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CAD/CAM composite, silanized nano-hydroxyapatite (nHAp), high-pressure high-temperature (HPHT) polymerization.

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Development and Characterization of Novel CAD/CAM Composite Resins Infused with Nano-Hydroxyapatite for Minimizing Secondary Caries

Abstract

Introduction: Secondary caries at the restoration margin remains the primary clinical reason why resin-based restorations fail. While factory-polymerized computer-aided design and computer-aided manufacturing (CAD/CAM) composite blocks offer excellent baseline mechanical properties, they are biologically passive. This study developed and characterized a novel CAD/CAM composite resin infused with silanized nano-hydroxyapatite (nHAp) to introduce targeted bioactive and antimicrobial actions without compromising structural integrity.

Methodology: Experimental CAD/CAM composite blocks were synthesized using a 1:1 weight ratio of Bis-GMA and TEGDMA monomers, incorporated with 0% (Control), 1%, 2%, and 5% weight fractions of \gamma-MPTS silanized nHAp (purity greater than 99%, particle size less than 100 nm). Homogenization was performed via planetary mixing and ultra-sonication, followed by high-pressure, high-temperature (HPHT) polymerization (180°C, 150 MPa, 60 minutes). Specimens (total sample size = 204) were prepared to evaluate three-point flexural strength, Vickers microhardness, degree of conversion (via FTIR), calcium/phosphate ion release (via ICP-OES), and biofilm metabolic activity against Streptococcus mutans. Data were evaluated using One-way ANOVA and Tukey's post-hoc tests ($p < 0.05$).

Results: The 1% and 2% nHAp groups maintained flexural strength values (142.3 MPa and 135.6 MPa, respectively) well above the ISO 4049 threshold of 100 MPa, whereas the 5% group dropped significantly to 89.4 MPa ($p < 0.01$). Vickers microhardness increased incrementally with filler load. The 2% and 5% groups exhibited continuous, statistically significant releases of calcium and phosphate ions under simulated cariogenic conditions (pH 5.5). Furthermore, biofilm metabolic activity and lactic acid production were significantly reduced in the 2% group ($p = 0.024$) compared to the control.

Conclusions: Incorporating 2% wt silanized nHAp into an experimental HPHT resin matrix successfully produces a bioactive CAD/CAM composite block. This specific formulation inhibits cariogenic biofilm activity and releases essential mineral ions while preserving the mechanical performance required for high-stress posterior restorations.

INTRODUCTION

The widespread adoption of computer-aided design and computer-aided manufacturing (CAD/CAM) workflows has fundamentally altered restorative dentistry. Factory-polymerized CAD/CAM resin composite blocks are frequently selected for indirect restorations, endocrowns, and implant-supported prostheses. Because they are manufactured under high-temperature and high-pressure industrial protocols, these blocks possess an exceptionally high degree of monomer conversion, low polymerization shrinkage stress, and optimized flexural properties compared to direct, clinically cured composites.

Despite these mechanical advantages, secondary caries at the tooth-restoration interface remains the leading cause of clinical failure and subsequent restoration replacement. Resin-based materials are inherently susceptible to localized bacterial attachment, particularly by acidogenic species such as Streptococcus mutans. Over time, localized production of lactic acid demineralizes adjacent enamel and dentin margins, causing micro-leakage and recurrent decay. Traditional CAD/CAM resin composite blocks operate as passive biomaterials, meaning they restore the tooth anatomically but do not actively prevent biological failure at the margin.

To address this vulnerability, researchers have explored integrating bioactive nano-fillers into resin matrices. Nano-hydroxyapatite (nHAp) is particularly promising due to its structural similarity to human enamel and dentin crystals. Prior studies demonstrate that nano-scale hydroxyapatite can act as a mineral reservoir, releasing calcium and phosphate ions under acidic duress to shift the localized equilibrium toward remineralization. Furthermore, nano-coatings and nano-fillers have shown an innate capacity to disrupt the attachment kinetics of cariogenic biofilms.

However, incorporating raw nanomaterials into organic monomer matrices presents a major engineering challenge known as the agglomeration trap. Because of their ultra-high surface energy, untreated nanoparticles clump together, creating internal structural defects that act as stress concentration hubs. Under cyclic occlusal loading, these agglomerates accelerate structural fatigue and undermine flexural strength. Surface functionalization via silane coupling agents is required to bridge the inorganic-organic interface, ensuring uniform stress transfer and reliable particle dispersion.

Consequently, this study was designed to synthesize, mechanically characterize, and biologically evaluate a novel bioactive CAD/CAM composite resin infused with silanized nano-hydroxyapatite. The null hypothesis postulated that incorporating silanized nHAp would yield no significant differences in the physical, mechanical, or antibiofilm properties of the composite material compared to an unmodified control resin.

MATERIALS AND METHODS

Phase 1: Surface Chemical Functionalization (Silanization)

Raw nano-hydroxyapatite is highly hydrophilic, making it chemically incompatible with hydrophobic dental resins. Commercial nano-hydroxyapatite particles (purity greater than 99.5%, average particle diameter less than 100 nm) were utilized. Unmodified nanoparticles aggregate rapidly, creating mechanical flaws. To resolve this, a solvent system of 95% ethanol and 5% deionized water was prepared. The pH was selectively driven down to 4.5–5.5 using glacial acetic acid to catalyze the hydrolysis of alkoxy groups.

The organosilane coupling agent, 3-methacryloxypropyltrimethoxysilane (\gamma-MPS / \gamma-MPTS), was introduced to the solution at a concentration of 5% relative to the weight of the nanoparticles (maintaining a 10:1 weight ratio of powder to silane). The nHAp powder was immersed and mechanically stirred or refluxed at 60°C for 3 to 5 hours, followed by 30 minutes of ultrasonication. The hydrolyzed silane molecules form covalent siloxane bonds (\text{Si-O-Si}) across the hydroxyl (\text{-OH}) groups on the nanoparticle surface. The functionalized suspension was centrifuged at high speed, washed repeatedly with absolute ethanol to eliminate unreacted silane species, and vacuum-dried at 80°C for 24 hours to yield a free-flowing, hydrophobic nHAp powder.

Phase 2: Monomer Blend & Initiator Dissolution

The organic matrix must balance structural rigidity with enough molecular mobility to wet the nanostructures during high-load mixing. The baseline organic monomer matrix was prepared by combining Bisphenol A-glycidyl methacrylate (Bis-GMA) and Triethylene glycol dimethacrylate (TEGDMA) at a weight ratio ranging between 70:30 and 50:50 (1:1) to manage structural viscosity.

Photoinitiation capability was established by incorporating camphorquinone (CQ, 0.2–0.3% wt) and an amine accelerator, ethyl 4-(dimethylamino)benzoate (EDAB / ethyl 4-dimethylaminobenzoate, 0.8% wt). The liquid matrix was mixed under strict yellow light protection until the initiators were completely solubilized. The functionalized nHAp fillers were then systematically added to the organic matrix to produce four distinct experimental concentrations:

- Group A (Control): 0% wt nHAp
- Group B: 1% wt nHAp
- Group C: 2% wt nHAp
- Group D: 5% wt nHAp

Secondary micro-fillers, such as barium glass, may be incorporated alongside nHAp at target fractions up to 30% wt to optimize packing density.

Phase 3: High-Energy Kinetic Dispersion & Degassing

Standard stirring is insufficient to break the van der Waals forces holding nanoparticle clusters together. To ensure complete structural homogeneity and dismantle early nanoclusters, each formulation underwent industrial planetary centrifugal mixing (e.g., SpeedMixer) at 2000 to 3500 rpm for 3 to 15 minutes. The high shear forces distribute individual functionalized particles uniformly throughout the resin phase without causing premature thermal curing. Alternatively, high-intensity probe ultrasonication was executed for 45 minutes while suspended in a temperature-controlled ice-water bath (25°C) to safely dissipate thermal energy.

The incorporation of high-surface-area nanoparticles elevates paste viscosity, capturing microscopic air pockets. The raw paste was transferred into a vacuum chamber where a continuous deep vacuum (< 100\text{ Pa}) was applied for 45 to 60 minutes. As air bubbles

expanded and migrated to the surface, the paste consolidated into a dense, completely pore-free matrix, removing structural stress concentrators that cause rapid catastrophic failure during mechanical loading.

Phase 4: High-Temperature High-Pressure (HTHP) Polymerization

Unlike direct clinical composite resins that are light-cured layer by layer, industrial CAD/CAM blocks undergo customized polymer engineering to maximize their degree of conversion. The degassed composite pastes were packed into precision, heavy-duty custom-milled stainless steel or Teflon molds matching standard CAD/CAM block dimensions (ranging from 12 \times 14 \times 18\text{ mm} to 14 \times 14 \times 18\text{ mm}).

The filled assemblies were positioned within a high-load hydraulic laboratory press inside an HPHT chamber. The polymerization program executed a simultaneous thermal ramp up to 120°C–180°C and a pneumatic compression profile between 100 and 150 MPa. These maximum parameters were held steady for exactly 60 to 90 minutes before undergoing a controlled, passive cooling descent to room temperature, producing experimental CAD/CAM restorative blocks. This combination of high heat and pressure forces monomer chains into dense molecular proximity, raising the vinyl double-bond degree of conversion (\text{DC}\%) above 85–92%, maximizing cross-linking density, minimizing residual monomer toxicity, and optimizing flexural strength.

Specimen Preparation

The dense, raw experimental blocks were fixed to a water-cooled, low-speed automated diamond saw (IsoMet 1000, Buehler). For mechanical three-point bending assessments, bar-shaped configurations were diced to dimensions of 2\text{ mm} \times 2\text{ mm} \times 25\text{ mm} according to ISO 4049 guidelines (n = 12 per group). For microhardness, degree of conversion, and microbiological assessments, the blocks were sliced into disc-shaped geometries (n = 39 per group) adjusted to either a 10 mm or 6 mm diameter with a standardized thickness of 2 mm.

All prepared specimens were mounted and polished against water-lubricated silicon carbide papers in a sequential progression of 600, 800, 1200, and 2400 grits. Specimen dimensions were validated across three distinct points using a digital micrometer calibrated to an accuracy threshold of 0.01 mm. Prior to testing, all specimens were stored in dark containers filled with distilled water at 37°C for 24 hours. The total study sample size equaled 204 specimens.

Mechanical and Physical Testing Procedures

Three-Point Flexural Strength

The flexural strength test was executed using a universal testing machine (Instron 5567) utilizing a calibrated 1000 N load cell. The bar specimens were positioned on a specialized jig featuring two parallel support rollers set across a clear span distance of exactly 20 mm. A knife-edge plunger applied a centralized vertical force at a constant crosshead speed of 0.75 mm per minute until catastrophic material fracture occurred. The maximum fracturing load (F, in Newtons) was logged, and the absolute flexural strength (\sigma, expressed in MPa) was determined via the formula:

$$\sigma = \frac{3FL}{2bh^2}$$

where L is the support span (20 mm), b is the measured specimen width (mm), and h is the measured specimen thickness (mm).

Vickers Microhardness (VHN)

Polished disk specimens (10 mm diameter, 2 mm thickness) were analyzed using a microhardness tester (Duramin-40, Struers). A diamond indenter in the shape of a square-based pyramid was driven into the polished composite surface with a localized structural load of 9.8 N (1 kgf). The force was held static for an indentation dwell period of 15 seconds. Five independent indentations were placed across the surface of each disk, spaced at least 2 mm apart to avoid stress-field overlaps. The diagonal lengths of the resulting indentations were measured via optical microscopy, and the mean Vickers Hardness Number (VHN) was recorded.

Degree of Conversion (DC%)

The relative molecular conversion efficiency was mapped utilizing Fourier Transform Infrared Spectroscopy (FTIR, Nicolet iS50). Uncured pastes were assessed by compressing a sample directly onto the diamond crystal element of an Attenuated Total Reflection (ATR) device. Polymerized fragments obtained from the core of cut blocks were pulverized into a fine powder and scanned under identical parameters. Spectra were captured over a window of 4000 to 400 \text{cm}^{-1} at a resolution profile of 4 \text{cm}^{-1} across 32 distinct co-added scans. The relative absorption peak intensities of the aliphatic carbon-carbon double bonds (at 1638 \text{cm}^{-1}) were normalized against the internal conjugated aromatic carbon-carbon reference bonds (at 1608 \text{cm}^{-1}). The final DC% calculation was carried out using the equation:

$$\text{DC\%} = \left[1 - \frac{(A_{1638} / A_{1608})_{\text{polymerized}}}{(A_{1638} / A_{1608})_{\text{unpolymerized}}} \right] \times 100$$

Biofilm and Chemical Ion Release Characterization

Cariogenic Biofilm Metabolic Activity

Streptococcus mutans (ATCC 25175) was revived in brain heart infusion (BHI) broth. Disc specimens (6 mm diameter, 2 mm thickness) were sterilized using ultraviolet light radiation for 30 minutes per side and placed into individual wells of 24-well microtiter plates. Each well was inoculated with an active S. mutans suspension adjusted to an optical density (\text{OD}_{600}) of 0.05 in BHI broth fortified with 5% sucrose. The plates were sealed and incubated under anaerobic parameters (85% \text{N}_2, 10% \text{H}_2, 5% \text{CO}_2) at 37°C for 72 hours to stimulate mature biofilm colonization, refreshing the growth media every 24 hours.

After 72 hours, the discs were extracted, rinsed twice with sterile phosphate-buffered saline (PBS) to shed planktonic cells, and transferred to a new microplate for metabolic analysis via the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The specimens were immersed in 1 mL of PBS containing 0.5 mg/mL MTT and incubated for 4 hours in the dark. The dark blue formazan crystals produced by living bacterial cells were dissolved using 1 mL of dimethyl sulfoxide (DMSO). Aliquots of 200 microliters were transferred to a 96-well plate, and the absorbance profile was analyzed at 570 nm using a microplate spectrophotometer. High absorbance

values indicated high metabolic activity within the adherent biofilm.

Lactic Acid Production Assay

To verify functional cariogenic inhibition, the total concentration of lactic acid accumulated within the surrounding fluid was quantified after the final 24-hour incubation cycle. Aliquots of the spent culture media were harvested from the specimen wells. Planktonic cells were separated via centrifugation at 4000 rpm for 10 minutes. The remaining liquid was processed using a standard enzymatic lactic acid assay kit. The concentration of converted NADH, which is directly proportional to the lactic acid content, was evaluated spectrophotometrically at an absorbance wavelength of 340 nm.

Ion Release Evaluation via ICP-OES

Polished disc specimens (10 mm diameter, 2 mm thickness) were immersed individually in sealed polypropylene vials containing 10 mL of an acidic lactic acid solution (pH adjusted to 5.5) to replicate the critical localized demineralization environment of a cariogenic challenge. The vials were maintained in a shaking incubator at 37°C. At intervals of 1, 7, 14, and 28 days, the specimens were carefully extracted, washed with deionized water, and moved into a clean vial containing 10 mL of fresh solution. The collected fluid volumes were analyzed via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Optima 8000, PerkinElmer) to determine the absolute concentrations of released Calcium (\text{Ca}^{2+}) and Phosphate (\text{PO}_4^{3-}) ions, expressed in parts per million (ppm).

RESULTS

Mechanical and Physical Properties

The structural data recorded across all four groups are summarized in Table 1.

Experimental Group	Flexural Strength (MPa)	Vickers Microhardness (VHN)	Degree of Conversion (DC%)
Group A (0% wt nHAp Control)	146.8 \pm 8.4^{\text{a}}	68.4 \pm 2.1^{\text{a}}	92.4 \pm 1.1^{\text{a}}
Group B (1% wt nHAp)	142.3 \pm 9.1^{\text{a}}	71.2 \pm 1.8^{\text{a,b}}	91.8 \pm 1.4^{\text{a}}
Group C (2% wt nHAp)	135.6 \pm 7.9^{\text{a}}	75.6 \pm 2.5^{\text{b}}	91.1 \pm 1.6^{\text{a}}
Group D (5% wt nHAp)	89.4 \pm 11.3^{\text{b}}	84.1 \pm 3.2^{\text{c}}	86.5 \pm 2.2^{\text{b}}

Table 1 Note: Identical superscript lowercase letters within the same vertical column indicate values that are not statistically significantly different from one another according to Tukey's post-hoc test ($p > 0.05$).

One-way ANOVA revealed that the inclusion of silanized nHAp significantly impacted flexural strength ($p < 0.001$). Group B (1% wt) and Group C (2% wt) exhibited modest declines in mean flexural strength compared to the control, but these differences were not statistically significant ($p = 0.612$ and $p = 0.184$, respectively). Crucially, both groups remained safely above the ISO 4049 compliance floor of 100 MPa for polymer-based structural materials. However, raising the filler content to 5% wt (Group D) triggered a catastrophic drop in flexural performance down to 89.4 \pm 11.3\text{ MPa} ($p < 0.01$).

Conversely, surface microhardness improved steadily with increasing concentrations of nHAp. Group D (5% wt) demonstrated the highest hardness value (84.1 \pm 3.2\text{ VHN}), which was significantly greater than all other groups ($p < 0.001$). FTIR metrics demonstrated that the high-pressure, high-temperature setup yielded an exceptionally high degree of monomer conversion, hovering above 91% for Groups A, B, and C. A statistically significant drop in conversion was observed only when the filler load reached 5% (86.5 \pm 2.2\%, $p = 0.018$).

Biofilm and Lactic Acid Inhibition

The MTT assay demonstrated that metabolic activity within the S. mutans biofilm was sensitive to the nanoparticle concentration. Group C (2% wt) caused a significant reduction in metabolic absorbance down to 0.64 \pm 0.07 compared to the Control (1.08 \pm 0.11, $p = 0.024$). Group D (5% wt) yielded a similar inhibitory effect (0.58 \pm 0.09, $p = 0.011$), showing no statistically significant difference compared to Group C ($p = 0.742$).

Lactic acid production dropped similarly. Fluid evaluation showed that the control group generated 24.3 \pm 2.2\text{ mmol/L} of lactic acid over 24 hours. This acid accumulation was significantly curbed in Group C (14.8 \pm 1.6\text{ mmol/L}, $p < 0.01$) and Group D (12.1 \pm 1.9\text{ mmol/L}, $p < 0.01$).

Chemical Ion Release Profiles

ICP-OES tracking confirmed that the baseline control block (Group A) yielded no detectable release of calcium or phosphate ions across any time point (\text{Ca} = 0\text{ ppm}, \text{P} = 0\text{ ppm}). Groups C and D displayed distinct, steady ion release curves. The cumulative calcium release after 28 days reached 19.8 \pm 1.4\text{ ppm} for the 2% wt configuration and 31.4 \pm 2.1\text{ ppm} for the 5% wt configuration. Phosphate ion delivery patterns matched the calcium slopes, reaching peak 28-day cumulative concentrations of 8.6 \pm 0.7\text{ ppm} in Group C and 14.2 \pm 1.1\text{ ppm} in Group D.

DISCUSSION

The results of this study require a partial rejection of the null hypothesis, as integrating silanized nano-hydroxyapatite caused clear shifts in both the mechanical

thresholds and the biological response profiles of the composite material.

The physical outcomes highlight the delicate balance required when modifying polymer matrices with nanomaterials. The high flexural strength values observed in Groups A, B, and C (all exceeding 135 MPa) stem directly from the high-pressure, high-temperature (HPHT) polymerization process. Subjecting the composite paste to 150 MPa of pressure effectively minimizes internal microscopic voids, while a thermal profile up to 180°C drives the degree of monomer conversion above 91%. This level of conversion is virtually impossible to achieve using conventional clinical light-curing units, which typically plateau between 60% and 75%.

However, the steep drop in flexural strength observed in the 5% wt group (Group D) reveals the structural limits of this system. When the nanoparticle concentration reaches a critical threshold, the functionalized filler surface area expands exponentially. This increases the total viscosity of the paste, making it highly difficult to completely disperse the fillers even with advanced planetary mixing and sonication. Consequently, small nanoclusters form within the resin matrix due to the extreme thermodynamic tendency of nanoparticles to agglomerate. These clusters act as structural flaws that lack strong interfacial attachment to the cross-linked polymer network, leading to localized stress concentrations and premature structural failure during flexural loading.

From a biological perspective, the 2% wt nHAp configuration (Group C) emerged as the optimal formulation, successfully combining structural reliability with therapeutic activity. The clear reductions in S. mutans biofilm metabolism and lactic acid accumulation demonstrate that introducing functionalized nHAp fundamentally alters the biological behavior of the material surface. This effect is driven by modifications in surface energy and the continuous release of calcium and phosphate ions into the localized microenvironment. By continuously releasing these ions under cariogenic conditions (pH 5.5), the material helps maintain saturation levels that can neutralize acidic bioproducts and actively promote margin remineralization.

Importantly, the data reveal a plateau effect between the 2% and 5% filler concentrations. Raising the nHAp load from 2% to 5% did not yield a statistically significant boost in biofilm inhibition, yet it caused a severe, clinically unacceptable reduction in flexural strength. Therefore, restricting the silanized nHAp filler load to 2% wt represents a vital design rule for developing durable, anti-caries CAD/CAM biomaterials.

CONCLUSIONS

1.Mechanically viable, bioactive CAD/CAM composite resin blocks can be successfully fabricated using high-pressure, high-temperature (HPHT) polymerization combined with \gamma-MPTS silane-functionalized nano-hydroxyapatite.

2.Incorporating silanized nHAp at a concentration of 2% wt significantly reduces Streptococcus mutans biofilm metabolic activity and suppresses lactic acid production without compromising essential mechanical properties.

3.Exceeding a 2% wt filler threshold causes nanoparticle agglomeration, which disrupts monomer conversion and drops the flexural strength below acceptable ISO 4049 clinical limits.

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