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Microwave assisted prepared interpenetrating hydrogels from guar-gum: chitosan IPN and guar gum hydrogels as novel functional materials: bonding, antioxidant and bioactivity

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ABSTRACT

The interpenetrating polymeric network (IPN) has emerged as one of the most useful novel biomaterial, which is entanglement of polymer networks of structurally and functionally different cross-linked molecular building blocks. The development of IPN is captivating because they provide free volume space for the easy encapsulation of drugs in the three-dimensional network structure, formulated by cross-linking of several distinct polymer network. Chitosan and guar gum are a natural, biodegradable, nontoxic, mucoadhesive, and biocompatible polymer, have found diverse pharmaceutical applications including functional biomaterials. Cytotoxicity of common dental resin components are well documented and is prone to cause the undesired biological responses such as oxidative damage of dental and related tissue as well as suppressing odontogenic differentiation of dental pulp cells. As antioxidants were found to protect cells from cytotoxicity of resin monomers in previous studies, we investigated the effects of common antioxidants, such as β-carotene, resveratrol, and propolis on anti-differentiation activity of bonding agents without compromising bond strength. Methacrylate monomers used in dentistry have shown to induce DNA double strand breaks (DSBs), a severe type of DNA damage. The formation of classical products of oxidative DNA damage, were investigated using the well established and reliable in vitro model, developed earlier by us which relies on correlation of BSA solubility and the amount of free radicals generated at the reaction site using UV-detection method. In this work, we hope to develop and evaluated novel functional biomaterials suitable for ameliorating dentin bonding system as well as asses in vitro the free radical protective properties of the novel materials by utilizing earlier developed model protein (BSA) probe was utilized to evaluate the chitosan-based carriers. Morphological behaviors, release behaviors (physiological pH and in acidic conditions) and stability guar gum: chitosan systems have been investigated. We considered worthwhile to study the antioxidant properties of β-carotene, resveratrol, and propolis as examples of the potential antioxidant additiives.Conclusion: Antioxidant containing chitosan hydrogels may reduce detrimental effects induced by common composite restorative agent in vitro and introducing additional therapeutic health benefits.

Keywords: *chitosan, guar gum, free radicals, hydrogels, functional biomaterials, bioadhesive dual functional materials.*

1. INTRODUCTION

In the recent years interpenetrating polymeric network (IPN) hydrogels has generated considerable interest as a biomaterial vehicle for drug delivery [1-4]. IPN hydrogels amalgamate the conventional dosage forms as well as novel drug delivery systems, by offering a biocompatible, convenient, and stable drug delivery system for molecules as small as non-steroidal anti-inflammatory drugs or as large as proteins and peptides. Hydrogels are the three-dimensional polymers that expand in aqueous solutions. In the swollen state, they are soft and gelatonous, resembling the living tissue therefor exhibiting excellent biocompatibility [5]. Polymeric hydrogels is of considerable interest as biomaterials in drug delivery research. IPNs represent a combination of two polymers in network form. [6,7]. The use of polymers as reactants in microwave-assisted reactions is less common than the use of monomers. Here, we demonstrated that microwave irradiation could serve as a valuable method of hydrogel synthesis by using a combination of polymeric reactants. This is the first report of the use of microwaves to form hydrogels in this way. Microwave irradiation is not ionizing and therefor cannot be used to form hydrogels from a single type of polymers.

Modern dental adhesive systems come in close and prolonged contact with vital dentin, their influence onpulp tissue is critical [1-3]. Thus, the biocompatibility of dentin bonding agents is a relevant aspect of the clinical success of these materials [1–3]. Dentin bonding agents alone proved to be cytotoxic [4], and it adverse biological effects are closely correlated with the type and quantity of leachable components significantly influence the biological behavior of resin restorations [5–10]. Cytotoxicity of dentin bonding agents in vitro has been examined using a variety of cell lines including primary human pulp and pulp-derived cells [4,5-8,11-14]. Chitosan is a natural cationic polysaccharide derived from chitin by partially deacetylating its acetamido groups in strong alkaline solutions [15]. Over the last two decades, chitosan has been widely used used for various biomedical and drug delivery applications because of its low toxicity and good biocompatibility and antimicrobial and bio-adhesive properties [16–18]. The main objective of this study was to evaluate the effect of microwave synthesized with guar gum:chitosan-antioxidant hydrogels on the antioxidant defense mechanism (resveratrol, propolis and β-carotene on in-vitro model of oxidative damage potentially generated by the model composite.

Secondly, we aimed to investigate the chemical nature of the defense on the interface between the composites and antioxidant/chitosan hydrogel layer formation by the use of SEM. In vitro model for DNA damage model for assessment has been evaluated. Bio-aspects, such as bio-adhesion and bioactivity have been accessed in vitro.

2. EXPERIMENTAL SECTION

Preparation of chitosan-guar-gum based IPN gels with resveratrol, β-carotene or propolis as potential substrate for bonding to dentin. Antioxidant (resveratrol, β-carotene, or propolis) gels were prepared by dispersion of corresponding antioxidant powder 0.02 grams in glycerol (5% w/w) using a mortar and a pestle. 1 milliliters of glacial acetic acid (1% w/w) was then added with continuous mixing and finally guar gum and chitosan:guar gum (1:1 w/w) polymer was spread on the surface of the dispersion and mixed well for 12 hours. The mixtures were subjected to microwave irradiation (CEM Discover Labmate) with set temperatures and held times. Processing conditions in the range 100-200^{o}C and 10-60 min were used with a pressure cut-off of 200 psi and a power of 200W. Corresponding antioxidant gel had been prepared with 5 %w/w concentrations of chitosan:guar gum or gum gelling agent. The summary of the newly prepared materials is highlighted in Table 1.

Table 1. Gel formulation prepared in the study, containing Guar Gum: Chitosan and Guar Gum as a functional backbone of the newly designed material.

Gel formulation		Chitosan / Guar Gum Concentration	Medium	pH
Chitosan/Guar Gum-H	Gel-1	5	1% acetic acid	5.12
Chitosan/Guar Gum-H + resveratrol	Gel-2	5	1% acetic acid	5.18
Chitosan/Guar Gum-H + propolis	Gel-3	5	1% acetic acid	4.98
Chitosan/Guar Gum-H + β-carotene	Gel-4	5	1% acetic acid	5.42
Guar Gum-H + resveratrol	Gel-5	5	1% acetic acid	4.89
Guar Guam-H + propolis	Gel-6	5	1% acetic acid	4.73
Guar Guam-H + β-carotene	Gel-7	5	1% acetic acid	4.62

2.1. Determination of gel pH.

One gram of the prepared gels was also weighed and dispersed in 10 ml of purified water. The pH of the dispersions was measured using a combination pH glass electrode coupled to a potentiometer (HANNA instruments, HI8417, Portugal).

2.2. Morphology of the gels

The interior and the surface morphology were observed in scanning electron microscope (SEM, Hitachi S4800, Japan).

2.3. Shear bond strength tests for dentine bonding.

Extracted non-carious, intact, human molars teeth stored in water containing a few crystals of thymol at 4°C were used within two months. Samples were checked before use for any damage caused by their removal. The teeth were embedded in PVC (Consjit Tubing, SA PVC, JHB, RSA) pipe containers with cold cure acrylic resin so that the ground occlusal surfaces projected well above the resin. 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. A standardized zig (Ultradent 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, Exp 2013-04). Two of these studs were then bonded to the polished dentine surface of each tooth via the bonding agent XP bond (Dentsply, New York, USA), as suggested by the manufacturer. The bonding agentcontains: carboxylic acid modified dimethacrylate (TCB resin), phosphoric acid modified acrylate resin (PENTA), urethane dimetacrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethylmethacrylate (HEMA), butylated benzenediol (stabilizer), ethyl-4-dimethylaminobenzoate), camphorquinone, functionalized amorphous silica, t-butanol. In this way were 72 teeth samples (each containing 2 studs) prepared and divided into 9 groups of 8 each, A, B, C, D, E, F, J, K, and L. (Table 2) and stored in a solution of artificial saliva. These groups were then treated as outlined in Table 1. After 24 hours one stud of each tooth was tested for shear bond strength and the other one after 6 months. 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 analysed using the non-parametric ANOVA test.

2.4. Morphology of the gels.

The interior and the surface morphology were observed in scanning electron microscope (SEM, Hitachi S4800, Japan).

2.5. 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 20^{o}C were examined on days (0, 15, 30 and 178).

2.6. Studies of equilibrium swelling in the alternative drug delivery systems.

The equilibrium swelling ratio (SR) was calculated using the following equation:

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

where Ws and Wd are the weights of the gels at the equilibrium swelling state and at the dry state, respectively [19]. Experiments

were repeated in triplicate for each gel specimen and mean value was obtained.

2.7. Bioadhesive study.

Bioadhesion studies were done using Chatillon apparatus for force measurement [20]. This method determines the maximum force and work needed to separate two surfaces in intimate contact [20]. The hydrogels (0.1g) were homogeneously spread on a 1cm^2 glass disk, the disks were attached to the support of the tensile strength tester using double side adhesive. The gel was brought into contact with the commercially available band aid, in order to simulate the skin attachment or the contact with slice of dentin was established in order to imitate adhesion of the gel to the tooth structure, after a pre-evaluated contact time (1 min) under contact strength (0.5N). The 2 surfaces were separated at a constant rate of displacement (1mm/s). The strength was measure 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.

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 (guar gum-H)
Group D	Gel2+primer+ Bonding immediately (guar gum-H + resveratrol)
Group E	Gel3+primer+ Bonding immediately (guar gum-H + propolis)
Group F	Gel4+primer+ Bonding immediately (guar gum-H + β-carotene)
Group J	Gel5+primer+ Bonding immediately (chitosan/ guar gum-H + resveratrol)
GroupK	Gel6+primer+ Bonding immediately (chitosan/ guar gum-H + resveratrol)
Group L	Gel7+primer+ Bonding immediately (chitosan/guar gum-H + resveratrol)

3. RESULTS SECTION

3.1. The characterization of antioxidants containing-Guar-Gum: Chitosan Gels: (Gel-1 to Gel-7).

The SEM images were obtained to characterize the microstructure of the freeze-dried 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.

3.2. Studies of equilibrium swelling in Guar-Gum-Chitosan IPN Gels (Gel-1-Gel-4) and Guar-Gum Gels (Gel-5-Gel-7).

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 2.

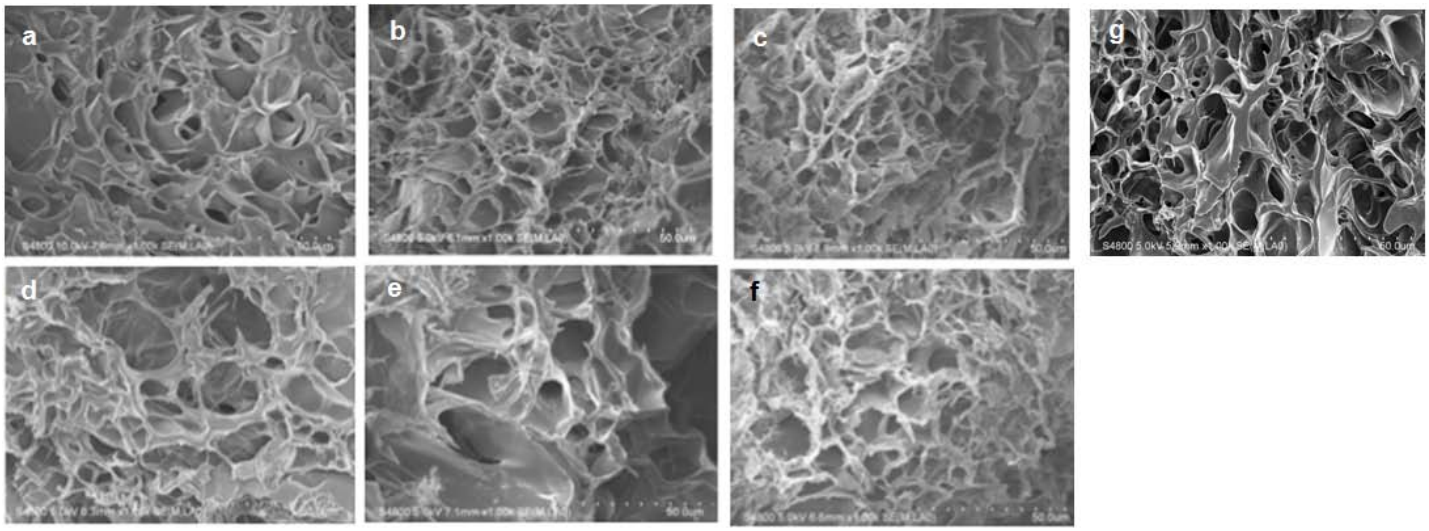

Figure 1. SEM photographs of the freeze dried interior morphology of the selected gels under investigation for (a) Gel-1, (b) Gel-2, (c) Gel-3, (d) Gel-4, (e) Gel-5 (f) Gel-6 and (g) Gel-7.

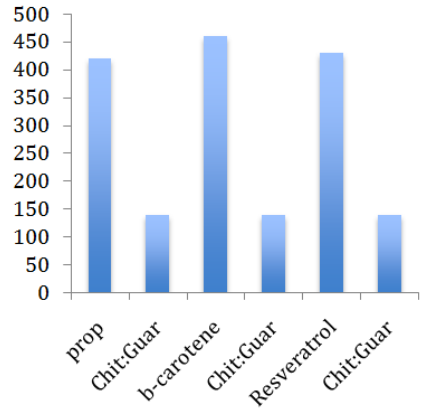

Figure 2. Water uptake degree of the gels: Gel1-Gel 4, (n=6, p<0.06).

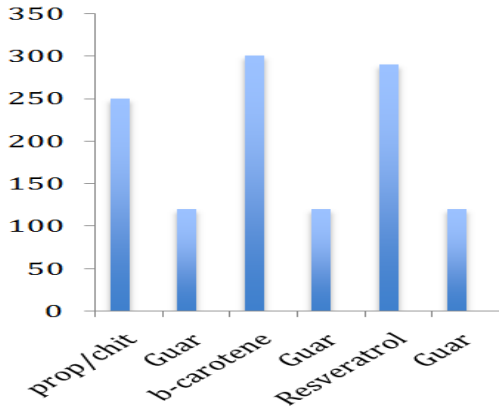

Figure 3. Water uptake degree of the gels: Gel5-Gel 7, (n=6, p<0.06).

Equilibrium swelling ratio (SR) of hydrogels directly influences the functional material release rates. The reduction in equilibrium swelling capacity is attributable to the formation of a tight network structure in high content. Environmental pH value in case of chitosan containing IPN hydrogels and presence of long hydrophilic chains 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 as well as the presence of polyol in the hydrogel makeup.

Namely, when the pH value of the buffer solution (pH 9.0) was far higher than the isoelectric point (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. Interestingly, the swelling trends of guar gum containing hydrogels prepared by microwave-assisted synthesis in our case are similar to the ones containing chitosan (non-charged versus charged groups containing functionality of the polymer and therefor warrant further investigation into this unique property.

3.3. Shear Bond Strength.

Mean shear bond strength values after 24 hours were listed in Figure 2. A significant increase in bond strength of the dentine treated with all 6 different gels (Figures 2) was found relative to the two groups with the conventionally bonded dentine (i.e. dentine not treatred with phosphoric acid). Interestingly the increase in bond strength was also observed in the groups treated with phosphoric acid and chitosan:guar gum hydrogels, suggesting that there additional benefits associated with IPN: antioxidant system are in need of further investigations.

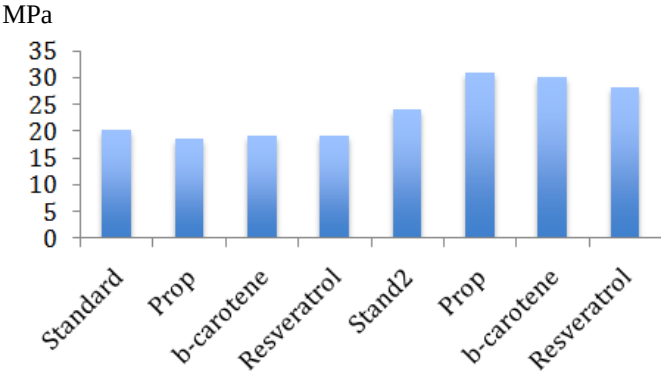

Figure 4. Shear bond strength (MPa) of 10 Groups after 24 hours of bonding to dentine.

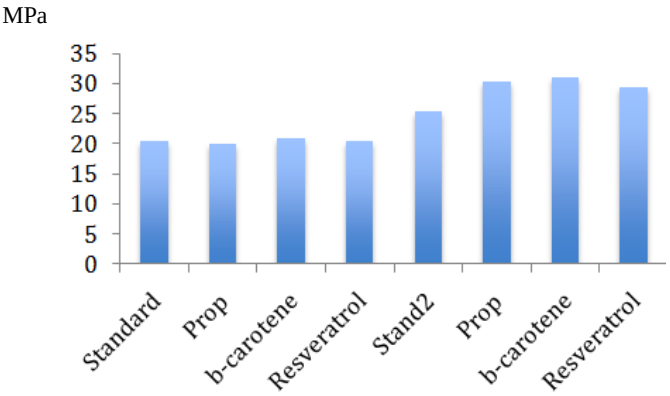

Figure 5. Shear bond strength (MPa) of 9 Groups after 6 month of bonding to dentine

It was found that chitosan: guar gum-H treated dentine gives higher values than dentine treated with guar-gum based hydrogel alone no phosphoric acid. Furthermore, the presence of the anti-oxidants (resveratrol, propolis and β- carotene) improved the shear bond strength without (D, E, F) phosphoric acid treatment.

In general, all 9 groups gave a relapse in the shear bond strength after a 6 months (Figure 5) storage period compared to 24 hours (Compare groups in Figure 4 to that in Figure 5).

The results of this study suggested that the optimum values for the strengthening of dentine can be achieved through the immediate treatment with resveratrol, beta-carotene and propolis newly designed hydrogels with the increase of dentine bond strength (Figure 2). The results further demonstrated that resveratrol, β-carotene and propolis showed synergistic effect with guar gum and guar gum: chitosan containing interpenetrating hydrogels on significant improvement in bond strength after 24h and 6 month (Figure 4 and 5). Additional benefits of chitosan:guar gum interpenetrating networks are currently being further evaluated in our laboratories as the in-depth understanding of the mechanism of action of this unique materials.

These results are in accordance with the previously reported observations by Upin [21] on the performance of sodium ascorbate hydrogels on the bond strength of composite material as well ability of the newly developed hydrogels to counteract the prohibitive oxygen layer formation on the interface of the

composite and adhesive material as well as allow the material to be easily applied in the desired area due to the higher viscosity and better handling properties in comparison to aqueous solution of corresponding active agents.

3.4. Solubility of BSA and free radical detection *in vitro*.

In the present work we adopted the method of Zs.-Nagy and Nagy [22] for recording changes in the water solubility of BSA exposed to the chemical source of hydroxyl free radicals to characterize the antioxidant efficiency of Propolis, Chitosan and BSA in a non-lipid protein system [22]. BSA, a completely water soluble protein, exposed to the above Fenton reaction system, was losing its water solubility depending on the concentration of the chelated. iron, as shown in Figure 7. Control experiments showed that omission of either chelated iron or H_2O_2+ascorbate from the reaction mixture gave no decrease in protein solubility (data not shown). Characteristic fluorescence of 325 (excitation)/415 (emission) was significantly increased in incubations of BSA with the Fenton system supported with 0.75 mM ferrous chelate, indicating the presence of bityrosine covalent bridges. The specific yield of bityrosine fluorescence was found to be strongly dependent on the initial concentration of BSA, which suggests an intermolecular character of bityrosine cross-links. On balance then, the results obtained in this study strongly indicated that the insolubilization of BSA induced by the Fenton system of Fe^{2+}/EDTA/H_2O_2/ ascorbate was caused by free $OH^{\cdot}$radical mediated polymerization giving rise to true covalent cross-links. The model system was found suitable for convenient testing of $^{\cdot}OH$radical scavenging ability of new antioxidants in a non-lipid environment.

3.5. % of BSA solubility.

We also wanted to examine the antioxidant properties of β-carotene, resveratrol and propolis also in a non-lipid environment of a pure protein. 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. In the present study we used the Fenton reaction system of Fe^{2+}/EDTA/ H_2O_2/ascorbate as a source of free radicals to prove the ability of β-carotene, Resveratrol or Propolis to protect BSA against free radical mediated cross-linking, in comparison with an. The reactive nature of the surface has been investigated using the SEM and comparison confirms the reactive nature of the transformation. (Figure 8)

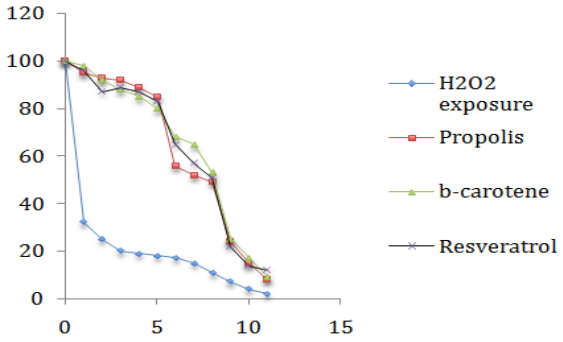

Figure 7. Free radical damage protection during the bonding process after 24 hours using BSA solubility as a probe, with Guar gum:Chitosan hydrogels, where Y-axix is % of BSA solubility and X-axis is time in hours

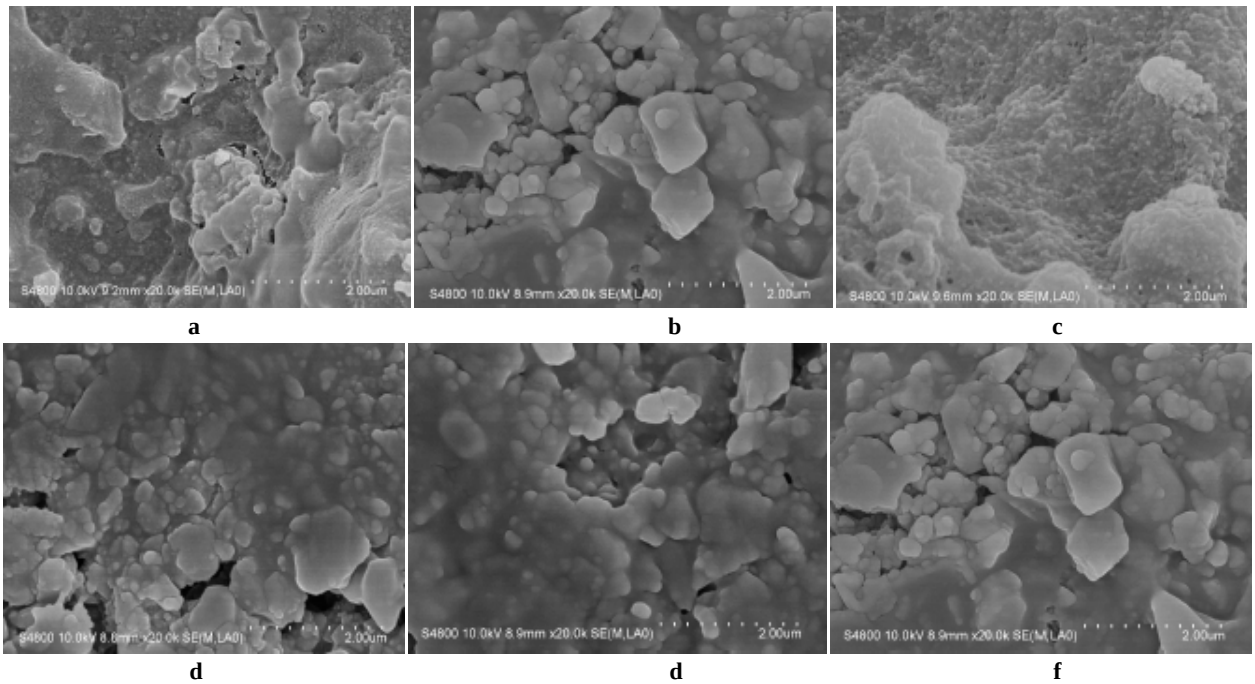

Figure 8. SEM images of the reactive surface of the composite under experimental conditions: a. Gel-2 b. Gel-3, c. Gel-3, d. Gel-4, e. Gel-5, f. Gel-6.

3.6. 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 2. 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 [23]. 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. Chitosan hydrogels showed the highest adhesive force and the work of adhesion this can be expected because of the well known intrinsic bio-adhesive 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. It is ell documented that hydration of the polymer causes mobilization of the polymer chains and hence influences polymeric adhesion [24]. Appropriate swelling is important to guarantee adhesivity; however, over hydration can form slippery non-adhesive hydrogels [25]. In addition the molecular arrangement of the polymeric chains, which are present in the new hydrogels, such as propolis, resveratrol, β-carotene 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 *in vivo* skin samples to evaluate the bio-adhesive capacity of the designer hydrogels.

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	1.25±0.40	1.09±0.35	3.35±0.48	2.92±0.34
Gel-2	0.97±0.25	1.17±0.42	3.19±0.52	3.49±0.42
Gel-3	0.99±0.30	0.99±0.40	2.85±0.41	2.94±0.29
Gel-4	1.12±0.34	1.09±0.24	3.31±0.31	3.38±0.31
Gel-5	1.18±0.28	1.11±0.26	3.49±0.31	3.35±0.28
Gel-6	0.89±0.45	0.96±0.41	2.55±0.46	2.98±0.21
Gel-7	1.12±0.29	1.23±0.30	3.48±0.46	3.81±0.28

The presented values are an average (n=5)

4. CONCLUSIONS

We have developed and evaluated novel class of biocompatible polysaccharide based hydrogels by exploiting microwave-assisted synthesis as an efficient and reliable method of preparation. The mechanical properties of the newly prepared gels are better then the corresponding hydrogels prepared using conventional gelation procedure. The functionality of the inter-penetrating hydrogels to deliver a "build in free radical protection mechanism" as well as increased bonding to dentine systems was demonstrated using all the newly prepared hydrogel. SEM was used to characterize all the aspects of structural features of unique materials as well as some insights into bonding capacities and capabilities of the materials.

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6. ACKNOWLEDGEMENTS

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