Antibacterial Dental Adhesive through Photocatalysis of Titanium Dioxide

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Degree project in applied biotechnology, Master of Science (2 years), 2009
Examensarbete i tillämpad bioteknik 30 hp till masterexamen, 2009
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# Table of Contents

Summary ................................................................. 2

Introduction ............................................................ 3

Results ................................................................. 10

Discussion ............................................................ 18

Conclusions and Future Experiments............................ 22

Materials and Methods ................................................ 23

Acknowledgements ..................................................... 28

References ............................................................. 29
Summary

The system of dental adhesive and restorative composite is considered to be the first choice in the treatment of dental decay. However, because of the shrinkage of the resin composite material and possible residual bacteria in the dental cavity, secondary caries occurs in about 40% of the cases. Much research has been done with the aim of achieving antibacterial properties with both the restorative resin composite and dental adhesives, using strategies such as agent releasing materials and particle mixtures. However, because of the problems of short release times, poor effectiveness in killing bacteria and reduction of bonding strength after incorporation of agents, commercial realizations of this research have been rare.

In this project, a novel dental adhesive was made by mixing TiO$_2$ nanoparticles with a commercially available dental adhesive. A photocatalytic reaction occurs when TiO$_2$ is irradiated under UV light. A series of radicals produced decompose organic molecules and can thus kill nearby bacteria. Several characterization tests were done to determine if this adhesive can kill the bacteria sufficiently and, at the same time, maintain its functionality as a bonding agent. Antibacterial tests showed that an adhesive composed of 20 wt% nanoparticles had a good bacterial killing effect. The mechanical properties of the nanoparticle/adhesive mixture were not significantly changed compared to the pure commercial adhesive. Additionally, the material was found to be not bioactive after one week of PBS treatment.
Introduction

**Dental caries treatment and the challenge**

Dental caries, also known as tooth decay, is a prevalent oral disease. The development of caries is a complex interaction between the tooth and the acid producing bacteria colonized in dental plaque. It is also influenced by host factors, like salivary dysfunction, dietary carbohydrates, and so on (Selwitz *et al.*, 2007). Without proper care, caries will progress until cavitations occur (Featherstone, 2000). In clinical practice, the dental cavity is treated by restorative dentistry. Dental amalgam has been used as restorative material for more than a century. Since the 1990s, system of adhesive and resin-based composite became the first choice for restorations instead of dental amalgam. The advantages of the adhesive and resin composite system include the reduced removal of dentin in cavity preparation, flexibility of the material to fit the cavity and better aesthetics through the control of the material color (Roeters, 2004). Many improvements in the material, technique and instruments have been made (Sarrett, 2005).

There are two main types of adhesives used today – total-etch and self-etch. With total-etch, the standard procedure for repair of caries is as follows: remove the infected dentin by means of drilling, etch the surface of the cavity wall and rinse, spread two thin layers of adhesive with a specific applicator, light cure the adhesive (polymerization of the resin components initiated by the radicals produced by the photoinitiator), press the restorative material into the cavity and light cure (or let set if self-curing), polish the surface of the restorative bulk and the interface of the dentin-adhesive-resin (3M ESPE, 2004; 3M ESPE, 1998).

Self-etch adhesives (see Fig. 1) are also popular in dental clinics because the acidic adhesive can perform the function of etchant. An advantage is that there is no problem of water retention in the etched dentine surface, which often occurs in the rinsing step when the dentist uses a separate etching step (Moszner, 2005).
However, there are significant problems with present dental restorations such as secondary (recurrent) caries and the resulting need for restoration replacement (Mjör, 1997; Peris, 2007). A main case is polymerization shrinkage, which is considered to result in the formation of a gap between the filling material and the tooth when the contraction stress exceeds the adhesive bonding strength in the dentin-adhesive-resin structure (see Fig. 2) (Davidson, 1997).
orthodontic applications. Evidence shows that the adhesive used to fix the bracket on the enamel actually help the adhesion and accumulation of bacteria (Imazato, 2003).

Although it cannot be conclusively shown that the microleakage along the cavity wall is the primary cause of secondary caries (Cenci, 2008), secondary caries are believed to be reduced by minimizing the marginal gap and ensuring better sealing of the exposed dentinal tubules due to the removal of the lesion (Sarrett, 2005; Bergenholtz, 2000; Love, 2002).

**Dental adhesives**

Dental adhesives have a wide range of applications, mainly in orthodontics and restoration. In a typical orthodontic device, a dental adhesive can be used to fix the brackets clamping the teeth. In restorative treatment, the use of dental adhesive and filling composite is the first choice for repair of dental caries. Dental adhesive can also be used for root surface desensitization and bonding of amalgam, porcelain and porcelain veneers on the surface of teeth (Nicholson, 1998; 3M ESPE, 2004).

The basic components of the dental adhesive include the resin matrix and photoinitiator. The photoinitiator produces initiator radicals to start polymerization of the resin matrix, which will form a solid network to supply required mechanical and bonding properties (Nicholson, 1998). A traditional photoinitiator used in dental material is camphoroquinone (CQ, see Fig. 3), a visible light sensitive diketone which can absorb light at a wavelength of about 470 nm and produce radicals for polymerization initiation (Cook, 1992). The adhesive exhibits good attachment to both enamel and dentine, of which the mineral phase (hydroxyapatite, Ca_{10} (PO_{4})_{6} (OH)_{2} ) is approximately 97% and 76%, respectively. Clinically, acidic etchant is used before adhesive application to create higher microporosity within the cavity surface to increase the available surface area for bonding. This etching step allows the adhesive to penetrate into the surface and form inter-locking “resin tags” after curing (Nicholson, 1998).
Figure 3. Left: Structure of the camphoroquinone (CQ), also known as 1, 7, 7-trimethylbicyclo (2.2.1) heptan-2, 3-dione. Right: the photoinitiating process of CQ (Picture resource: sci-toys.com/scichem/jqp066/25208.html; Moszner, 2005)

The adhesive used in this project was Adper™ Scotchbond™ 1XT Adhesive, manufactured by 3M ESPE. The resin matrix components include HEMA (hydroxyethylmethacrylate), BisGMA (bisphenylglycidyl dimethacrylate), and dimethacrylates (see Fig. 4). A patented photoinitiator system produces initiator radicals that start the polymerization when irradiated under high intensity light at about 470nm (3M ESPE, 2004).

Figure 4. Structure of commonly used monomers for dental adhesives (Picture resource: Moszner, 2005)

**Antibacterial property of dental adhesive**

Because of the high probability of secondary caries, much attention has been focused on improvement of the filling composite and dental adhesive (Imazato, 2003), including the antibacterial effect of the material and inhibition of demineralization (Ahn, 2009).
Self-etching dental adhesives contain an acidic functional group in the resin (See Fig. 1), leading to a pH of around 2 to 3 that provides an antibacterial effect due to the low pH (Feuerstein, 2007). However, it is not sufficiently effective to kill the bacteria.

A common way to achieve an antibacterial effect is to use an agent-releasing material (Imazato, 2003). Many kinds of agents have been incorporated into the adhesive resin, such as sodium fluoride, dodecylamine, bipyridine and so on (Imazato, 2003). The following paragraphs describe some agents that have been recently investigated.

Silver nanoparticles (with diameter less than 5nm) have been added to an orthodontic adhesive. As an antibacterial agent, silver ions are selectively toxic to prokaryotic microorganisms without harming eukaryotic cells. Due to the reduced surface free energy (SFE), bacteria adhesion is decreased and bacterial suspensions containing a silver filled adhesive showed slower bacterial growth (Ahn, 2009). Silver colloids have also been added to restoration composites and showed antibacterial effects (Kassaee, 2008).

Chlohexidine (Fig. 5) has proved to inhibit the host-derived degradation of collagen, which is the main organic component of the dentin and enamel. By adding 2 wt% CDA (chlorhexidine diacetate) to the resin adhesive, the adhesive can slowly release chlohexidine and has shown good effect in adhesive-dentin interface preservation (Hiraishi, 2008).

MDPB (methacryloyloxy dodecyl pyridinium bromide, see Fig. 5) has been successfully used in adhesives for arresting the residual bacteria after the cavity preparation and for adhesives that penetrate into the gap between the restoration material and cavity wall. The bonding strength of the adhesive was not significantly influenced by the addition of MDPB (Imazato, 2003). The commercially available adhesive Clearfil Protect Bond contains 5% MDPB (Imazato, 2006).

CPC (cetylpyridinium, see Fig. 5) is a well known antiplaque agent, which can destroy the cell wall of the bacterial by interrupting the negatively charged cell wall due to the positive charge of CPC. CPC has been added to an adhesive and showed good antibacterial effect. However, this strategy suffers from short release time, about 180 days, with the highest release in the first week (Al-Musallam, 2006). To solve this problem, CPC has been immobilized in a resin and tested. The results showed that bacteria on the surface of the CPC-resin were completely inhibited. However, there is no effect on bacteria not attached to the surface (Namba, 2009).
Although several agents have shown antibacterial properties, none claim to have long-lasting effect or completely killing of bacteria. Furthermore, some of the agents even decrease the bonding strength of the adhesive.

![Figure 5. Several antibacterial agents that have been investigated in previous research.](image)

**Photocatalysis of Titanium dioxide**

The portion of the electromagnetic spectrum containing ultraviolet light (UV) is subdivided into different regions. In the near to middle UV region we have UVA (400nm-320nm), UVB (320nm-280nm) and UVC (280nm-100nm). Although extensive exposure to UVA can lead to damage of collagen fibers, it is generally harmless under limited dosages, and does not cause sunburns like UVB does. UVB and shorter wavelengths cause direct damage to DNA and are therefore much more harmful to living organisms. The short wave UVC is generally blocked by ozone in the upper atmosphere, and is widely used for sterilization purposes in medical facilities and disinfection of drinking water as the high energy of the light results in damage to organisms.

For the anatase crystalline form of titanium dioxide, irradiation with UVA light having a wavelength less than 385nm can excite electrons above the material’s band gap of 3.2eV and thus generate an electron-hole pair. Reacting with a suitable electron acceptor, like oxygen, the excited electron will produce superoxide ions (O$_2^-$). The positive hole in the valence band can react with H$_2$O or OH$^-$ to produce hydroxyl radicals (·OH). Other reactive oxygen species, like hydroxyl peroxide H$_2$O$_2$ and singlet
oxygen can also be generated. The reactive oxygen species and radicals can diffuse and decompose nearby organic molecules into CO₂ and H₂O. This photocatalytic property has been used in solar cells, anti-fouling surfaces, self-cleaning coatings and so on (Maness, 1999).

Due to the high redox reaction ability of the photocatalysis products, TiO₂ can be used as a bactericide for the drinking water and indoor environments (Li, 2008). The mechanism is the destruction of the cell membrane or cell wall and causing leakage or structural damage of the cell (Maness, 1999). Research has demonstrated the killing of viruses, Gram-positive and Gram-negative bacteria, and even cancer cells by the photocatalysis of TiO₂. TiO₂ can even be metal doped to change the band gap energy and render TiO₂ photocatalytic under visible light (Li, 2008).

**Aims of this project**

The aim of this project was to investigate a novel dental adhesive made by mixing TiO₂ nanoparticles with a commercially available dental adhesive, Adper™ Scotchbond™ 1XT Adhesive manufactured by 3M ESPE.

Initially, three different candidate nanoparticles (NP) were assessed for photocatalytic activity. Included in this goal was the selection of an appropriate assessment technique for photocatalytic activity. Then the TiO₂ nanoparticles showing the best photocatalytic properties were mixed into the commercial dental adhesive. The antibacterial effect was tested using *Staphylococcus epidermidis*. Characterization of the nanoparticle/adhesive mixture (referred to as NP/adhesive in this report) was performed, including bioactivity and mechanical strength (tensile bond strength).
Results

Photocatalytic reaction rate of three types of TiO\textsubscript{2} nanoparticles

In figures 6 and 7, the photocatalytic reaction rate of three types of TiO\textsubscript{2} nanoparticles is shown. Figure 6 shows the decreasing rate of rhodamine B concentration in the first hour and figure 7 shows that of the overnight reaction. The plots show the absorbance of rhodamine B and are a direct measure of the degradation of this dye due to the photocatalysis induced by the TiO\textsubscript{2} nanoparticles. UV irradiation was the same for all three nanoparticle types.

Figure 6. Photocatalytic reaction rate over short time range (1 hour). (P25: TiO\textsubscript{2} nanoparticles from Degussa; NFP: mesoporous TiO\textsubscript{2} particles; GW: 20-30nm anatase nanoparticles with high degree of aggregation)

Figure 7. Photocatalytic reaction rate over long time range (18 hours)
In the test of commercial TiO₂ nanoparticles (P25), the pink color of rhodamine B disappeared in about 30 minutes while other two types of particles (GW and NFP) did not show obvious optical changes during the same time interval.

Under the reaction conditions of 50 mg nanoparticles, 45 ml of 5×10⁻⁶ M rhodamine solution, 0.6 mW/cm² UV strength and room temperature, P25 nanoparticles showed a rhodamine degradation rate of about 7.2 μM·g⁻¹·hr⁻¹ (i.e. 1 gram of nanoparticles degrade 7.2 micro moles of rhodaminB in 1 hour). The NFP TiO₂ nanoparticles showed a steady reaction rate of about 0.13 μM·g⁻¹·hr⁻¹. The GW TiO₂ nanoparticles didn’t show any effect in the first three hours, but subsequently showed a reaction rate of 0.24 μM·g⁻¹·hr⁻¹ in the following 15 hours.

**Photocatalytic reaction rate of the NP/ adhesive mixture**

Figure 8 displays the photocatalytic activity of nanoparticle/adhesive mixtures through the degradation of rhodamine B. Mixtures of 5%, 10%, and 20% NP/adhesive showed a good photocatalytic reaction rate, with the 10% NP/adhesive sample giving the highest reaction rate.

![Figure 8. Photocatalysis reaction rate of sample plates.](image)

A glass plate was used as control. Here the rhodamine B concentration increased over time, which might be caused by the evaporation of water due to the relative higher temperature under the UV lamp (38°C). A TiO₂ coated Ti plate was also used as a control. It gave a result similar to glass plate, which meant that the TiO₂ coated Ti plate had no significant photocatalytic effect under the UV irradiation.
The adhesive sample containing no TiO₂ nanoparticles showed a decrease in the rhodamine concentration for about 250 minutes and remained steady. After the test this pure adhesive sample was observed to have a bright pink color. Therefore it was hypothesized that the decrease in rhodamine B concentration could be due to two reasons: it may have been absorbed by the adhesive component or degraded through the photocatalytic reaction of TiO₂. Thus the following three stage experiment was performed to assess these two possibilities.

Figure 9. Three stage experiment to compare the adsorption and degradation of rhodamine B by the NP/adhesive.

Stage 1 is the absorption stage. An adhesive sample containing 20 wt% TiO₂ was submerged in 5 ml rhodamine solution and UV was not applied. After 3 hours of shaking, the solution was somewhat lighter in color (mostly pink still, see Fig. 9) while the sample plate turned from white to bright pink. Thus it is clear that the adhesive absorbs some dye molecules. In stage 2, the photocatalytic effect was examined by replacing the solution with fresh rhodamine B and applying UV light. After 3 hours of UV irradiation, the solution was much clearer compared to the control and to the solution at end of stage 1. Additionally the sample was less pink. This showed that more dye molecules were degraded due to photocatalysis than the amount that was absorbed into the sample. Finally, in stage 3 the rhodamine B solution was replaced
with water and UV light was again applied. After 2 hours under UV irradiation, the pink color in the sample totally disappeared and the water remained clear.

**Antibacterial test with low intensity UV irradiation**

Figure 10 displays the antibacterial effect of the nanoparticle/adhesive mixture. A clean glass plate and a TiO$_2$ coated Ti plate were used as controls. Bacteria collected from the samples were cultured on agar to assess the viability of the bacteria after the following conditions: 1) No UV irradiation; 2) 30 minutes UV irradiation; and 3) 2 hours UV irradiation. The UV intensity was 1.2 mW/cm$^2$, which is approximately the same intensity as the UV irradiation from the sun felt on a sunny day.

Figure 10. Antibacterial test result with low intensity UV irradiation

Good bacteria killing ability was observed with NP/adhesive mixtures of 10 and 20 wt% TiO$_2$ nanoparticles after 2 hours UV irradiation. The 10% sample had 12 colonies while the 20% sample had only one colony; thus 20% NP/adhesive is more effective than 10%. Neither the glass plate nor the TiO$_2$ coated plate showed a sterilization effect.

Interestingly, after 12h culture of the agar, the 10% and 20% samples with only 30 minutes of UV irradiation (II-2, III-2) didn’t show obvious bacteria growth. However, after 18 hours culture, the growth on II-2, III-2 showed up. This growth delay phenomenon occurred in the other bacteria tests as well.
**Antibacterial test with high intensity UV irradiation**

The results showed good bacteria killing effect with the 20 wt% NP/adhesive (only 20 wt% was tested). Only 7 colony forming units (CFU) showed up on the blood agar after 10 minutes UV irradiation (see Fig.11). When irradiated for 7 minutes, the quantity of the survived bacteria was still large compared to the sample that received 10 minutes irradiation (in Fig. 11 one can observe a light spread of bacteria on the agar). However, there was only one healthy colony and other colonies were very small compared to the well spread growth on the glass control or the 20% NP/adhesive plate that was not exposed to UV light.

![Antibacterial test result with high intensity UV irradiation](image)

**Bioactivity test & Surface morphology**

Figure 12 shows a scanning electron microscopy (SEM) image of a TiO₂ coated Ti plate after incubation in PBS for one week. The hydroxyapatite (HA) crystal growth is clearly evident as “leaves” growing up from the otherwise smooth TiO₂.
X-ray diffraction analysis also showed typical hydroxyapatite peaks, indicating good bioactivity according to the hydroxyapatite crystal formation on the surface. Conversely, all the NP/adhesive mixture sample plates were not bioactive. NP/adhesive with 0%, 5%, 10%, 20%, 30% and 40% TiO₂ nanoparticles were incubated in PBS solution for one week without any HA growth. However, the PBS seemed to change the surface morphology of the sample somewhat. The same phenomenon observed with 20 wt% NP/adhesive, shown in Fig. 13, was also observed on samples with 5% and 10% TiO₂ nanoparticles.

![Figure 12. SEM image of TiO₂ coated Ti plate showing bioactivity.](image1)

![Figure 13. SEM images of 20%NP/adhesive before and after 1 week of PBS treatment.](image2)
Mechanical strength testing of NP/adhesive

The loaded force at which the bonds of the HA-NP/adhesive-composite sandwich structures (see Fig. 19) failed was measured using a material tester and recorded automatically with the accompanying software. Figure 14 shows the plot of the mean tensile bond strength of the five sample types while Table 1 presents the corresponding statistical data. The bonding area was circular with a diameter of 4 mm.

Figure 14. Plot of the mean tensile strength of the adhesive bonds using pure adhesive, 10%, 20%, 30% and 40% NP/adhesive. The error bars represent ± 1 standard deviation.

Table 1. Statistical data for the tensile tests.

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<th></th>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
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<td>5.32</td>
<td>6.26</td>
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<tr>
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<td>34.80</td>
<td>48.71</td>
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<td>249.99</td>
<td>230.36</td>
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<td>15.00</td>
<td>13.00</td>
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<tr>
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<tr>
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<td>6.55</td>
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<tr>
<td>Variance</td>
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<td>150.11</td>
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<tr>
<td>Std Error</td>
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<tr>
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<td>-1.46</td>
<td>-1.03</td>
</tr>
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The tensile test results were analyzed using a one-way analysis of variance (ANOVA) to determine if the mean values of tensile bonding strength differed significantly from each other. The ANOVA analysis gave an F value of 0.1080 and the probability of the null hypothesis (i.e. that there is no effect of percentage of nanoparticles in adhesive on the mean tensile strength) of 0.98, which means that the percentage of nanoparticle in the adhesive does not significantly influence the bonding strength.
Discussion

**Photocatalytic reaction rate of three types of TiO₂ nanoparticles**

Under the same UV irradiation conditions, P25 nanoparticles showed the best photocatalytic reaction rate with rhodamine B degradation, which was about 30 times that of GW and 50 times that of NFP. Therefore P25 was used in all subsequent tests.

NFP is a mesoporous TiO₂ material, with an average pore size of 3.67nm and average particle size of 750nm. However, the size distribution of the particles is extremely large (see Fig. 17). The lower photocatalytic ability of NFP might be because of the SiO₂ in the crystal of anatase, which is added for the purpose of functionalization. Another reason could be due to the lower surface area exposed to the UV light.

The photocatalytic reaction ability of GW was improved after 3 hours of stirring and UV irradiation. This delay might be due to the some de-aggregation of the particles. The particles had been stored for 1.5 years and had a high degree of aggregation. From the SEM pictures (see Fig. 17), we can see the high degree of aggregation of GW nanoparticles, likely resulting in the reduced photocatalytic reaction rate.

P25 is the mixture of anatase and rutile crystalline phases (3:1) produced by Degussa. It is named P25 because the particle size of anatase crystal is 25nm (see Fig. 17). P25 has been observed to be more photocatalytically active than the pure crystalline phases of anatase or rutile (Ohno, 2001). Degussa P25 is recognized as a standard photocatalyst used in many photocatalysis researches. (Ohno, 2001)

**Photocatalysis reaction rate of P25/ adhesive mixture**

The nanoparticle/ adhesive mixture showed a high reaction rate with rhodamine B. Although the nanoparticles were encased in the network of the dental adhesive polymer network (see Fig. 15), the photocatalytic reaction was not totally suppressed. This might be due to the porosity of the polymer. The nanoparticles obviously still produced radicals, which likely diffused out the bulk of NP/adhesive until they came in contact with a dye molecule. Figure 15 shows the surface morphology of the 20% NP/adhesive.

The TiO₂ coated Ti plate did not show significant photocatalytic ability, although X-ray diffraction showed the TiO₂ coating to be predominately anatase crystalline. Reactive magnetron sputtering with increasing oxygen content was used to make the TiO₂ coating and resulted in a TiO₂ thickness of about 40nm with a 70nm gradual transition from TiO₂ to Ti. The lack of photocatalytic ability may be due to the thinness of the
TiO$_2$ layer. (Engqvist, 2008) The close proximity of the metallic Ti may serve to quench the generation of electron-hole pairs after UV photon absorption in the TiO$_2$.

![Figure 15. The surface morphology of the 20% NP/adhesive.](image)

Although the adhesive absorbs rhodamine resulting in a decrease of rhodamine concentration, the photocatalytic reaction was found to be the predominant factor for the decrease in rhodamine B concentration. The absorption of dye in adhesive can be explained by the charge of the rhodamine B. The color part of Rhodamine B is positive charged and balanced by chloride anions (see Fig. 16). The adhesive polymer components (see Fig. 4) with repeated ester bonds, give the possibility to form hydrogen bonds with the dye molecule, thus leading to the observed absorption.

![Figure 16. Structure of Rhodamine B](http://en.wikipedia.org/wiki/File:Rhodamine_B.svg)
**Antibacterial test with low intensity UV irradiation**

Gram positive *Staphylococcus epidermidis* bacteria were used in the Antibacterial tests. Although these bacteria are different from the anaerobic bacteria more commonly associated with tooth decay, they served as a good model for testing the Antibacterial effect since gram positive bacteria have a thicker cell wall and thus are generally more difficult to kill using photocatalysis. The main reason that anaerobic bacteria were not used was the difficulty in culturing such bacteria after the UV irradiation.

The photocatalytic ability of 10% and 20% NP/adhesive is strong enough to kill almost all the bacteria after 2 hours of irradiation under low intensity UV (1.2mW/cm²). 30 minutes of UV irradiation will kill bacteria, but to a much smaller degree. The slower growth of bacteria with 30 minutes UV irradiation on the surface of 10% and 20% NP/adhesive could be due to non-fatal damage of the bacteria and a smaller population at the start of the post-irradiation incubation.

**Antibacterial test with the high intensity UV irradiation**

The results showed good bacteria killing effect with the 20% NP/adhesive within 10 minutes. As we used a fan for air circulation and an ice bath to keep the temperature from rising, the water in the bacteria suspension was prevented from evaporating. Thus the influence of dryness and heat was greatly suppressed.

Considering that the number of bacteria put on the sample plate was about $10^6$, the survival ratio ($10^{-6}$) was quite small after 10 minutes UV irradiation on 20% NP/adhesive.

For the 7 minutes of irradiation sample, the quantity of the survived bacteria was large compared to the sample exposed to 10 minutes of irradiation. However, there was only one healthy colony and other colonies were very small. There was no good spread growth as with the glass control or 20% NP/adhesive sample without UV irradiation. This suggests that the bacteria cells were partially damaged but not enough to kill them (The gram positive bacteria have a thick cell wall for protection).

**Bioactivity test**

Bioactivity, which is critical factor for implant materials, refers to the desirable formation of hydroxyapatite on the surface of the material. Hydroxyapatite can be specifically recognized by growth factors or signaling receptors that results in extracellular matrix or other tissue growth on the material. In our case, if the adhesive is bioactive, the micro gap between the tooth and the restoration could be sealed by hydroxyapatite, the main inorganic component of dentin and enamel. So far, no resin
based dental adhesive has been reported to be bioactive. However, a mixture of 55.6% TiO$_2$, 14.8% polymethyl methacrylate (PMMA) and 29.6% methyl methacrylate (MMA) is bioactive, according to Kokubo, 2007.

The sample plates of 0%, 5%, 10%, and 20% NP/adhesive did not show bioactivity. There are two possible reasons for this result: The P25 nanoparticles cannot provide a uniform surface for the hydroxyapatite growth, or the resin surrounding the nanoparticles affects the environment for hydroxyapatite growth.

As can be seen in Fig. 13, the PBS treatment changes the surface morphology of the NP/adhesive sample plates. Consequently one could expect that the photocatalytic ability would be changed if the photocatalysis depends on the porous surface. Therefore it is desirable to test the long term antibacterial effectiveness of the NP/adhesive in future studies.

**Mechanical strength testing of NP/adhesive**

The result of the ANOVA analysis showed that the influence of percentage of nanoparticles in the adhesive did not significantly change the bonding strength of the adhesive. This means that the adhesive maintains the bonding function of composite-on-dentin.

As mentioned in the introduction, decrease in bonding strength is a major limitation for application of many kinds of incorporated agents, like CPC and chlohexidine. The good bonding strength of NP/adhesive could be due to that “resin tags” that can form in the case of NP/adhesive. Inclusion of nanoparticles in the adhesive definitely changes the mechanical properties of the bulk of adhesive. However, the bulk of NP/adhesive is still stronger than the interface of dentin-adhesive. Results from the tensile testing showed that with both pure adhesive and NP/adhesive, the bonding failures always happen in the interface of the hydroxyapatite tablets and adhesive, and not within the adhesive or composite structures.
Conclusion and Future Experiments

In this project, a novel dental adhesive was designed by mixing TiO$_2$ nanoparticles into a commercially available dental adhesive.

Tests of the photocatalytic reaction rate showed that 10 to 20 wt% P25 nanoparticles was sufficient to achieve good photocatalytic activity. The antibacterial effect of the P25 nanoparticle/adhesive mixture using UV light was found to be sufficient to kill all bacteria on the surface of the NP/adhesive by irradiating with a low intensity UV lamp (1.2 mW/cm$^2$) for 2 hours. A higher intensity UV lamp (7.5 mW/cm$^2$) decreased the time to achieve similar antibacterial effect to less than 10 minutes.

The bonding strength of the adhesive is a critical factor. In this project, we tested the bonding strength of the pure adhesive compared with NP/adhesive mixtures of 10%, 20%, 30%, 40% nanoparticles. The adhesive samples were used to bond a dental composite to a sintered hydroxyapatite tablet (tooth mimic) and tensile strength testing was performed. It was found that the percentage of nanoparticles did not influence the adhesive bonding strength.

From the results of SEM and XRD, NP/adhesive mixture did not show bioactivity after one week of PBS treatment.

Considering the high risk of secondary caries with the application of resin-based dental adhesive and filling composite, the antibacterial effect of the dental adhesive is highly desirable. We observed good antibacterial effect without negatively affecting the bonding strength of the adhesive, which an improvement compared to commercially available solutions for antibacterial adhesives. However, the UV dose needs to be optimized (both concerning UV strength and irradiation duration) to achieve a safe, but short UV application time. It is suggested that strong UV diodes should be used in future tests.

The bioactivity of the dental restorative material is a means by which bacterial colonization on the tooth surface (i.e. dental plaque, biofilms) can be greatly reduced. Future work should be directed to making the NP/adhesive surface bioactive as this would provide a significant advantage over present antibacterial dental materials.
Material and Methods

**Photocatalytic reaction rate of three types of TiO\textsubscript{2} nanoparticles**

Three types of TiO\textsubscript{2} nanoparticles were investigated in order to find the nanoparticles with the best photocatalytic activity. The first type, designated P25 in this study, was purchased from Degussa Company. The second type, designated NFP in this study, is a mesoporous nanoparticle synthesized at the division of nanotechnology and functional materials at Ångström Laboratory, with an average size of 750nm. The third nanoparticle type investigated, designated GW, is primarily in the 20-25nm size range. The nanoparticles were highly aggregated. SEM images of all three nanoparticles types are shown in Fig. 17. For photocatalytic activity studies, 0.05 ±0.005 g of powdered sample was dispersed in 45 mL of rhodamine B solution (5 \(\times\) \(10^{-6}\) M, pH ~6, Sigma). The dispersion was irradiated with UV light (1 mW/cm\(^2\), 360-380 nm) while stirring with a magnetic bar. Aliquots were collected at various time intervals to monitor the degradation of rhodamine concentration using a UV-VIS spectrophotometer (UV-1650pc, SHIMADZU). Samples were centrifuged to remove the nanoparticles and the absorbance at 554nm was measured. The reaction rate of rhodamine B degradation was obtained from a first order plot.

![Figure 17. SEM pictures of three types of TiO\textsubscript{2} nanoparticles. From left to right: NFP, GW, P25](image)

**Photocatalysis reaction rate of P25/ adhesive mixture**

The antibacterial adhesive was made by adding P25 TiO\textsubscript{2} nanoparticles directly into the commercially available dental adhesive Adper\textsuperscript{TM} Scotchbond\textsuperscript{TM} 1XT Adhesive (3M ESPE, St. Paul, Germany) at the w/w P25 percentages of 5\%, 10\%, and 20\%. Control samples included a glass plate, adhesive without P25 and a TiO\textsubscript{2} coated Ti plate (a titanium plate with increasing degree of oxidation towards the surface giving a TiO\textsubscript{2} layer of approximately 40 nm thickness.)
To determine the photocatalytic reaction rate of our nanoparticle/ adhesive mixture, the mixture was spread over an area of 4 cm² and placed into 25mL of rhodamine B solution (5 × 10⁻⁶ M, pH ~6). The sample plates were irradiated with the UV light (0.6 mW/cm², 360-380 nm, Philips, TL/10 UV-A, 15W) while gently shaking. Aliquots were collected at various time intervals to monitor the degradation of rhodamine concentration by measuring the absorbance at 554nm. The rate constant for degradation was obtained from a first order plot.

**Antibacterial test with low intensity UV irradiation**

The UV lamp (360-380nm) produced a UV intensity of about 1.2 mW/cm² at the sample surface, similar to the UV strength of sunlight at the earth’s surface. The samples and UV irradiation times used in the bacterial test were as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface</th>
<th>Sample</th>
<th>UV irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Glass plate (no TiO₂, no NP/adhesive)</td>
<td>I-1</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-2</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-3</td>
<td>2 h</td>
</tr>
<tr>
<td>II</td>
<td>10% P25 TiO₂ NP in adhesive</td>
<td>II-1</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II-2</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II-3</td>
<td>2 h</td>
</tr>
<tr>
<td>III</td>
<td>20% P25 TiO₂ NP in adhesive</td>
<td>III-2</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III-3</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III-3</td>
<td>2 h</td>
</tr>
<tr>
<td>IV</td>
<td>Plates with TiO₂ coating</td>
<td>IV-1</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV-2</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV-3</td>
<td>2 h</td>
</tr>
</tbody>
</table>

The growth of anaerobic dental bacteria *Streptococcus mutans* was not successful, so resilient Gram positive bacteria with a thick cell wall was selected to test the antibacterial effect. Gram positive *Staphylococcus epidermidis* was used in the bacterial killing test. *S.epidermidis* was inoculated into 40 ml of LB broth culture medium and placed in a 200mL flask (sterilized) and overnight cultured while gently shaking in 37 ºC incubator. Bacteria were collected by centrifugation (5000 rpm, 8 minutes) and re-suspended in 300 µl of sterile distilled water. Bacterial suspensions (30 µl each) were spread on the 3 sample plates for each group. UV irradiation was applied for 30 min or 2 hr, while the controls without UV irradiation were only exposed to air for 2 hours. For the 2 hr irradiated samples, every half an hour a drop of water was spread on the bacteria area to compensate for loss of water due to the higher
temperature (about 38°C) under the UV light. After the test (30 min or 2 hr), the bacteria were collected using 20 μl water to wash and re-suspend the bacteria from the surface. Every surface was washed and collected twice. The collected bacteria were then transferred to a piece of blood agar culture and cultured overnight in a 37 °C incubator prior to a check for colony formation units (cfu).

**Antibacterial test with high intensity UV irradiation**

![Diagram of high intensity UV lamp apparatus]

The antibacterial test was performed in the same manner as with the low intensity UV lamp tests except for the use of a stronger UV lamp (Philips, HPA S400, 400W). The emission spectrum of the lamp is shown in figure 18. A filter was used to block short wavelength light (UV under 320nm). Higher intensity UV (7.5mW/cm2) was applied by putting the sample plates under the filter inside the UV lamp chamber. The ice in the
chamber was used to preventing the temperature from increasing rapidly and excluding the possibility of bacteria death caused by dryness or heat. UV irradiation time was set at 0min, 7min and 10min. The tests were done with 20 wt% P25 TiO$_2$ nanoparticles in the adhesive with a glass plate as a control sample. Table 3 describes the tests performed.

Table 3. The samples and UV irradiation time used in the high intensity UV antibacterial test.

<table>
<thead>
<tr>
<th>Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Glass plate (no TiO$_2$, noNP/adhesive)</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>II</td>
<td>10% P25 TiO$_2$ NP in adhesive</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10min</td>
</tr>
</tbody>
</table>

**Bioactivity test**

Bioactivity refers to a material’s interaction with or effect on cell tissues in the human body. In our case it is bone bioactivity that is of interest, and thus it refers to the ability of the material to promote bone tissue growth or bone bonding. Testing was done by placing NP/adhesive mixture samples, as well as a TiO$_2$ coated Ti plate in a simulated body fluid, phosphate buffered saline (PBS), and checking for subsequent apatite growth on the surface.

The sample plates were cleaned and placed in PBS (pre-warmed in 60°C for 2 hours) and incubated at 37°C for one week. Scanning electron microscopy and X-ray diffraction were used to analyze the hydroxyapatite growth on the sample surface.

**Mechanical testing of tensile bonding strength of NP/adhesive**

The functional adhesion property of the adhesive mixed with P25 TiO$_2$ nanoparticles was tested through tensile testing of samples composed of a hydroxyapatite tablet bonded with the adhesive mixture to a dental filling composite (Filtek Z250 produced by 3M ESPE, St. Paul, Germany). This arrangement mimics the bonding of a dental cavity filling to the tooth. Figure 19 displays a typical test sample.

Hydroxyapatite (HA) is the main inorganic component of the enamel and the dentin, and therefore HA tablets were used instead of real teeth in the tensile test experiment. One gram of hydroxyapatite was pressed into a tablet (Φ12mm) under the pressure of 90 MPa. This green body was sintered in air at 1100 °C for 2 h, at a furnace ramp rate of 2 °C/min. The final density of the HA tablet was 2.16 g/cm$^3$. 
The adhesive used was Adper Scotchbond 1XT produced by 3M ESPE. Before tensile testing, the sample adhesive mixture was made by mixing 10%, 20%, 30% and 40% (w/w) of P25 TiO$_2$ nanoparticles with the adhesive. The sample adhesive was spread over a circular area of 4 mm diameter in the middle of the HA tablet. The polymerization via light curing of the adhesive and the dental filling material were performed according to the instructions of the manufacturer. The bonded structures were stored in water for at least half an hour before the tensile test.

Figure 19. The sample adhesive with 0%, 10% or 20%, 30% or 40% TiO$_2$ nanoparticles were used to bond the HA tablet (lower portion) and the dental filling material (upper portion).

The tensile testing of the bonded structure was performed using the two custom made holders, which hold the HA tablet and the composite filling material separately. (See Fig. 20). A universal mechanical tester Instron 5544 performed the tensile testing using a cross head speed of 1 mm/min. The loaded force at which the structure failed was recorded automatically by the software Merlin.

Figure 20. Left: the tensile test instrument, Instron 5544, with a sample loaded in the holder. Right: The cross section of the two holders (grey), which were used to fix the bonding structure.
Acknowledgement

I still remember how excited I was at the moment I knew there was a possible project for me in this wonderful group, Nanotechnology and Functional Materials.

Firstly, I want to thank Professor Maria Strømme and my supervisor Dr. Ken Welch to give me this golden opportunity to work in this multidiscipline group. Also thank Dr. Håkan Engqvist, Associate Professor, to bring me the world of dental materials and the basic idea of my project.

Ken, my super supervisor, gave me lots of good instruction on the experiments; made me tools needed in the project, taught me techniques like SEM, and encouraged to do my own ideas. Working with him, I always try to think “why is it” and “how can we prove it”. He never pushed me when I had bad results and always said encouraging words when I got some good results. In sum, he provided me a scientific but relaxing work atmosphere.

Specially, I want to thank Group of Polymer Chemistry, led by Professor Jöns Hilborn, for providing me the instrument for tensile test, which is a big part of my project. Jonas Mindemark, Ph.D. student in polymer chemistry group, taught me the operation of the instrument and helped me to explain my result.

Ylva Molin in Section of Infectious Diseases helped me with bacteria strain, culture medium and sterile stuff, with which the bacterial tests were successful and always under control. Göte Swedberg in Department of Medical Biochemistry and Microbiology provided me strains of oral bacteria which would be a focus of future experiment.

I really appreciate the help from those, who spent their own time and helped analyzing my samples; Albert did SEM for me and Johan did XRD for me. Babu and Aamir, who always work in the weekend, made me feel my enthusiasm to work. My good friends in this group shared not only their knowledge, experience but also good activities, tasty food and office. Thank you all, Aamir, Albert, Alfonso, Babu, Chunfang, Erik, Gustav, Johan, Mattias, Natalia, Teresa!


