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Research Article

Bioactive Materials for Temporary Restorations: From Design to Applications

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Abstract

Objective: To examine the effect of Protemp4:chitosan:nanodiamond bioactive containing modified materials and compare the protective activity of the propolis (Brazilian) and copaiba oil as bio-active additives to compare with N-acetyl cysteine *in vitro*.

Materials and Methods: The bioactive modified temporary materials were prepared by dispersion of the corresponding component with the addition of commercially available temporary restorative material (Protemp 4). The surface morphology (SEM) of the freshly prepared temporization restorative materials. The physicochemical properties such flexural and compressive strength for the bio-active modified materials have been measured and are reported. The effect of the bioactive addition to the commercially available temporary materials and nanodiamond modified variants of bioactive modified temporization materials such as Protemp 4 swelling capacity, free radical quenching ability of the material as well as release of bio-active of copaiba oil and Propolis (Brazilian) were evaluated.

Results: The SEM images were obtained to characterize the microstructure of the freeze-dried gels. The surface of the modified and of the Protemp 4 materials is structurally consistent and the collapse of the surface pores may be due to artifacts (freeze-drying process). Antioxidant capacity of the Propolis (Brazilian) and Copaiba Oil have been compared in the investigation and are showed superior properties in their ability to quench the excessive free radical formation in the well calibrated BSA solubility system. The additional benefits of using Propolis (Brazilian) and Copaiba Oil lies in the well documented effects of bioactives on altering the surface of the temporary restoration by a deposition of bio-active resin of propolis or copaiba oil and therefore possibly as a barrier to Candida species and also prevents formation of the biofilm. Mean fracture strengths of the four provisional materials ranged from 75 to 160 MPa. The value for Protemp 4 material was measure to be 110±12 MPa, which is well within manufacture specification. Mean compressive strengths ranged from 180 to 300 MPa. The value for Protemp 4 material was measure to be 230±15 MPa, which is well within manufacture specification. Bioactive cumulative release profile of bioactives from copaiba and Propolis (Brazilian) containing Protemp 4 modified materials samples were investigated and quantified in PBS and pH 4. The correlation between the force and work of adhesion in all modified materials and is noticeable in all the modified Protemp 4 variants.

Conclusion: The materials were tested using effective *in-vitro* development of "dual function temporary restorative materials. We quantified the effects of functional designer biomaterials on the dentin bioadhesion, measure compressive and flexural strength of the bio-active nano diamond containing materials in comparison to the commercially available Protemp 4 temporary material. Within the limitations of the study design chitosan: nanodiamond based additives are suitable materials for functional temporary restorative applications *in vitro*. Cytotoxicity data is currently being evaluated in our laboratory.

Keywords: Protemp 4; Propolis; Copaiba oil; Nanodiamonds; Chitosan

Introduction

Temporary restorations or interim restorations as clinical protective measure used in fixed prosthodontics as they act as temporary measure to protect the remaining tooth structure during the treatment [1]. There importance is especially crucial in the instances of prolonged treatment, laboratory protocols and complex prosthodontics treatment [1,2]. The ideal temporary restoration must comply with the following specifications: i.e. to maintain structural/ functional integrity for the duration of the treatment to achieve maximum therapeutic benefit as well as desired protection of the remaining tooth structure [2,3].

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Provisional restorations are fabricated using resin based provisional crown and bridge materials [4,5]. Interim fixed restorative materials can be divided into four groups according to composition: polymethyl methacrylate (PMMA), polyethyl or butyl methacrylate, microfilled bisphenol A-glycidyl dimethacrylate (Bis-GMA) composite resin, and urethane dimethacrylate (light-polymerizing resins) [6,7]. While ethyl methacrylates have poor aesthetics and wear resistance, methyl methacrylates and bis-acryl resin composites are superior with regard to those properties [8,9].

Nanodiamonds (ND), are becoming one of the most widely studied nanomaterials due to their unique properties as hardness, thermal conductivity, dopability or optical transparency over a wide spectral range [10]. Considering that the application of nanoparticles as fillers in polymeric matrices is well documented in the strengthening of the materials, it could be expected that the incorporation of ND nanoparticles into dental polymeric materials could have an enhancing effect on the mechanical properties of the resulting functional biomaterials [11].

Several studies have described the adverse effects of acrylate based resin at the cellular and tissue levels [12]. The mechanisms of these adverse effects involve direct toxicity from released or residual MMA monomer and oxidative stress [13]. The potential usefulness of N-acetyl cysteine in preventing the adverse biological effects of resin has been demonstrated in fibroblastic cells. NAC also acts as a strong oxidant scavenger and a pro-acetyl cysteine (NAC), an anti-mediated redox cycle is the most important removal system for free radicals [14].

Our objective was to examine the effect of Protemp4:chitosan:nanodiamond bioactive containing modified materials and compare the protective activity of the propolis (Brazilian) and copaiba oil as bio-active additives to compare with N-acetyl cysteine *in vitro*.

Methods

Preparation of various hydrogels

The bioactive containing gel was prepared by dispersion of 0.2gm in glycerol (5%w/w) (1ml) using a mortar and a pestle following the earlier reported generic protocol [15]. Ten milliliters of glacial acetic acid (3%w/w) was then added with continuous mixing and finally chitosan (10% chitosan w/w) polymer was spread on the surface of the dispersion and mixed well to form the required gel and then mixed into a Protemp 4. The strength of the prepared gel (10gm) is 0.2g of propolis or copaiba oil (ND/propolisor ND/copaiba oil) in each gram of the base.

Swelling/weight loss tests and bioactive release

The swelling/weight loss tests were performed when triplicates of each samples composition (approximately 2cm^2, weight normalized) were immersed in 2 mL of different fluids at 37°C for each time interval studied (1, 2, 4, 24, and 96 h). Two different media were used in accordance with the ISO 10993-9 standard. The first media was Phosphate Buffered Saline (PBS, Sigma Aldrich), intended to mimic the inorganic phase of human plasma [16]. The other media was PBS with a reduced pH which was intended to simulate the local inflammatory environment of the wounds [17,18]. This is termed Solution pH 4.0. The pH was lowered using Lactic Acid (Sigma Aldrich). The fluid absorption of each sample was calculated according to equation (2) to obtain their swelling degree (SD). WS is the weight of the sample at each time interval (swollen weight) and WD is the dry weight before swelling [19]. After 4 days of immersion, the samples were dried and weighed in order to calculate their weight loss (WL) [equation (3)], where WD and WDS are the weight of the dried samples before and after swelling tests, respectively;

$$SD = 100\frac{W_S - W_D}{W_D}(\%)$$

$$WL = 100\frac{W_D - W_{DS}}{W_D}(\%)$$

To analyze the bio-active release (propolis (Brazilian) and Copaiba oil) based on the total phenolic concentration; the swelling media was analyzed after 1, 2, 24, and 96 h of immersion via UV–V is spectrometer, from 300 to 800 nm, using polystyrene cuvettes [20]. For quantification of the amount of propolis released, a standard curve was created by diluting the original propolis and Copaiba oil) in isopropanol resulting in several aliquots of known concentration, which were then analyzed in the same wavelength range. The area of the peak of these aliquots (of known concentration of bio-additive) was calculated and used to compare with those of the bio-additive released by the samples.

Total phenol concentration in propolis (Brazilian) and copaiba oil used in the study

Total phenols content was estimated by a colorimetric assay based on the same procedure realized by Rocha et al. [21] and described by Waterman & Mole [22] with some modifications. The samples were diluted in distilled water to obtain a concentration of 5 µg/mL of total phenols. The concentration of 5% v/v of Folin-Ciocalteu reagent and 10% v/v sodium carbonate (35% w/v) reagents were added to the samples. After the addition of the reagents, the solutions were kept in the dark at room temperature for 30 min and the absorbance was read at the wavelength of 760 nm in a spectrophotometer UV mini-1240 (Shimadzu Co., Kyoto, Japan). Gallic acid (Aldrich, Australia) was used as a standard. The analyses were performed in triplicate.

Bioadhesion studies: Bioadhesion studies were done using Chatillon apparatus for force measurement [23]. This method determines the maximum force and work needed to separate two surfaces in intimate contact. The bioactive "protemp derivatives" (0.1g) were homogeneously spread on a 1cm^2 glass disk and then the disks were fixed 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 dent in was established in order to imitate adhesion of the modified "protemp derivatives" to the tooth structure, after a preset contact time (1min) under contact strength (0.5N) the 2 surfaces were separated at a constant rate of displacement (1mm/s). The strength was recorded as a function of the displacement, which allowed to determine the maximal detachment force, F max, and the work of adhesion, W, which was calculated from the area.

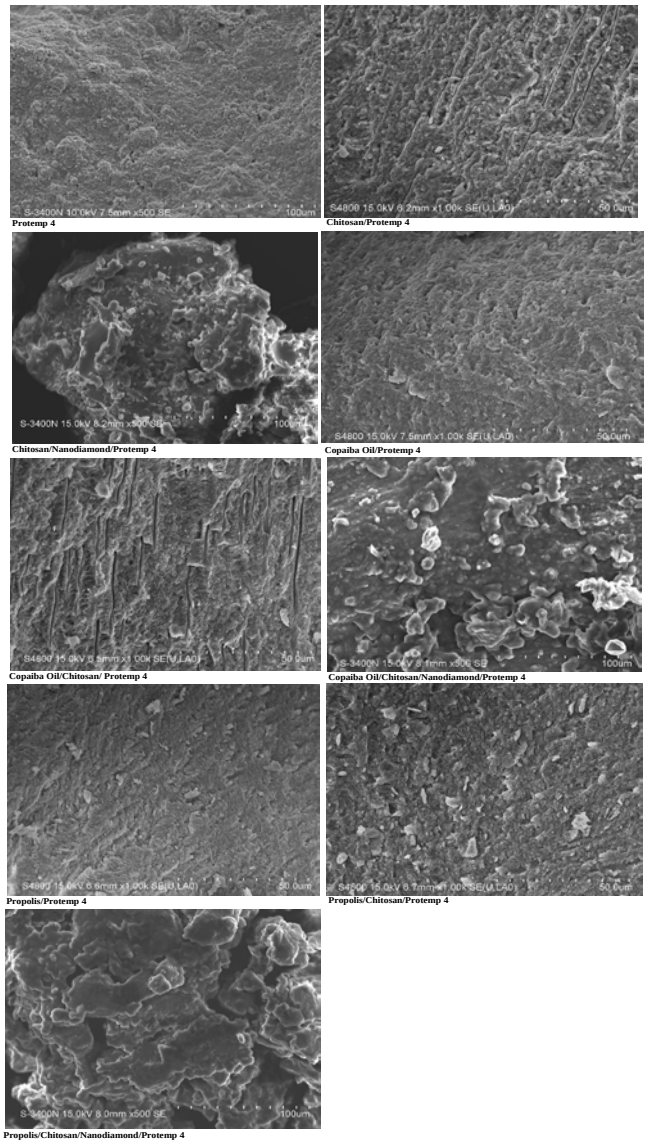

Figure 1: SEM of the surfaces of the materials after polymerization where a ...

Results and Discussion

Scanning electron microscope characterization of the bio-active restorative materials

The SEM images were obtained to characterize the microstructure of the freeze-dried gels and are presented in Figure 1. The surfaces of the modified and of the Protemp 4 materials are structurally consistent and the collapse of the surface pores may be due to artifacts (freeze-drying process).

Free radical defense of the system

Although the mechanisms underlying the adverse effects of resin bis-acrylate materials such as Protemp 4 materials are unknown, there are two possible explanations: genetic damage and an oxidative stress, resulting from an imbalance between the ROS (reactive oxygen species) and anti-oxidant redox defensive system. ROS generated from resins is known to reduce the intercellular level of anti-oxidant

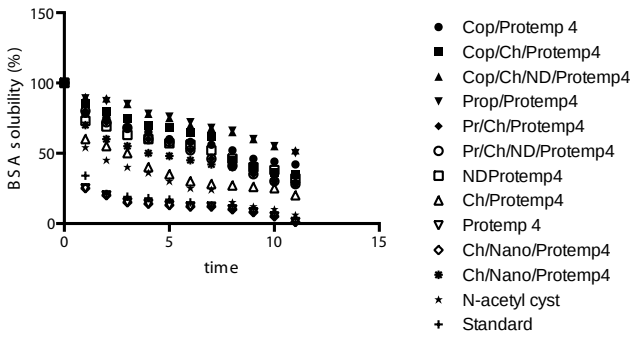

Figure 2: Free radical defense and biomaterials as a function of the BSA solubility (%) at pH 6.7.

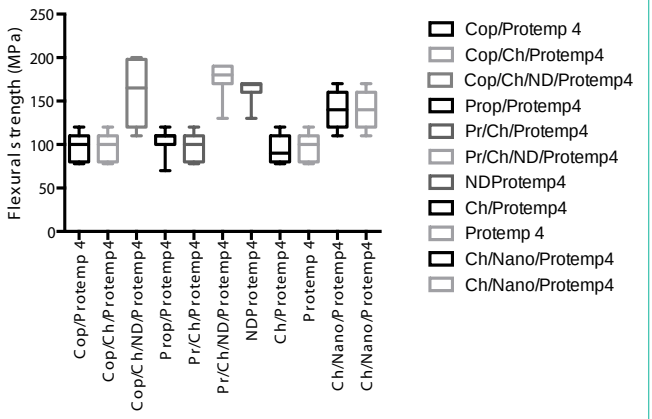

Figure 3: Flexural strength of the Bio-active modified Protemp 4 derivatives.

molecules such as glutathione, a direct ROS scavenger [25,26]. The increased ROS after glutathione depletion may induce cytotoxicity by modulating the signaling pathways leading to apoptosis [27]. In addition, ROS may directly damage the cellular structure [28,29]. The direct scavenging effect and the promotive effect on the glutathione system, as well as unknown protective mechanisms of NAC, may have contributed to reducing the cytotoxicity of bis-acrylate resin [30].

Antioxidant capacity of the Propolis (Brazilian) and Copaiba Oil have been compared in the investigation and are showed superior properties in their ability to quench the excessive free radical formation in the well calibrated BSA solubility system [31] (Figure 2).

The additional benefits of using Propolis (Brazilian) and Copaiba Oil lies in the well documented effects of bioactives on altering the surface of the temporary restoration by a deposition of bio-active resin of propolis or copaiba oil and therefore possibly as a barrier to Candida species and also prevents formation of the biofilm [32]. Although propolis and copaiba oil release their bioactive components and it could even prolong its benefits to inflamed mucosa, considering its anti-inflammatory and skin healing properties [33].

Flexural strength and bioactive Protemp 4 derivatives: The mean values and standard deviations of the four provisional materials are shown in Figure 3. Mean fracture strengths ranged from 75 to 160MPa. The value for Protemp 4 material was measure to be 110±12 MPa, which is well within manufacture specification [34].

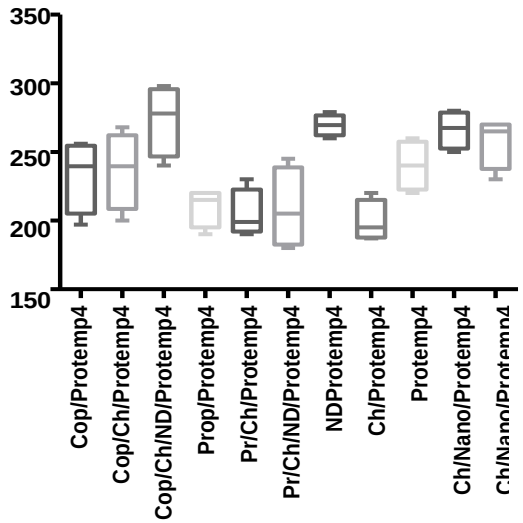

Figure 4: Compressive strength summary for bioactive ND containing Protemp 4 derivatives.

Compressive strength and bioactive Protemp 4derivatives: The compression behavior for the temporary restorations represents one of the most important mechanical properties. The compression strength values from the compression test that carried out on the bio-active modified Protemp 4 derivatives have been summarized in Figure 4. Mean compressive strengths ranged from 180 to 300 MPa. The value for Protemp 4 material was measure to be 230±15MPa, which is well within manufacture specification [35].

Swelling and bio-additive release for the modified Protemp 4 derivatives

The swelling tests in PBS and in Solution pH 4.0 for 4 days revealed that when reaching the equilibrium swelling degree, all samples swelled in accordance with (Figure 5a and 5b).

The amount of bio-actives (such as Brazilian propolis and Copaiba oil) release in swelling media was analyzed after 1, 2, 24, and 96 h of immersion (Figure 6a and 6b). The propolis release by polymeric systems usually occurs in two steps: the release of certain amounts of propolis or copaiba oil derivatives in the first day of swelling as well as a prolonged release in some cases. 37 A trend could be observed in all curves after 4 days of immersion: there was a high bioactive release in the initial hours and the cumulative release reached constant values up to 1 day of immersion. No prolonged release was observed.

The amount of bioactive release in swelling media was analyzed after 1, 2, 24, and 96 h of immersion (Figure 5b). The bioactive release by polymeric systems usually occurs in two steps: the release of certain amounts of propolis in the first day of swelling as well as a prolonged release in some cases. 49 Nonetheless, the samples released more bioactive to PBS than to Solution pH 4.0, probably indicating that the propolis and copaiba oil release can be influenced by the media pH.

Bioadhesive study: Chitosan is a natural cationic polysaccharide derived from chitin with the structural composition being heavily influenced by the pH factors. The unique property of the chitosan lies in its ability to form controlled bio-functional interface with the dentin, enamel, restorative materials as well as muco-adhesion to the

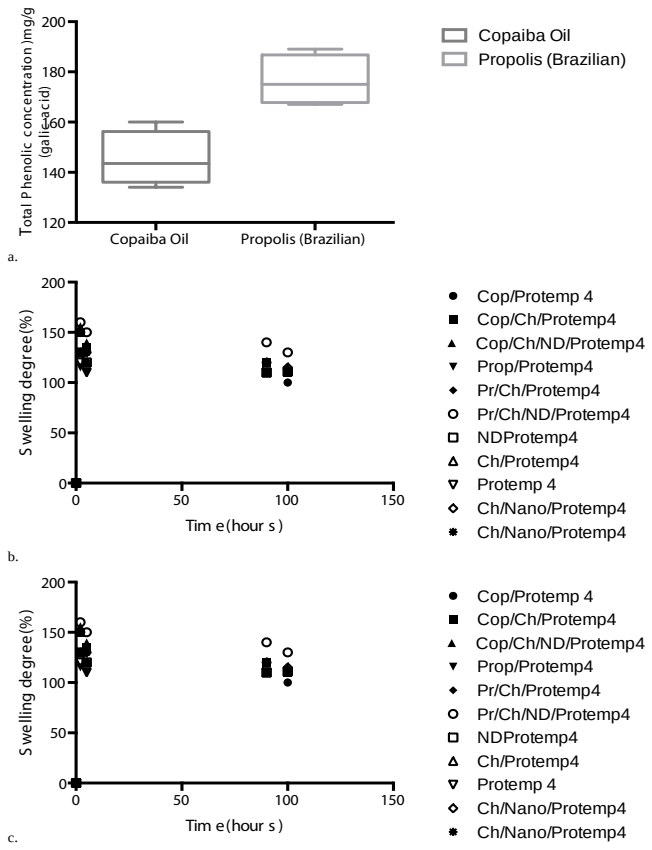

Figure 5: (a) Total phenolic concentration of the Copaiba oil and Brazilian propolis used in the incorporation into the bio-active Nanodiamond containing hydrogels (b) Swelling degree (SD) results after regular time intervals (1, 2, 4, 24, 96 h), when immersed in (b) PBS and (c) Solution pH 4.0 for 4 days.

skin and other oral tissues, through favorable chemical and structural compatibilities of the bio-active material. The correlation between the force and work of adhesion is presented in table 1 and is noticeable in all the modified Protemp 4 variants.

Chitosan is a natural cationic polysaccharide derived from chitin with the structural composition being heavily influenced by the pH factors [36]. The unique property of the chitosan lies in its ability to form controlled bio-functional interface with the dentin, enamel,

Table 1: Summary of Adhesive Force and Work of Adhesion for the bioactive containing Nano-diamond Pro-temp 4 derivatives.

Protemp 4 Bioactives	Adhesive Force (N) ± SD (Dentin)	Work of Adhesion (Ncm) ± SD (Dentin)
Cop/Protemp 4	1.09±0.24	3.38±0.31
Cop/Ch/Protemp4	1.09±0.35	2.92±0.34
Cop/Ch/ND/Protemp 4	1.17±0.42	3.49±0.42
Prop/Protemp4	0.99±0.40	2.94±0.29
Pr/Ch/Protemp4	1.09±0.24	3.38±0.31
Pr/Ch/ND/Protemp4	1.09±0.35	2.92±0.34
NDProtemp4	1.17±0.42	3.49±0.42
Ch/Protemp4	0.99±0.40	2.94±0.29
Protemp 4	1.09±0.24	3.38±0.31
Ch/Nano/Protemp4	1.09±0.24	3.38±0.31

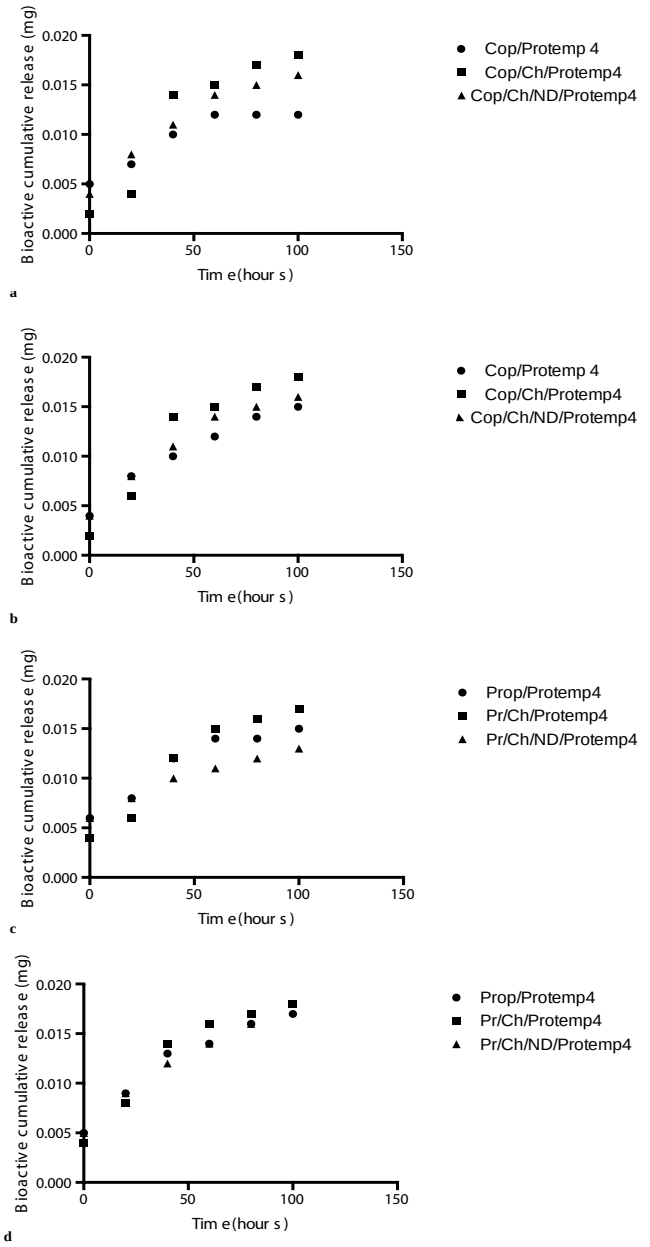

Figure 6: Bioactive cumulative release profile of bioactives from copaiba containing Protemp 4 modified materials samples. Copaiba containing Protemp 4 modified materials–bioactive samples were immersed in (a) PBS, (b) Solution pH 4.0 and the copaiba oil delivered from Protemp 4 modified materials was quantified after regular intervals of time for 4 days, (c) PBS and the propolis from Protemp 4 modified materials delivered was quantified after regular intervals of time for 4 days, (b) Solution pH 4.0 and the propolis Protemp 4 modified materials delivered was quantified after regular intervals of time for 4 days.

restorative materials as well as muco-adhesion to the skin and other oral tissues, through favorable chemical and structural compatibilities of the bio-active material [37]. This can be expected due to 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 the dentin structure, can also explain these results. The results are summarized in Table 1.

It is well established that there is a correlation between the hydration of the polymeric materials and the bio-adhesion. Appropriate swelling is important to guarantee adhesiveness; however, over hydration can form slippery non-adhesive hydrogels [38].

Conclusion

The materials were tested using effective in-vitro development of "dual function temporary restorative materials. We quantified the effects of functional designer biomaterials on the dentin bioadhesion, measure compressive and flexural strength of the bio-active nano-diamond containing materials in comparison to the commercially available Protemp 4 temporary material. Within the limitations of the study design chitosan: nanodiamond based additives are suitable materials for functional temporary restorative applications *in vitro*. Cytotoxicity data is currently being evaluated in our laboratory.

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