Biomimetically- and Hydrothermally-grown HAp Nanoparticles as Reinforcing Fillers for Dental Adhesives

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Purpose: Differently prepared hydroxyapatite (HAp) nanoparticles were incorporated into the adhesive solution of a commercial adhesive system in order to evaluate the effect on microtensile bond strength to dentin.

Materials and Methods: HAp nanoparticles (20 to 70 nm) were prepared by different processes (biomimetic and hydrothermal) and incorporated into the adhesive of the Adper Scotchbond Multi-Purpose (SBMP) system at various concentrations. Control (unfilled) and experimental groups (filled) were applied onto flat mid-coronal human dentin. Composite crowns were built up and cut into beams with a cross-sectional area of 0.65 ± 0.05 mm². Specimens were fractured in tension and examined with a scanning electron microscope (SEM) for fractographic analysis. Microtensile bond strength (μTBS) data were analyzed using a two-way ANOVA and modified LSD test at α = 0.05. Analysis of the nanofiller distribution and ultramorphological characterization of the interface was performed by transmission electron microscopy (TEM).

Results: HAp nanoparticle incorporation into the adhesive of SBMP significantly influenced μTBS to dentin depending on the fillers and the concentration used. A significant increase of the mechanical strength was obtained for the adhesives containing 1% (wt/vol) biomimetic and 5% hydrothermal silanized HAp particles, while the other particle fractions did not influence μTBS significantly. 10% (wt/vol) HAp particles significantly lowered the μTBS irrespective of the particle type used. TEM micrographs revealed nanoparticle dispersion through the adhesive layer but no deposition on or penetration into the hybrid layer.

Conclusions: HAp nanoparticle incorporation into SBMP increased bond strength to dentin by cohesively reinforcing the interface adhesive layer. At a concentration of 10% (wt/vol), nanofiller incorporation had a negative effect on bond strength.

Keywords: HAp, biomimetic and hydrothermal HAp preparation, silanization, adhesion, nanoparticle filler, dentin bonding, tensile bond strength.


In polymers, particle incorporation has shown a reinforcing and toughening effect by crack deflection and local plastic deformation around particles. Reducing the particle size down to the nanoscale, closer to the monomer chain length, chain/filler interactions are affected by the increased surface-to-volume ratio of the fillers, with a direct impact on polymerization dynamics and internal stress development. Under loading, nanoparticles have the ability to reorient in a stress-dissipation mechanism in order to inhibit crack extension in semi-crystalline and amorphous polymers. Filler incorporation into dental adhesives is used to increase viscosity, increase radiopacity, minimize shrinkage, or improve the mechanical properties of the neat polymer blend.

For dentin bonding, it is essential that dental adhesive systems provide effective bonding between the tooth structure on the one side and the filling material on the other side. As dentin is a hydrated biological composite, bonding to it is much more complex than adhering...
to enamel.\textsuperscript{22} Bonding to dentin is based on penetration of amphiphilic molecules into the acid-etched dentin,\textsuperscript{8,42} resulting in micromechanical retention between the resin and dentin surfaces,\textsuperscript{26,41} thus forming a hybrid layer.\textsuperscript{26,38,45} Presumably, incorporation of fillers into a dental adhesive system increases the elastic modulus to provide a flexible intermediate stress-relieving layer that can resist the polymerization shrinkage stress of the overlying composite resin and distribute the stresses induced by the occlusal loads.\textsuperscript{16,17,27} Significant increases in flexural and tensile strength by incorporating 1 wt\% to 10 wt\% silica nanofillers or hydroxyapatite nanofibers (at 10 wt\% and 20 wt\%) into adhesive resins have been reported.\textsuperscript{3,4,17} Recently, it has been shown that the addition of hydroxyapatite nanorods significantly increased diametral tensile strength and flexural strength of an experimental adhesive when added in amounts of 0.2 wt\% to 0.5 wt\%.\textsuperscript{32} Addition of up to 20 wt\% spherical zirconia particles resulted in a significant increase in microtensile bond strength.\textsuperscript{24} An adhesive loaded with 0.5 wt\% PMMA-grafted nanoclay resulted in higher shear bond strength (SBS) to dentin.\textsuperscript{1} Recent results by Sun et al\textsuperscript{37} showed that TiO\textsubscript{2} nanoparticles treated with acrylic acid were able to increase SBS to dentin at a concentration of 0.1 wt\%.\textsuperscript{37}

At the dentin/composite interface, cohesive failure within the adhesive resins have often been attributed to higher dentin-composite bond strength values.\textsuperscript{6,12} Strong adhesion to tooth substrates shifts the fracture plane from the interface to the second weakest element, so that stronger adhesives should render stronger bonds between tooth and composite resins. However, different studies yield differing conclusions regarding nanoparticle incorporation into adhesive resins and dentin bond strength increase. Miyazaki et al\textsuperscript{25} found a significant shear bond strength increase with the addition of up to 20 wt\% filler addition and a bond strength decrease when the filler content exceeded 50 wt\%. With hydroxyapatite nanofillers, bond strength increased significantly at 0.2 wt\% content, but decreased to the initial level with further packing.\textsuperscript{32} Conversely, filled adhesives have failed to increase dentin bond strength in comparison to their unfilled counterparts in several other studies.\textsuperscript{17,21,27} These divergent results clearly show that the influence of nanofillers in an adhesive system depends strongly on the nature of the fillers, their morphology and surface modifications, as well as their distribution.\textsuperscript{17,21,25,27} Each kind of filler has different specific surfaces and surface energies and therefore shows different agglomeration behavior in an adhesive system. As the tendency to agglomeration depends on the zeta potential of particles, the adhesive system used with its specific composition and pH plays another pivotal role. The same particle used in different adhesive systems may therefore play different roles.

Hydroxyapatite and other calcium phosphates have been studied as fillers for mineral-releasing dental composites.\textsuperscript{3} As hydroxyapatite is the major component of the inorganic material of teeth and shows good biocompatibility,\textsuperscript{34} it might be a promising material for the preparation of new filled dental adhesives with improved mechanical and biological properties.\textsuperscript{32} Sadat-Shojai et al\textsuperscript{33} showed that different preparation conditions result in HAp nanoparticles of different forms and surface charges, which in turn play a pivotal role for dispersion stability of HAp particles in a dental adhesive.\textsuperscript{33}

The agglomeration of nanoparticles may adversely affect the mechanical properties of a nanoparticle-filled system by changing the size of the filler phase from nanometer to micrometer. Surface modifications can decrease the surface bonding energy of nano-apatite particles, weaken their agglomeration,\textsuperscript{31} and improve the interfacial strength between the fillers and the matrix.\textsuperscript{7} Silane coupling agents have been examined for surface modification of nano-apatite particles.\textsuperscript{7,23} Labella et al\textsuperscript{18} showed that silanization of HAp fillers in composites enhances flexural strength, diametral tensile strength, and Vickers hardness.\textsuperscript{18}

The purpose of this study was to evaluate the effect of adding two different HAp nanofillers with or without silane surface modification to the adhesive resin of a commercial three-step etch-and-rinse adhesive on dentin bond strength and to correlate it with interfacial morphological characteristics. The null hypotheses tested were that 1) the incorporation of nanofillers does not affect bond strength to dentin; 2) the amount of filler incorporated has no influence on bond strength to dentin; 3) there is no difference in incorporating differently synthesized and functionalized fillers into the adhesive resin.

**MATERIALS AND METHODS**

**Preparation and Silanization of HAp Nanoparticles**

HAp powders were obtained by precipitation and subsequent maturation. HAp with a molar Ca/P ratio of 1.67 was synthesized according to the reaction:\textsuperscript{19}

\[
5 \text{Ca(NO}_3\text{)}_2 \times 4\text{H}_2\text{O} + 3 \text{(NH}_4\text{)}_2\text{HPO}_4 + 4 \text{NH}_4\text{OH} \rightarrow \text{Ca}_5\text{(PO}_4\text{)}_3\text{OH} + 10 \text{NH}_4\text{NO}_3 + 23 \text{H}_2\text{O}
\]

100 ml of a 1 mol/l Ca(NO\textsubscript{3})\textsubscript{2} x 4H\textsubscript{2}O (98%, Roth; Karlsruhe, Germany) solution was diluted with 100 ml of a 1 mol/l (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} (32%, Roth) solution were added under constant stirring to start the calcium phosphate precipitation. Addition of 20 ml of 32% NH\textsubscript{4}OH (32%, Roth) resulted in a final pH value of 11. After the initial formation of an amorphous calcium phosphate, the resulting precipitates were exposed to different maturation conditions.

Biomimetic maturation took place at 25°C for 24 h. Particles from this group are referred to as “Bio”. For hydrothermal processing, sample solutions were transferred in a 600 ml stainless steel pressure vessel of a high pressure reactor (Mini Bench Top Reactor, Parr Instruments; Frankfurt/Main, Germany). Samples were heated to 200°C in this airtight container at a rate of approximately 6°C/min. After this maturation time, they were cooled to 50°C at a rate of approximately 50°C/min. Nanoparticles of the hydrothermally processed group are referred to as “Hyd”.
After maturation, both particle fractions were vacuum filtered and thoroughly washed with deionized water. The filter cake was dried in air at 75°C for 24 h, and the dry residues were pulverized using an agate mortar.

Particle morphology and crystal habit of the prepared samples were examined by transmission electron microscopy (TEM) (HRTEM 3010, JEOL; Tokyo, Japan). The phase composition of the precipitated powders was determined by x-ray powder diffraction (XRD) (Bruker D8 DISCOVER GADDS; Madison, WI, USA) using an area detector. Monochromatic Cu Kα radiation (λ = 1.5418 Å) at an angular velocity of 0.6 degrees/min was applied in the range of 2θ = 20 to 60°C with increments of 0.02°C at an angular velocity of 0.6 degrees/min was applied in the range of 2θ = 20 to 60°C with increments of 0.02°C (U = 40kV, I = 40 mA).

The length of the coherent domains of apatite along the c-axis (d002) was calculated, using the line broadening of the (002) peak, according to Scherrer’s equation:

$$X_C \approx 1 - \left( \frac{V_{112}}{I_{300}} \right)$$

Equation 1 was used to estimate the degree of crystallinity. $I_{300}$ corresponds to the intensity of the (300) reflection and $V_{112}$ corresponds to the valley between the (112) and (300) reflection which completely disappears in non-crystalline samples.

Five grams of the Bio and Hyd HAp powders were silanized with 3-aminopropyltrimethoxysilane (APTMS, 97%, Alfa Aesar; Karlsruhe, Germany) at room temperature for 24 h under ultrasonication. After thorough washing with ethanol, the powders were dried and analyzed by IR spectroscopy (ALPHA FT-IR, Bruker Optics) (Fig 2 and Table 1). Silanized particles of the Bio group are referred to as “Bio-Si”, the respective particles of the Hyd group are referred to as “Hyd-Si”.

Nanoparticle Incorporation into the Adhesive System

The HAp fillers were incorporated into the adhesive solution of the commercial adhesive system Adper Scotchbond Multi-Purpose (SBMP, 3M ESPE; St Paul, MN, USA) in 0.2%, 1%, 5%, and 10% (wt/vol), corresponding to 0.06, 0.32, 1.56, and 3.16 vol%, respectively. After being weighed with a high accuracy balance (YDK01, Sartorius; Göttingen, Germany), the fillers were added to the resin solutions and mechanically stirred by a motorized mixer (Roti-Speed handpiece with conical micropestle adapter, Roth). To further increase the dispersion of the fillers, the resin mixtures were ultrasonicated for 30 min (Elma ultrasonication bath, Elma; Singen, Germany) and subsequently directly applied on the etched dentin.

Bonding Procedures, Specimen Fabrication, and Microtensile Testing

Sound human third molars were stored in 0.5% chloramine solution and used within 3 months of extraction. Using a low-speed diamond saw in an Isomet cutting machine (Buehler; Lake Bluff, IL, USA), the occlusal third was removed and flat surfaces were prepared in mid-coronal dentin ensuring that the remaining dentin thickness was in the range of 2.5 ± 0.2 mm. The occlusal dentin surface was ground with a 600 grit SiC paper in order to produce a clinically relevant smear layer. In the control group, the unfilled commercial adhesive system SBMP was applied according to the manufacturer’s instructions. Briefly, Scotchbond etchant (35% H3PO4, 3M ESPE) was applied on the dentin surface for 15 s, rinsed for 30 s, and left slightly wet. The primer solution (2-hydroxyethyl-methacrylate, polyalkenoic acid polymer, water) was applied, gently air dried for 10 s, and reapplied until a shiny surface was achieved. Subsequently, the adhesive solution (bisphenol-A-diglycidyl-methacrylate, 2-hydroxyethyl-methacrylate, tertiary amines, photoinitiator) was applied on the surface, gently spread with air to remove excess, and light cured for 20 s under a halogen light-curing unit (EliparTrilight, 3M ESPE) with an output intensity of 750 mW/cm². Experimental groups were prepared the same way after nanoparticle incorporation into the adhesive. Resin composite crowns (Grandio, VOCO; Cuxhaven, Germany) were incrementally built up in 1 mm increments up to 5 mm under the same curing conditions as described above and stored in distilled water for 24 h at 37°C.

Twenty-four h after the bonding procedure, the restored teeth were longitudinally sectioned in both x and y directions across the bonded interface with a low-speed diamond saw under constant water cooling (IsoMet low-speed saw with a 300-μm diamond wafering blade, Buehler). Each beam had a cross-sectional area of 0.65 ± 0.05 mm². The specimens were then stored in distilled water for 24 h at 37°C. After 24 h, the specimens were fixed to Geraldis’s testing jigs with cyanoacrylate glue (Loctite 401, Henkel; Garching, Germany) and loaded in tension using a universal testing machine (Z2.5, Zwick; Ulm, Germany) with a 100-N load cell traveling at a crosshead speed of 0.5 mm/min, following the non-trimming microtensile testing method. After beam fracture, the dimensions of the cross-sectional area were measured using a digital caliper (Insize, Conrad; Hirschau, Germany) and used to calculate the microtensile bond strength (μTBS) by dividing the imposed force (in N) at the time of fracture by the bonded area (in mm²). μTBS data were analyzed using a two-way ANOVA and modified LSD test at α = 0.05 (SPSS Version 20, IBM; Chicago, IL, USA).

SEM Analysis of Failure Mode

The failure modes were evaluated for each sample using a light microscope (SV 6, Zeiss; Oberkochen, Germany) and classified as “cohesive” (failure entirely within dentin substrate or resin composite), “mixed” (failure at dentin/resin interface including cohesive failure of one of the substrates), or “adhesive” (failure at the dentin/resin interface). The cohesive failures were not included in the mean bond strength calculation. Five representative specimens of each group were randomly selected, sputter-coated with gold and examined under a scanning electron microscope (SEM) (SR 50, Leitz ISI; Tokyo, Japan).
Specimen Preparation for Transmission Electron Microscopy (TEM)

Specimen preparation for TEM was conducted as described above, but no composite was placed over the adhesive resin. Following the adhesive treatment, the teeth were cut into 1 mm disks and demineralized in 0.54 M EDTA (Roth) solution for 7 days at room temperature and a constant stirring rate of 60 rpm. The further treatment has been described elsewhere.42,43

Single ultrathin sections of about 90 nm were cut perpendicular to the bonded surface by means of a 45-degree diamond knife (Diatome; Bienne, Switzerland) in an ultramicrotome (Ultracut UCT, Leica; Wetzlar, Germany), mounted on single Pioloform-coated copper slot grids (oval hole, Plano; Wetzlar, Germany) and stained with saturated uranyl acetate (Science Services; München, Germany) for 12 min and lead citrate (Science Services) for 8 min. Stained and unstained sections were prepared. The ultrathin sections were examined using TEM (Zeiss 906).

Table 1  Technical data of the biomimetic (Bio) and hydrothermal (Hyd) HAp particles

<table>
<thead>
<tr>
<th></th>
<th>Bio</th>
<th>Hyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25°C, 24 h</td>
<td>200°C, 24 h</td>
</tr>
<tr>
<td>Particle size</td>
<td>20 nm</td>
<td>73 nm</td>
</tr>
<tr>
<td>Crystallite size</td>
<td>24.5 nm</td>
<td>55.8 nm</td>
</tr>
<tr>
<td>Crystallinity</td>
<td>5%</td>
<td>71%</td>
</tr>
<tr>
<td>Hydroxylation</td>
<td>33%</td>
<td>100%</td>
</tr>
<tr>
<td>Carbonate cont.</td>
<td>1.5 wt%</td>
<td>0.5 wt%</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>92.5 m²/g</td>
<td>41.7 m²/g</td>
</tr>
<tr>
<td>Silane load after silanization</td>
<td>0.99 (10⁴ Mol/g)</td>
<td>0.62 (10⁴ Mol/g)</td>
</tr>
<tr>
<td>Silane load per surface area</td>
<td>0.64 x/nm²</td>
<td>0.90 x/nm²</td>
</tr>
</tbody>
</table>

Summary of most important characteristics of the two differently prepared HAp samples as well as silane load of the respective samples after silane activation.

RESULTS

Particle Characterization

Hydroxypatite powders were obtained either by a wet chemical precipitation reaction (Bio samples) or by a subsequent hydrothermal treatment at 200°C (Hyd samples). Figure 1 shows the dimensions of the differently prepared nanoparticle powders. The majority of primary particles of the Bio sample (Fig 1 A) were evenly distributed and tended to form weak agglomerates. The particles had a size of 20 nm and an irregular geometrical form. An increase in maturation temperature to 200°C and subsequent aging for 24 h (Hyd sample) resulted in particle growth with a mean particle size of 73 nm; particles showed regular geometrical, rod-like forms (Fig 1B). For that reason, these particles were considered to be single crystalline. The slight elongation is due to a preferential growth along the crystallographic c-axis. The aspect ratio was found to be 2 to 2.5 for both samples. Due to their irregular geometrical form, the biomimetically-grown nanoparticles showed a higher specific surface than did the hydrothermally-manufactured nanoparticles (Table 1).

X-ray analysis confirmed that the prepared powders were single phase HAp. Hyd nanoparticles showed an increased definition of diffraction lines compared to the Bio nanoparticles, indicating a higher crystallinity of the former (71% vs 5%). Increasing the maturation temperature also resulted in a major increase in crystallite size (Table 1).

IR spectroscopic analysis revealed the chemical composition of the samples. Hydrothermally-produced nanoparticles showed an increase in absorption intensity due to crystal growth as well as an even stronger increase in absorption due to hydroxy ion content (liberation mode 3568 cm⁻¹, bending mode 634 cm⁻¹) compared to the Bio nanoparticles. The former also showed a much higher degree of hydroxylation (100%) than the biomimetically-grown particles (33%) (Fig 2 and Table 1).

The number of amino groups introduced to the HAp surface was determined by potentiometric titration and resulted in 0.99 x 10⁴ and 0.62 x 10⁴ mol/g for the Bio and Hyd powders, respectively. This corresponds to 0.64 and 0.9 silanes/nm² for Bio and Hyd powders, respectively (Table 1). The resulting HAp particle fractions were referred to as “Bio-Si” and “Hyd-Si”.

Fig 1  TEM photomicrographs of biomimetically (Bio) (A) and hydrothermally (Hyp) (B) grown HAp nanoparticles. Bar = 200 nm.
Microtensile Bond Strength Testing and Analysis of Fracture Mode
Incorporation of HAp nanoparticles into the adhesive solution of SBMP significantly increased μTBS to dentin for biomimetically matured, nonsilanized HAp particles in a final concentration of 1% (wt/vol) (Table 2). The control group had a mean μTBS of 52.1 ± 16.6 MPa, while the experimental adhesives with 1% (wt/vol) Bio particles resulted in significantly higher means (59.5 ± 11.9 MPa; p = 0.008). In contrast, in the other groups, up to 5% (wt/vol) nanofiller incorporation resulted in no significant differences in μTBS compared to the unfilled control. Further increase to a filler level of 10% (wt/vol) led to a significant decrease of bond strength irrespective of the kind of nanoparticles incorporated. This effect was more pronounced in groups Hyd and Hyd-Si than in groups Bio and Bio-Si. No pre-test failures were recorded during specimen preparation.

Analysis of Failure Modes by SEM
Figure 3 shows representative SEM micrographs of specimens’ fractured surfaces. Fractographic analysis revealed a predominance of adhesive failure for the control and the experimental groups. Typically, fracture initiated at the bottom of the hybrid layer and propagated to the adhesive layer, occasionally deflecting at the top of the hybrid layer. With increasing filler degree, there seemed to be a tendency towards cohesive failure within the adhesive resin.

Distribution of HAp Nanoparticles in the Adhesive/Dentin Interface
TEM revealed that the hybrid layer extended 2 to 5 μm into the dentin showing a sharp transition to the underlying intact dentin in the stained sections (Fig 4). Open dentinal tubules were filled with adhesive resin, thus forming resin tags. A highly stained, electron-dense layer could be observed in all stained sections. As reported by Van Meerbeek et al.,44 this layer corresponds to the

Table 2  Microtensile bond strength in MPa [mean ± SD (n)]

<table>
<thead>
<tr>
<th></th>
<th>0 wt%</th>
<th>0.2 wt%</th>
<th>1 wt%</th>
<th>5 wt%</th>
<th>10 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio</td>
<td>51.3 ± 12.5 (55)A</td>
<td>59.5 ± 11.9 (51)B</td>
<td>49.0 ± 13.6 (56)D</td>
<td>41.3 ± 11.5 (73)E</td>
<td></td>
</tr>
<tr>
<td>Bio-Si</td>
<td>50.5 ± 12.9 (60)D,E,A</td>
<td>47.6 ± 11.6 (65)D,C</td>
<td>49.9 ± 12.7 (69)D,E,D</td>
<td>42.3 ± 11.1 (63)D,E</td>
<td></td>
</tr>
<tr>
<td>Hyd</td>
<td>52.5 ± 13.4 (64)E,h,A</td>
<td>48.5 ± 9.4 (61)E,h,C</td>
<td>53.1 ± 14.6 (71)E,D</td>
<td>33.1 ± 12.4 (64)E,F</td>
<td></td>
</tr>
<tr>
<td>Hyd-Si</td>
<td>48.1 ± 10.6 (56)K,A</td>
<td>48.8 ± 13.0 (49)K,I,C</td>
<td>55.9 ± 13.4 (73)K,I,D</td>
<td>36.0 ± 12.5 (66)K,F</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>52.1 ± 16.6 (43)A,B,C,D,E</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Means followed by the same superscript letters are not statistically different (2-way ANOVA, mod. LSD, p < 0.05, lowercase letters for same filler type, uppercase letters for same filler loading). The unfilled control shows no statistical difference to any group, except to 1 wt% Bio nanoparticles (p = 0.008).
polyalkenoic acid polymer found in the primer solution. In general, nanoparticles were hard to distinguish from the electron dense layer in stained sections, but could be easily identified when no staining was performed. Biomechanically-grown particles (Bio and Bio-Si; Figs 4A to 4C) were harder to differentiate because they were smaller (20 nm) than the hydrothermally manufactured particles (particles Hyd and Hyd-Si; 73 nm; Fig 4D to 4F).

The distribution of nanofillers in the adhesive solution is shown in representative stained TEM micrographs (Fig. 4) and varied depending on the filler content. At lower concentrations, nanoparticles tended to disperse throughout the adhesive thickness and to infiltrate dentin tubules. At higher concentrations, the nanoparticles tended to agglomerate. This resulted in nanoparticle clusters within an adhesive layer with otherwise dispersed filler particles (Fig 3). Common to all groups, nanoparticles showed a tendency to agglomerate; they easily penetrated dentin tubules, but rarely infiltrated into the collagen matrix.

Bio-Si HAp particles showed a higher tendency to agglomerate than the corresponding nonsilanized samples (Bio). Huge agglomerates could already be seen with only 1% (wt/vol) of Bio-Si particles (Fig 4A), but only rarely with 1% (wt/vol) Bio nanoparticles. More and larger agglomerates could be found in TEM micrographs of either 10% (wt/vol) Bio or Bio-Si (Fig 4C). No difference could be seen between these two groups.

No differences were detectable between nonsilanized or silanized hydrothermal HAp particles. Hardly any agglomerates were apparent at 1% (wt/vol), while more and larger...
agglomerates were detected in TEM micrographs of 5% and 10% (wt/vol) specimens. Experimental adhesives containing Hyd and Hyd-Si nanoparticles showed lower propensities to build large clusters than did groups Bio and Bio-Si. In general, agglomerates of Hyd and Hyd-Si particles seemed to be smaller and less dense than those of Bio and Bio-Si (Figs 5B and 5C vs 5A), with adhesive resin being enclosed within the agglomerates (Fig 5B). SEM observations confirmed this observation (see above).

**DISCUSSION**

Two differently produced types of HAp particles were used in this study in order to assess the role of specific particle properties on the reinforcing mechanisms of nanoparticle-filled dental adhesives. An increase in maturation temperature of the calcium phosphate precipitate led to an increased definition of diffraction lines, indicating a much higher degree of crystallinity for the hydrothermally-grown HAp particles (71%) than for the biomimetically produced ones (5%). Higher crystallinity implies higher structural order and therefore higher density, so that Hyd particles should present improved mechanical properties, such as hardness and strength. As a consequence of the maturation period under higher temperatures and pressure, Hyd particles grew larger, reaching a more defined, rod-like form as well as a much higher hydroxylation degree (100% vs 33%). Biomimetic maturation resulted in smaller, irregular-shaped particles with a high surface-area:mass ratio.
Both the smaller specific surface and higher hydroxyl-
gation grade of Hyd particles could contribute to a lower ag-
glomerate tendency than that observed for Bio particles. This
is attributed to lower van der Waals forces between
particles and a high electrokinetic potential that tends to
maintain dispersion stability. The irregular shape of the
Bio nanoparticles is disadvantageous regarding particle
dispersion, since it increases surface area and reactivity
between particles. Our TEM micrographs confirmed the
existence of secondary large agglomeration centers for
Bio nanoparticles after simple mechanical and ultrasonic
mixing already at relatively low filler concentrations,
with an increase in the number of agglomerates with rising
filler content. Sadat Shojai\(^3\) obtained similar results com-
paring HAp nanoparticles prepared by either hydrother-
mal or solvo-treatment processes. The higher colloidal
stability of the hydrothermally-grown nanoparticles in an
experimental dental adhesive was attributed to its higher
surface charge and lower aggregation.

As agglomeration of nano-apatite fillers can adversely
affect the mechanical properties of the filled material,\(^7\)
silanization of the different particles was carried out to
decrease the surface energy\(^3\) and improve their wetting
properties.\(^15\) Particles matured at 25°C showed a specific
silane load of 0.64/nm\(^2\), while the silane load of particles
matured at 200°C was 0.90/nm\(^2\). This corresponds to a
sub-monolayer coverage.\(^4\) As silanol condensation takes
place on hydroxyl groups on the surface of HAp particles,
the higher hydroxylation grade of the Hyd than the Bio par-
ticles probably accounts for the higher silanization grade
of the former. However, for the hydrothermally synthesized
HAp nanoparticles, no difference in agglomeration could
be detected between silanized and nonsilanized particles.
For the biomimetically matured HAp nanoparticles, the
silanized species tended to agglomerate already at 1%,
while for the corresponding nonsilanized particles, ag-
glomerates were only detected at a filler degree of ≥ 5%,
which is contradictory to the supposed function of silane
functionalization. As neither the hydrophobic environment
nor the high viscosity of the adhesive resin support ag-
glomerate,\(^13,35\) it is probable that huge secondary clus-
ters are built prior to suspension of the nanoparticles in
the adhesive solution. SEM observations of the fractured
surfaces revealed that silanization was successful in im-
proving the bonding between fillers and the resin matrix,
whereas nonsilanized particles often demonstrated gaps
at the interface (Figs 3C and 3D). The improvement in bond
quality between particles and matrix, however, brought no
benefits regarding bond strength reinforcement and has
even shown to be detrimental for biomimetic particles at
the 1% loading level.

TEM and SEM have shown that the potential for particle
reinforcement of the adhesive bond is closely related to
how the particles disperse in the medium. Agglomerations
with different sizes were present for all groups with no det-
imental effect for bond strength up to 5%. Fractographic
evaluations showed that their fracture morphology was
mainly brittle, with the crack passing through them without
being deflected in a way that would result in an increase in
energy for failure. Figure 3A shows a typical fractographic
pattern occurring for all groups with a concentration ≤ 1%,
in which the particle agglomeration acts as a flaw at the
front of the crack tip due to the concentration of stress.

Here, only at concentrations over 10% do agglomerates
start to act as primary flaws, as has been suggested by
other authors.\(^3,4,15,17\) leading to failure at lower stress
levels, thus significantly decreasing bond strength. This
was shown for secondary clusters in contrast to primary
clusters.\(^14,17\) In that sense, it seems that the fraction
of particles not agglomerating is responsible for a po-
tential reinforcing effect. However, such potential seems
to lie within a narrow range of particle concentration, as
shown for nonsilanized Bio particles. Rather than density
or shape, size and surface properties seem to play a
more important role in particle reinforcement of dental
adhesives. As the present TEM micrographs showed that
the agglomerates were embedded within the adhesive

\[ \text{Fig 5} \quad \text{Stained TEM photomicrographs of HAp-particle agglomerates of different groups. The agglomerates of the 10% Bio-Si group (A) show high density, the particles seem to be compactly packed. Bar = 2 \mu m. (B) TEM photomicrograph of a stained specimen of the 5% Hyd group. Bar = 1 \mu m. (C) Stained section of the 10% Hyd-Si group. Bar = 1 \mu m. Agglomerates of the 5% Hyd- (B) and 10% Hyd-Si groups (C) show a less dense packing compared to those of the 10% Bio-Si group (A).} \]
layer and were not deposited on top of the hybrid layer, prevented penetration of adhesive monomers into the demineralized dentin can be excluded as a weakening mechanism at high filler content, which has been suggested previously.1,17,27

CONCLUSIONS

The reinforcing effect of HAp nanoparticles on a dental adhesive is strongly dependent on specific particle properties, concentration, and dispersion within the adhesive resin. Among the particle properties, size and surface-area:volume ratio seem to be of more importance than shape, silanization, or density of agglomerates.

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REFERENCES

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**Clinical relevance:** Including experimental HAp nanoparticles in a commercial adhesive exhibits limited clinical benefit. The bond strength to dentin, however, can be increased within a narrow range of nanoparticle fractions.