was performed in triplicate. Chemical characterization was conducted to asses overall antioxidant capacity and total phenolic concentration as described later. At no stage were individual components of the tea infusions, were attempted to be separated and the work is currently underway to identify the single components of the mixtures and the antioxidant capacity.

Preparation of the gels of the various tea-infused hydrogels

Chitosan hydrogels have been prepared using the methodology previously described.[10] Briefly, the corresponding antioxidant mixtures extracts from the corresponding teas, were incorporated by dispersion of corresponding antioxidant powder 0.02 g in glycerol (5% w/w) using a mortar and a pestle. A volume of 1 ml of glacial acetic acid (3% w/w) the corresponding antioxidant mixtures were incorporated into the mixture and the summary of the newly prepared materials were highlighted in Table 1.

Determination of gel pH

A total of 1 g of the prepared gels was accurately weighed and dispersed in 10 ml of purified water. The pH of the dispersions was measured using pH meter (HANNA instruments, HI8417, Portugal).

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 (SEM, Hitachi S4800, Japan).

Gel stability

Stability of the gel formulations was also investigated. The organoleptic properties (color, odor), pH, drug content and release profiles of the gels store at 20°C were examined on days (0, 15, 30 and 178). The performance of the hydrogels was not affected by the storage conditions, suggesting remarkable stability of the novel biomaterials under investigations.

Studies of equilibrium swelling in the alternative drug delivery systems

The known weight tea infused-containing dry gels were immersed in pH 4.0, pH 9.0 buffer solutions, respectively and kept at 25°C 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 W_s and W_d are the weights of the gels at the equilibrium swelling state and at the dry state, respectively.[11-14] Experiments were repeated in triplicate for each gel specimen and the mean value calculated.

Shear bond strength tests for dentine bonding

Extracted non-carious, intact, human molars stored in water containing a few crystals of thymol at 4°C were used within 2 months. Samples were checked before use for any damage caused by their removal. All teeth were thoroughly washed with water to remove residual thymol and transferred into an artificial solution of saliva for 48 h prior subjecting the teeth to the experimental solution.

The roots of the teeth were removed with a separating disc and the occlusal enamel removed by grounding wet on 60-grit silicon carbide (SiC) paper. The teeth were embedded in PVC (Consjit Tubing, SA PVC, JHB, RSA)

Table 1: Gel formulation prepared in the study

Gel formulation	Gel number	Chitosan (w/w%)	Additive green tea extract (w/w%)	Additive rooibos tea extract (w/w%)	Additive black tea extract (w/w%)	pH
Chitosan-H	Gel-1	5	0	0	0	5.10
Chitosan-H-TG1	Gel-2	5	1	0	0	5.64
Chitosan-H-TG2	Gel-3	5	1	0	0	5.54
Chitosan-H-TR1	Gel-4	5	0	1	0	4.94
Chitosan-H-TR2	Gel-5	5	0	1	0	5.84
Chitosan-H-TB1	Gel-6	5	0	0	1	5.74
Chitosan-H-TB2	Gel-7	5	0	0	1	5.34

Where TR1 and TR2 are extracts from rooibos teas 1 and 2, TB1 and TB2 are extracts from black teas 1 and 2, TG1 and TG2 are extracted from green tea 1 and 2

pipe containers with cold cure acrylic resin and hence that the ground occlusal surfaces projected well above the resin. The 10 mm length pipes were put on a glass surface with one end blocked by the glass and the embedding done through the open-end. Immediately after embedding the occlusal surfaces were ground wet with 180-grit followed by 600-grit SiC on a polishing machine to expose the superficial dentin. The samples were washed under a stream of tap water. Two of these studs (next to each other) were then bonded to the polished dentine surface of each tooth through the bonding agent XP bond (Dentsply, New York, USA), as suggested by the manufacturer. A standardized gig (Ultra dent ISO A2-70) with an internal diameter of 2.5 mm and height of 3 mm was used to shape the composite resin stud (SDR, DENTSPLY, CA, USA, Batch number 1105000609, Ext 2013-04), which was cured, as suggested by the manufacturer. The bonding agent XP Bond contained: Carboxylic acid modified methacrylate (TCB resin), phosphoric acid modified acrylate resin (PENTA), urethane dime acrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethylmethacrylate (HEMA), butylated benzenediol (stabilizer), ethyl-4-dimethylaminobenzoate), camphorquinone, functionalized amorphous silica, t-butanol.

In this way were 48 teeth samples (each containing 2 studs) prepared and divided into 9 groups of 8 each, A, B, C, D, E, F, K, L and M [Table 2] and stored in a solution of artificial saliva in order to replicate the oral cavity conditions. These groups were then treated as outlined in Table 2. After 24 h one stud of each tooth was tested for shear bond strength and the other one after 6 months. An Instron Universal Testing Machine (5900 System, Instron) at a crosshead speed of 0.5 mm/min was used to test the de-bonding strength. All data were analyzed using the non-parametric ANOVA test.

RESULTS

Properties of antioxidant-chitosan gels

Antioxidant content in 0.3 g of the different gel formulations from the prepared formulae was presented in Table 1. The prepared gel formulations have a uniform distribution of drug content, homogenous texture and color. The pH of the formulations was ranging from 4.94 to 5.84. Table 1 represents the summary of the tea infused-chitosan Gels prepared in this study.

Table 2: Groups tested (8 teeth/groups)

Groups	Experimental conditions
A	37% of phosphoric acid+primer+bonding immediately (negative control)
B	Self-etching primer+bonding immediately (positive control)
C	Gel1+primer+bonding immediately
D	Gel2+primer+bonding immediately
E	Gel3+primer+bonding immediately
F	Gel4+primer+bonding immediately
K	Gel5+primer+bonding immediately
L	Gel6+primer+bonding immediately
M	Gel7+primer+bonding immediately

The characterization of tea infused containing-chitosan gels (Gel-1 to Gel-7)

The SEM images were obtained to characterize the microstructure of the freeze-dried tea infused composite gels and are presented in Figure 1. It could be seen that the gels displayed a homogeneously pore structure. 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 "skin" of the gels can be seen and the collapse of the surface pores may be due to freeze-drying process.

Studies of equilibrium swelling in tea infused-chitosan gels (Gel-1 to Gel-7)

The hydrogels remain in the cylindrical form after swelling. Compared with dry state hydrogels, the swollen state hydrogel volume display significant increases and are summarized in Figure 2.

Equilibrium SR of hydrogels exerts an influence on their release rates. The reduction in equilibrium swelling capacity is due to the formation of a tight network structure in high content. Environmental pH value has a large effect on the swelling behavior of these gels. From Figure 2, it is clear that the SR value increases with the increase of pH. Such pH dependent properties of the hydrogels come from the polyelectrolyte nature of chitosan segments in the hydrogel network. Namely, when the pH value of the buffer solution (pH 9.0) was far higher than the PI of GEL (PI 4.0-5.0), the carboxyl groups were de-protonized to carry negative charges, which made molecular chains repulsed to each other. The network became looser and it was easy for the water molecules to diffuse into the cross-linked network

DISCUSSION

It is well-established that HO can be generated from a reaction known as the biologic Fenton reaction and this reaction requires the presence of H_2O_2.[15,16]

The generation of HO from the biologic Fenton reaction has been shown to be a critical factor in various reactive oxygen species-induced oxidative stresses.[17,18] H_2O_2 and HO might be related to apoptosis in atherosclerosis.[19] Godley *et al.* also reported that blue light induces mitochondrial deoxyribonucleic acid damage and cellular aging.[20]

The reactive nature of the surface has been investigated using the SEM and comparison confirms the reactive nature of the transformation [Figures 3 and 4].

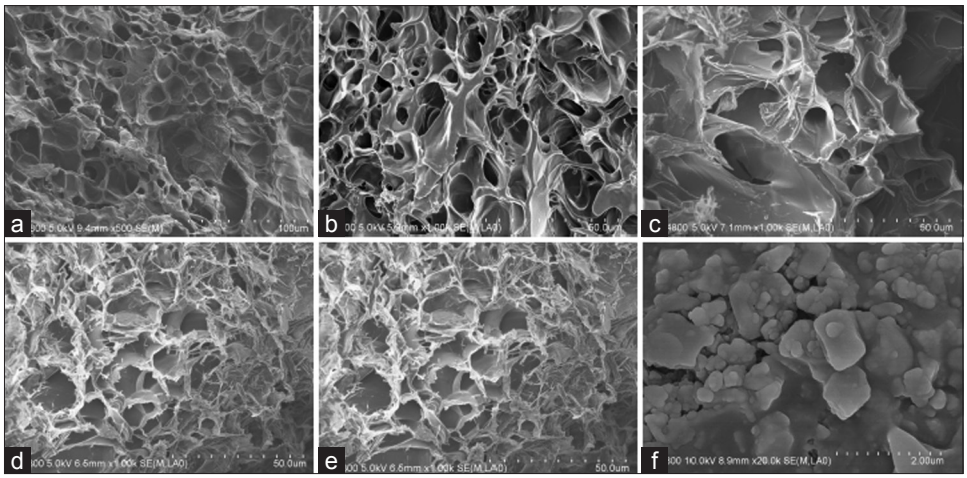

Figure 1: Scanning electron microscope photographs of interior morphology of the selected gels under investigation for (a) Gel-2, (b) Gel-3 (c) Gel-4, (d) Gel-5, (e) Gel-6, (f) Gel-7

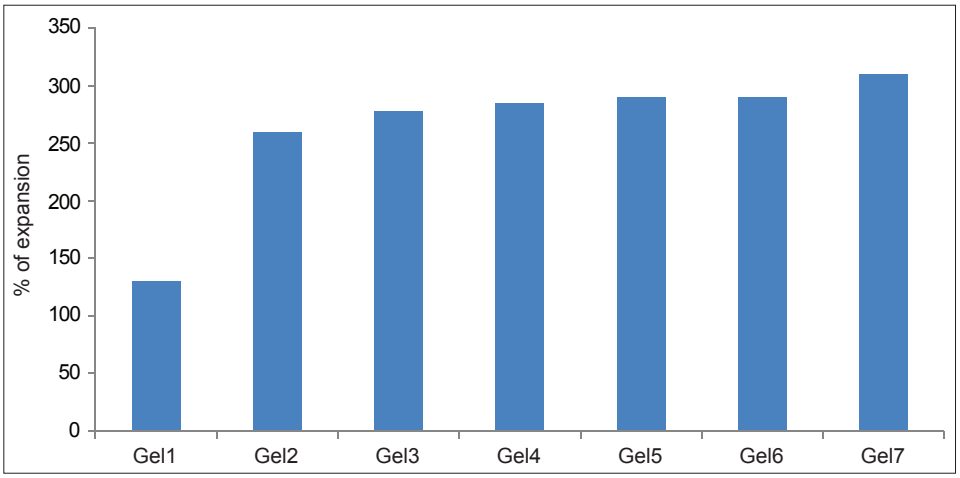

Figure 2: Water uptake degree of the tea infused chitosan-hydrogels: Gel 1-Gel 7, ($n = 6$, $P < 0.05$)

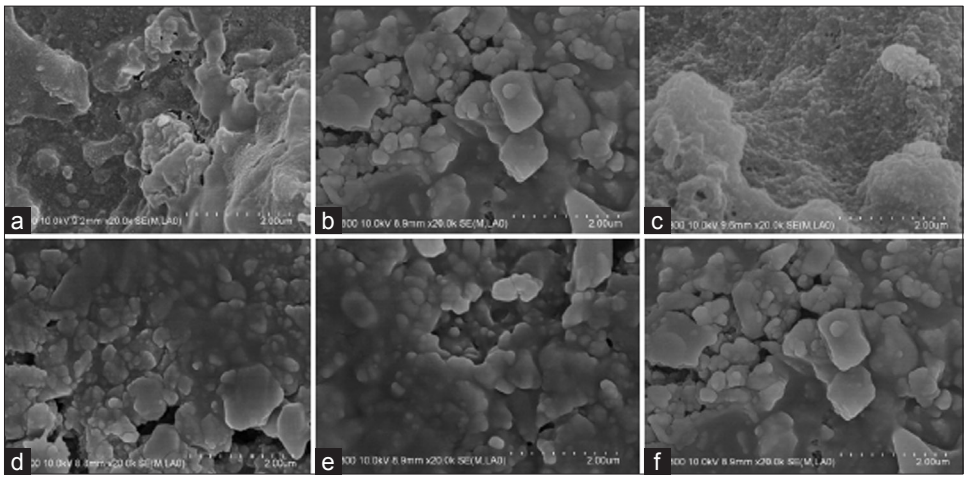

Figure 3: Scanning electron microscope images of the reactive surface of the composite under experimental conditions after 24 h: a. Gel-2 b. Gel-3, c. Gel-4, d. Gel-5, e. Gel-6, f. Gel-7

The visible surface deterioration of the surface of the composites used in the experiments indicated that the surface exposed to conventional "dentistry related chemical exposure" is significantly affected over time (24 h vs. 6 month). The chemical nature of this transformation is currently under investigation in our laboratory.

Mean shear bond strength values are summarized in Figure 5 for bonding to dentine after 24 h and Figure 6 for bonding to dentine after 6 month. The increase in bond strength of the dentine treated with the antioxidant containing hydrogels in Groups C to Group K compared with the bond strength of the conventionally bonded teeth such as Group A and Group B. Interestingly the increase in bond strength was also observed in groups of hydrogen peroxide exposed samples such as samples in Group A or Group K suggesting that there additional benefits associated with chitosan: Antioxidant system are in need of further investigations. There was no significant difference in the shear bond strength after 24 h and we are currently in the process of evaluating the shear bond strength after 6 months of storage of the sample in the artificial saliva.

The results of this study suggest that the optimum results for the strengthening of dentine bonding can be achieved through the immediate treatment with tea infused: Chitosan. The additional advantage of the system may

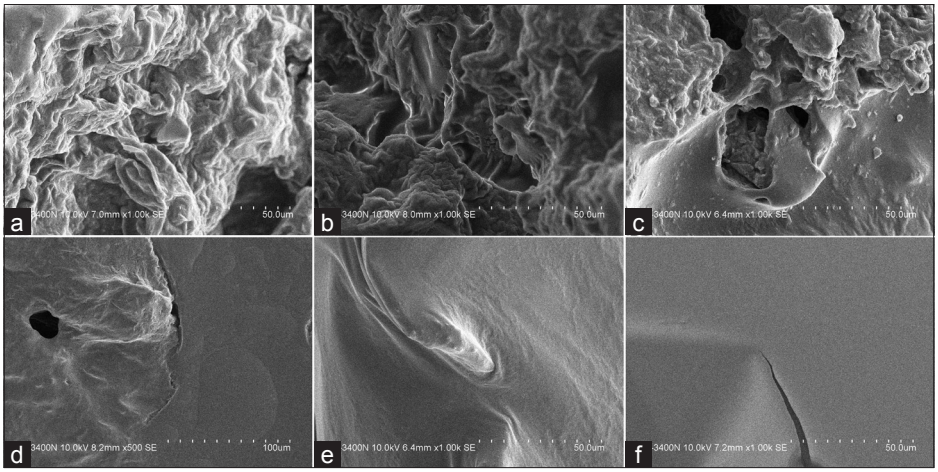

Figure 4: Scanning electron microscope images of the reactive surface of the composite under experimental conditions after 6 month: a. Gel-2 b. Gel-3, c. Gel-4, d. Gel-5, e. Gel-6, f. Gel-7

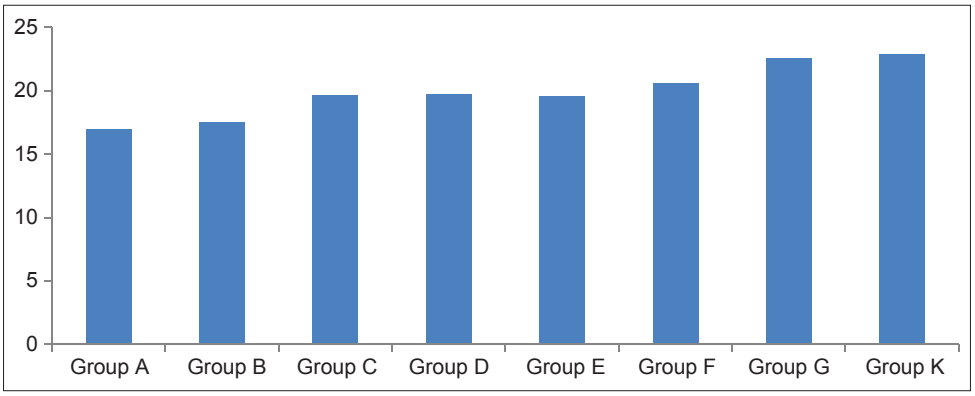

Figure 5: Shear bond strength of hydrogels after 24 h of bonding to dentine MPa

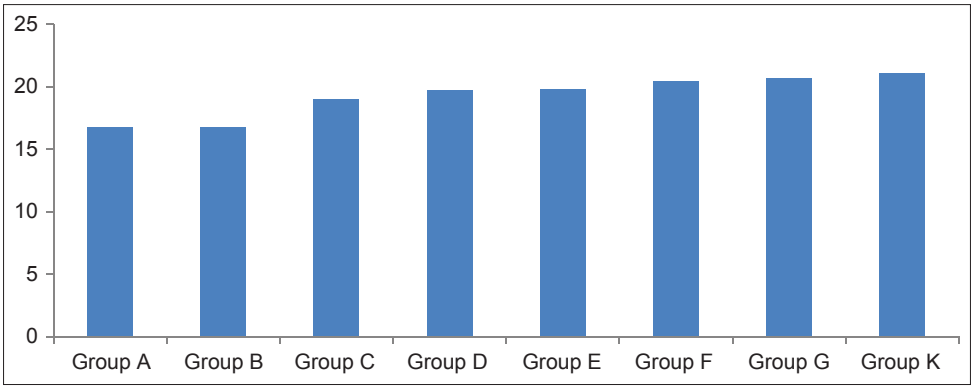

Figure 6: Shear bond strength of hydrogels after 6 month of bonding to enamel MPa

suggest that, antioxidant release from chitosan gel depends on the physical network structure (open cell like structure) as well as pH properties and flexibilities of the material. Antioxidant release occurs through the pores of the low polymer concentration while chitosan concentration increment resulted in more cross-linking of the network structure; consequently slower antioxidant release from the gel base was achieved and there for weaker adhesive properties of materials such as Gel-1 in case of the other groups.[21]

A statistically significant difference (decrease) was found between C (24 h) relative and C after 6 month groups ($P < 0.02$). Groups D, E and F after 24 h were statistically significantly different (higher) than D, E and F after 6 months ($P < 0.01$). Group C also differed significantly from J after 24 h and 6 month ($P < 0.01$) as well significant difference was found to be between groups J and K, L and M (after 24 h and 6 month) ($P < 0.01$).

It was shown by others and us earlier,[21] that the swelling properties and antioxidant release from gels were increased under acidic conditions due to the protonation of the primary amino group on chitosan.[21] Chain relaxation due to protonation of amino groups leads to a faster hydrogen bond dissociation and efficient solvent diffusion. Thus, the appreciable increase in water uptake at lower pH values can be attributed to the high porosity of the gels, which seems to govern the diffusion of the solvent in the gel matrix and thus, the release of the antioxidant from the gel.[21] The additional benefit of using chitosan: Antioxidant system as a bonding/pre-bonding to the 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). 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. Therefore the newly developed chitosan: Antioxidant systems might be able to address the shortfalls in the current perspectives for improving bond durability through understanding factors affecting the long-term bonding performance of modern adhesives and addresses the current perspectives for improving bond durability.

CONCLUSIONS

We have developed and evaluated several functional chitosan hydrogels with several targets as therapeutic restorative materials, the added benefits of their unique functionality involve increased dentin adhesive bond strengths (after 24 h and after 6 month), concept of using functional materials as carriers for pro-drugs as well as display certain degree of defense mechanism for a free radical damage.

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